Microbiology lec. 9 + lec.10

Bacillus and Clostridium

By:

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Learning Objectives:

After this lab. You must be able to:

- Distinguish between G +ve rods genera.
- Describe each species of Gram positive rods microscopically and culturally.
- Differentiate between *Bacillus anthracis* and other saprophytic species.
- Differentiate between *Clostridium* spp.
- List types of clinical infections these organisms produce
- Predict G +ve causative agents causing clinical cases.
- Discuss the principles of identifying tests.
- Know prevention ways of some organisms.

The family Bacillaceae consists of rod-shaped Gram-positive bacteria that form endospores. The family includes:

Spore-forming G positive bacilli

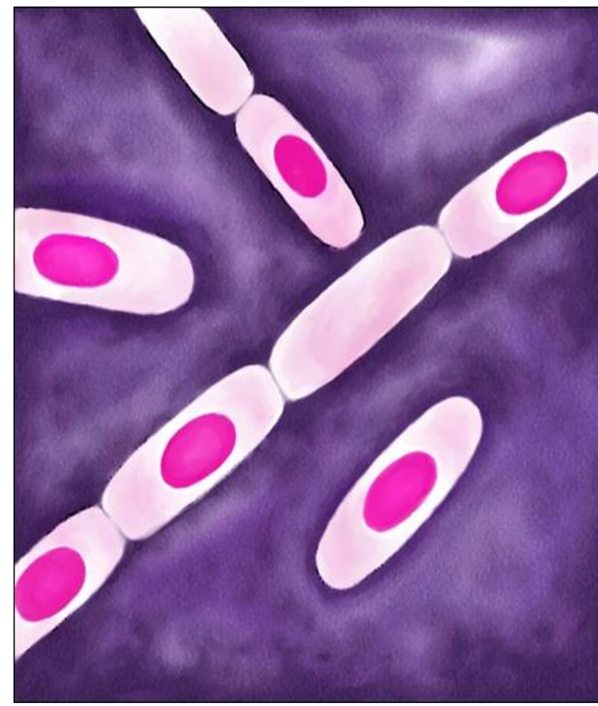
Bacillus

1- Aerobic spore forming: *Bacillus*

2- Anaerobic spore-forming: *Clostridium*

Bacillus

- **Large G +ve rods arranged in chains.**
- aerobic
- saprophytic prevalent in soil, water, air & on vegetation.
- Non motile spore forming (the location of the spore is either central, terminal, or subterminal according to species).



Human infections caused by Bacillus sp.

Bacteria	Diseases
Bacillus anthracis	**Anthrax** (cutaneous, gastrointestunal, and inhalational) and anthrax meningitis
Bacillus cereus	**Gastroenteritis**, intravenous catheter septicemia, and endocarditis
Bacillus lechniformis	Gastroenteritis
Other Bacillus sp.	Opportunistic infections

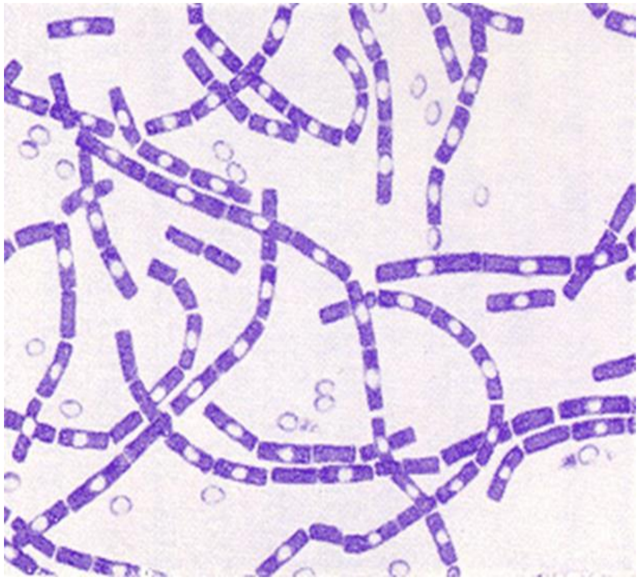
Bacillus anthracis

The causative agent of **Anthrax**, which is a primarily a disease of animals.

- **Morphology:**

- Large G+ve rods, arranged in long chain, with square end (**bamboo-stick**) appearance.
- Capsulated (**polypeptide**)
- Non motile
- Non hemolytic.
- Spore-forming (**central spore, the same width of the cell**).

Bacillus anthracis



❑ **Cultural characteristics:**

- **On nutrient agar:** medusa head.
- **On blood agar:** non hemolytic, ground glass appearance, and some times have tail.
- **On penicillin- containing media:** strings of pearl phenomenon.
- **On gelatin medium:** inverted fir tree appearance

❑ **Antigenic structure:**

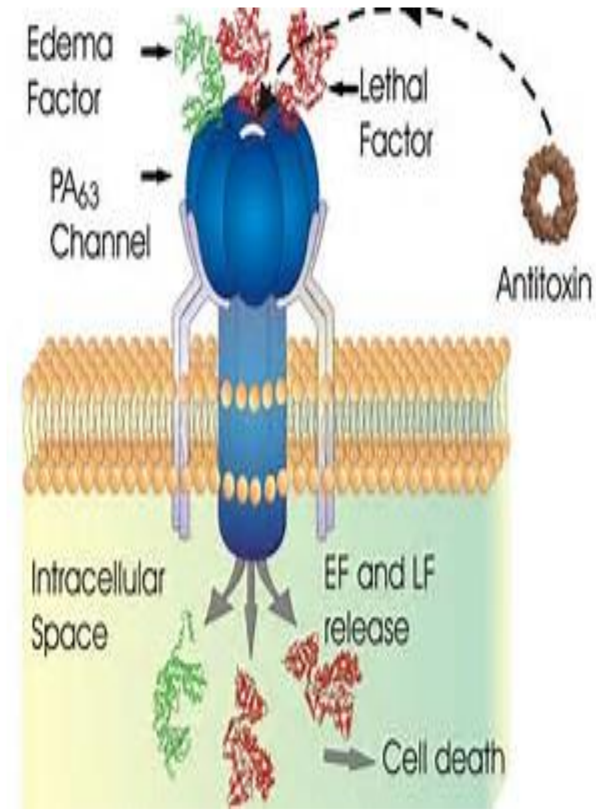
- Capsular antigen
- Cell wall antigen
- Somatic antigen

Bacillus anthracis

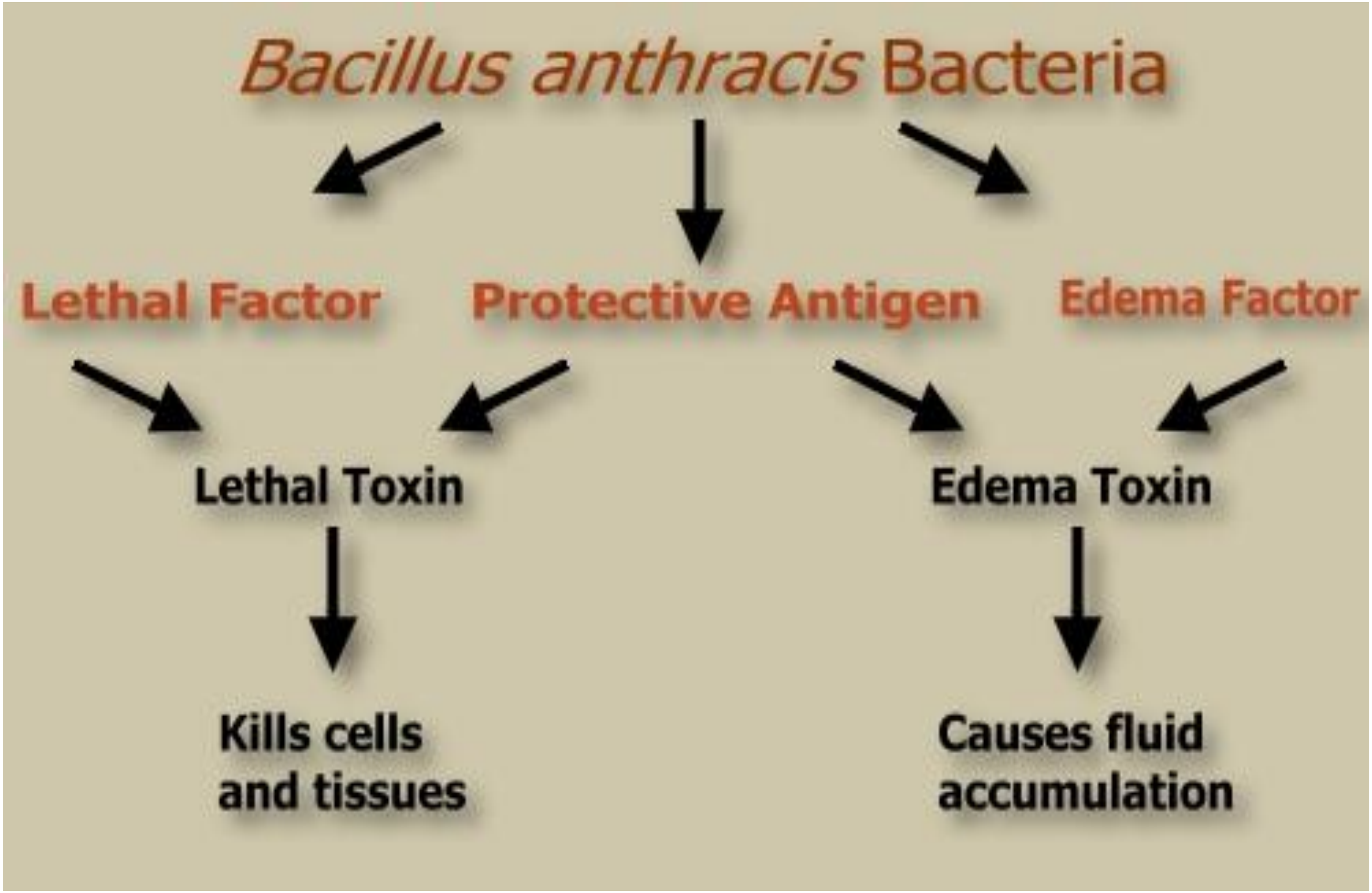
❑ Virulence factors:

- **Capsule:** poly- D- glutamic acid capsule, antiphagocytic.
- **Anthrax toxin- complex:** composed of three components:
  - **Protective antigen (PA):** is the binding domain of anthrax toxin binds to specific cell receptors forming a membrane channel that mediate entry of EF & LF into the cell.
  - **Edema factor (EF):** causes cellular edema within the target tissue and inhibit neutrophil function.
  - **Lethal toxin (LT):** the major virulence factor and causes death in infected animals.

Virulence factors of *B. anthracis*

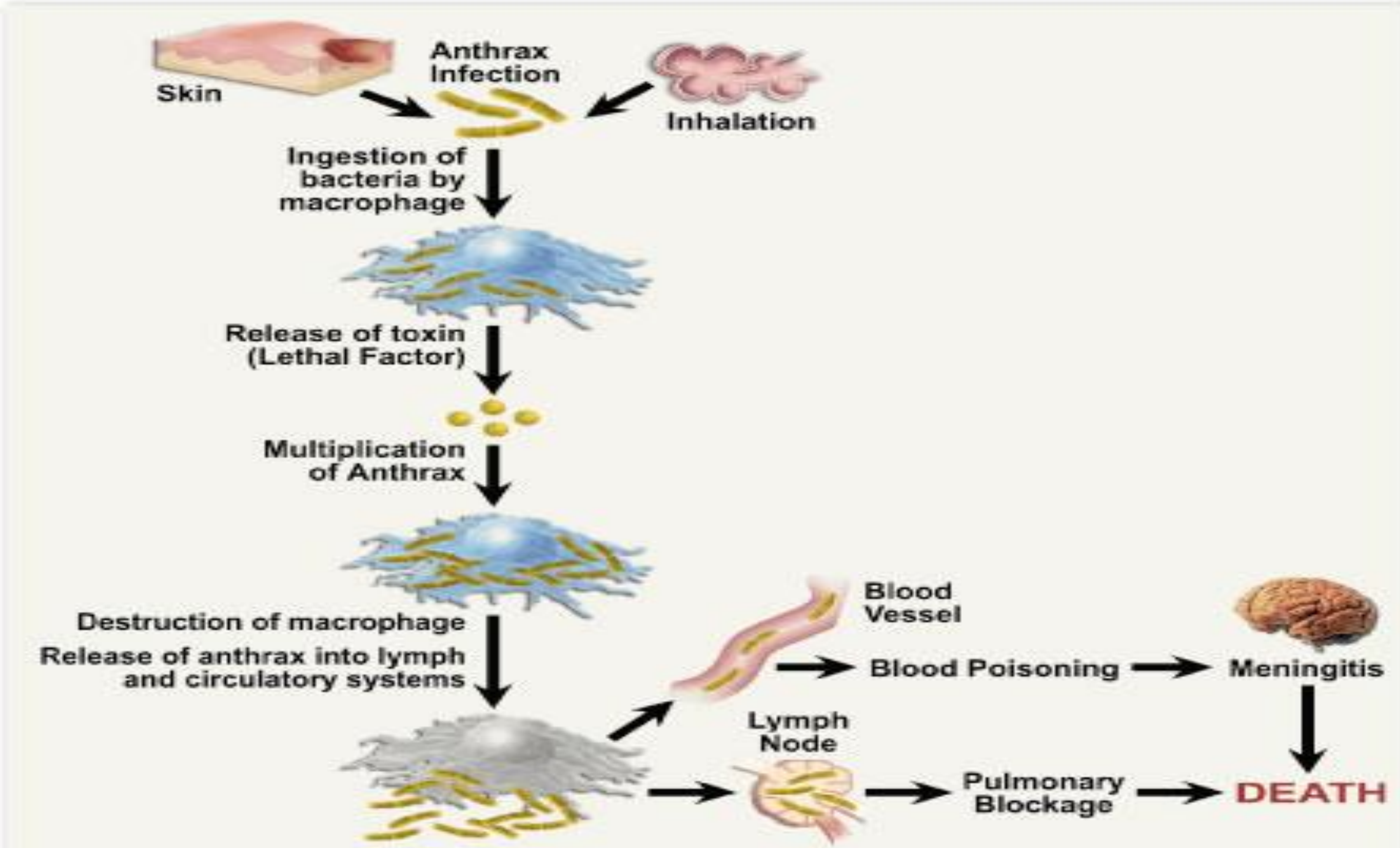


❑ Pathogenicity



❑ Pathogenesis of *B. anthracis*

How the Bacterial Toxin "Lethal Factor" Results in the Fatal Spread of Anthrax



Source: Dixon et al., Anthrax. New England Journal of Medicine 341:815-826, 1999.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- **Clinical findings:**

- In human, 95% of cases are cutaneous anthrax & 5% are inhalation anthrax.

- **Cutaneous anthrax:** *generally occur on arms* or hands & less frequently on face & neck.

Stages of cutaneous anthrax:

- **Papule** at the site of entry (wound or scratches).
- **Vesicle**
- **Eschar:** malignant pustule

- **Gastrointestinal anthrax:**

- Ulcers
- Bloody diarrhea, due to necrosis and ulceration which produces GI hemorrhage.
- Renal failure due to anthrax toxin.
- Death in untreated cases (MR more than 50%).

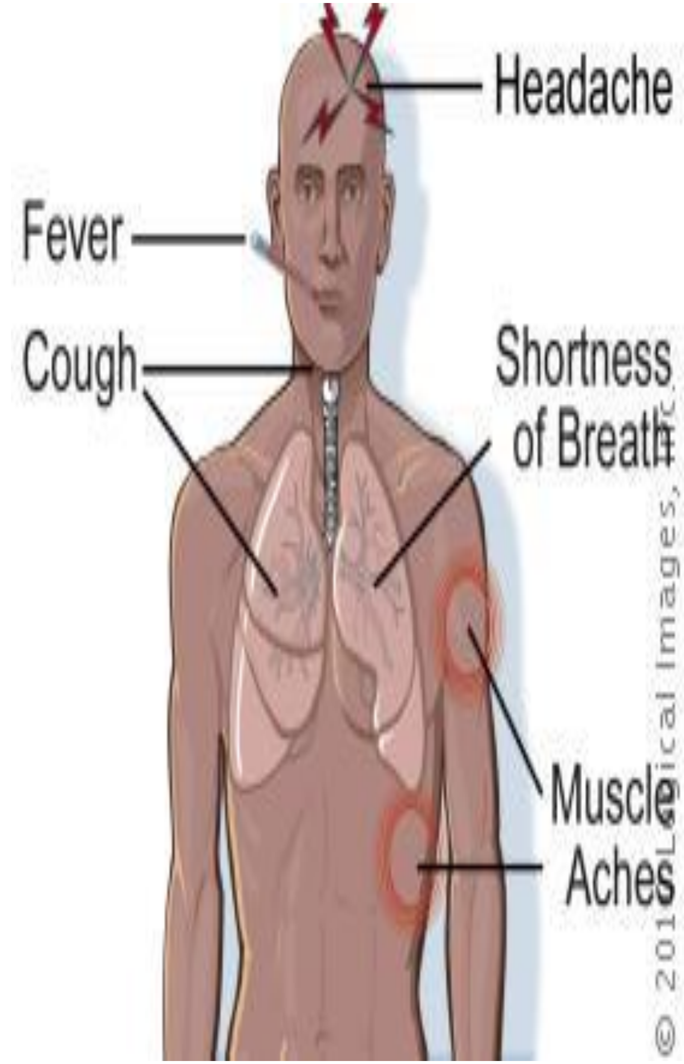
- **Oropharyngeal anthrax**

❖ **Inhalation anthrax: wool sorter's disease**

- Non specific symptoms: after 1-6 days incubation period low grade fever and non productive cough.

- Second phase: The early clinical manifestations of inhalation anthrax is marked after 1-2 days hemorrhagic necrosis & edema of the mediastinum & substernal pain, high fever, shortness of breath, tachypnea, and hematemesis.

Sepsis may occur. Spread to the meninges causing hemorrhagic meningitis. The fatality rate is 85-90%.

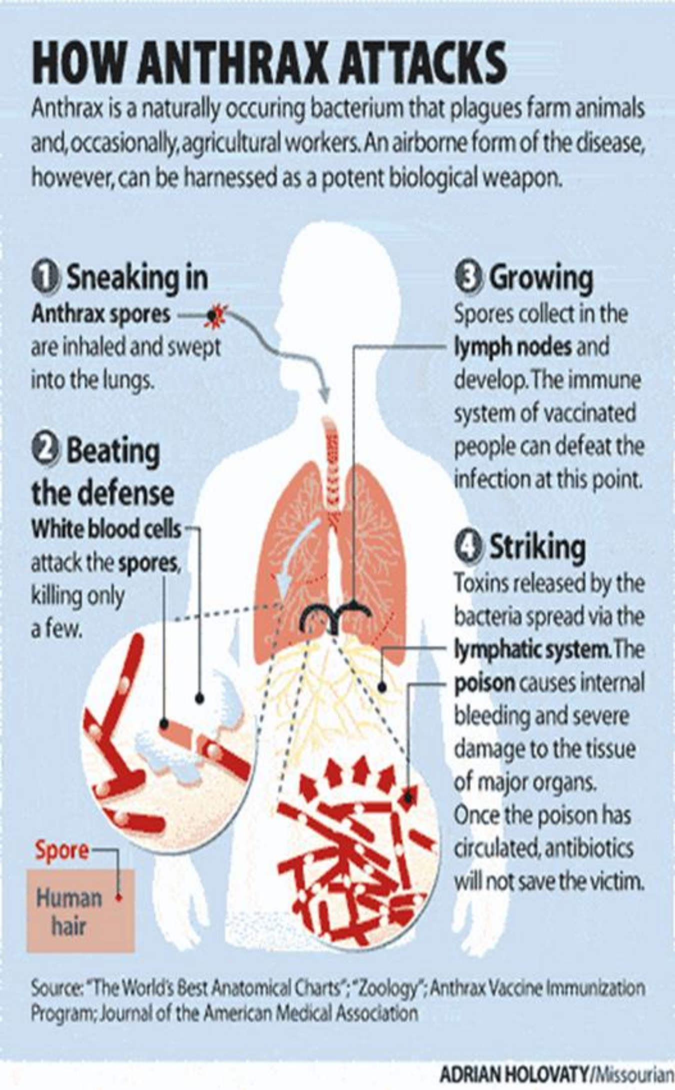


❑ **Immunity:** specific antibodies to anthrax toxin (primarily against PA), and capsular antigen.

- **Epidemiology:** soil is contaminated with anthrax spores from the carcasses of infected animals. These spores remain viable in soil for decades.

- **Prevention and control:**

- **Chemoprophylaxis** with antibiotics: is indicated for people who have been exposed to anthrax but do not have symptoms of the disease (ciprofloxacin and tetracyclines).
- **Immuno-prophylaxis**: A noncellular human anthrax vaccine called anthrax vaccine adsorbed (AVA).
- **Decontamination of animal products:** Chemical disinfection, Burn carcasses.



B. cereus

- Causes **food poisoning** which has two types:

- **emetic type:** associated with fried rice, is manifested by nausea, vomiting, abdominal crumps, it is self-limited, recovery occur within 24 hrs.
- **diarrheal type:** associated with meat & sauces, (1-24 hrs incubation period) manifested by profuse diarrhea with abdominal crumps & pain
- **eye infection**

Spore-forming G positive bacilli

B. cereus

Differentiating features between *Bacillus anthracis* and *Bacillus cereus*

Characteristics	Bacillus anthracis	Bacillus cereus
Motility	Nonmotile	Motile
Capsule	Capsulated	uncapsulated
Medusa head colony	Present	Absent
Hemolysis on sheep blood agar	Absent	Present
Gelatin liquefaction	slow	rapid

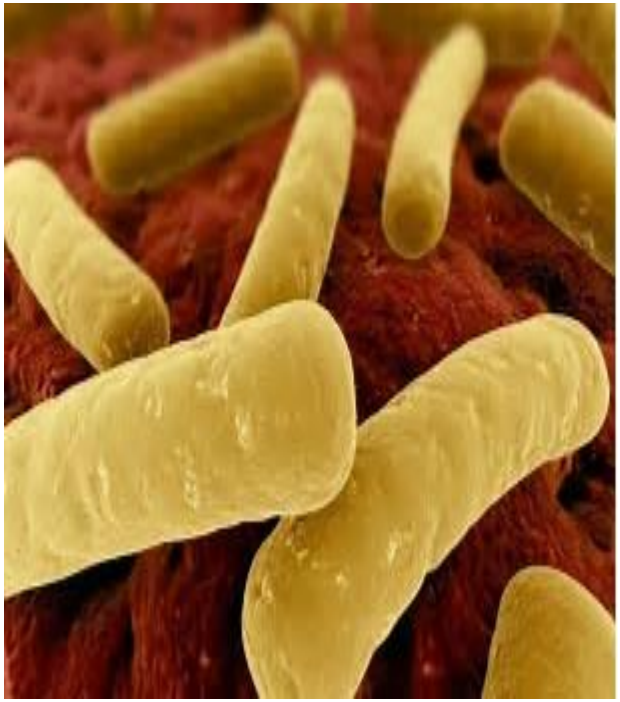
Clostridium

Clostridium:

❑ **Morphology & identification:**

- Large **G+ve rods**.
- Strict **anaerobes**
- **Spore forming**: spores wider than the diameter of the rod (bulging). They may be centrally, subterminally or terminally located.
- Most species are **saprophyte**, a few are **commensals** residing in the intestine of human and other animals
- Most *Clostridium* species with few exception are motile due to the presence of peritrichous flagella.

Anaerobic spore- forming Clostridia



Clostridia of clinical importance:

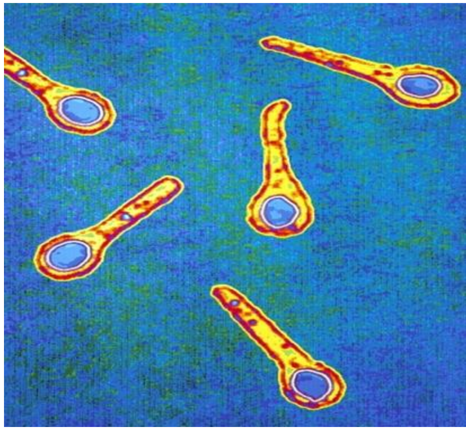
- ***C. tetani***
- ***C. botulinum***
- ***C. perfringens***
- ***C. difficile***

Clostridium tetani:

The causative agent of **tetanus (lock jaw).**

Cl. tetani

- ❑ **Morphology:**
- G+ve, straight rods with rounded ends.
- Has round, terminal, and bulging spores giving **drumstick** appearance to the rod.
- Most strains are motile
- Capsulated.

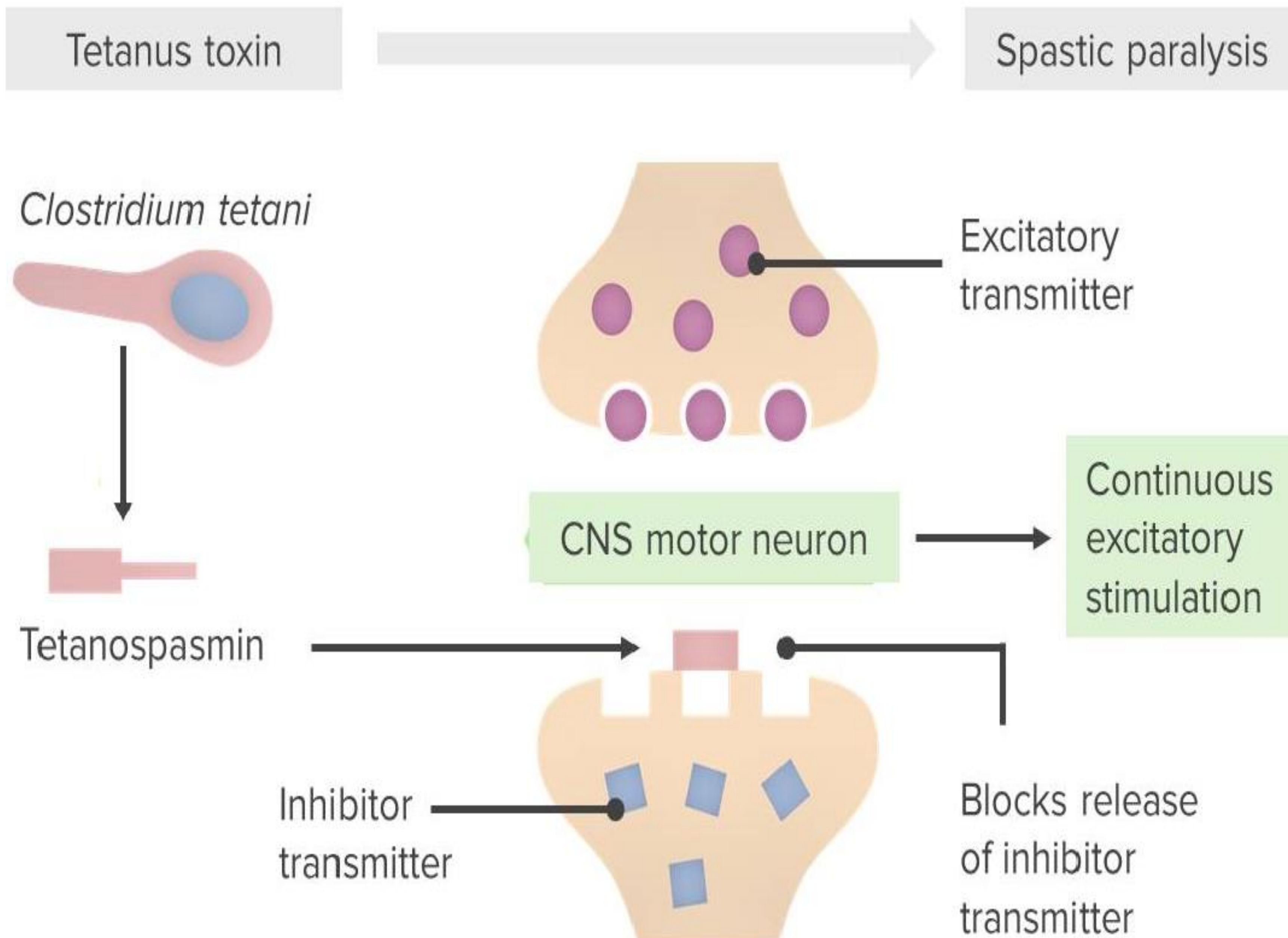


- ❑ **Cultural characteristics:**
- **Robertson cooked meat:** turbidity, gas production and blackening meat.
- **Blood agar:** α- hemolytic ⟶ β- hemolytic, Surface colonies tend to swarm over the entire surface of the agar
- **Nutrient agar slope:** Fildes technique (a routine method for isolating pure colonies of C. tetani).

Virulence factors of Clostridium tetani

- **Virulence factors:**
  - *C. tetani* produces tetanolysin, tetanospasmin, and neurotoxin (nonspasmogenic toxin). **Tetanospasmin** is the toxin responsible for the clinical manifestations of tetanus

Virulence factors	Biological functions
Tetanospasmin (Tetanus toxin TT)	Potent heat-labile toxin; prevents the release of neurotransmitters (e.g., GABA, glycine, etc.), hence blocks specific synaptic inhibition in the spinal cord. Motor neurons are left under no inhibitory control and undergo sustained excitatory discharge
Tetanolysin	Hemolysin, unknown significance in pathogenesis of tetanus.
Neurotoxin	Nonspasmogenic and peripherally active neurotoxin of unknown significance


Tetanus toxin
Spastic paralysis
Clostridium tetani
Excitatory
transmitter
CNS motor neuron
Continuous
excitatory
stimulation
Tetanospasmin
Inhibitor
transmitter
Blocks release
of inhibitor
transmitter

Pathogenesis:

- ***C. tetani*** is a noninvasive bacillus and causes disease only by **production of toxins**.
- The infection remains localized in the area of devitalized tissues (wounds burns, umbilical stump, surgical suture) into which the spores have been introduced.
- Germination of spores & vegetative cells produce toxin. Toxin production are aided by (Necrotic tissues, Calcium salt, **Associated pyogenic infections**).
- The toxin reaches the CNS & binds to receptors in the spinal cord & brain. **This binding is irreversible**.
- Unregulated spread of impulses, inhibited anywhere in the central nervous system (CNS) due to the action of toxin

Clinical findings:

Cl. tetani

- **Incubation period:** 5 days to many weeks.
- Rigidity & spasm of voluntary muscles of the jaw (Lockjaw, trismus)
- **Risus sardonicus** (grinning).
- Gradually, other voluntary muscles are involved result in tonic spasm (**Opisthotonos**).
- Death occurs due to **interference with respiration.**
- The mortality rate in generalized tetanus is high.

Tetanus neonatorum is an enormously important medical problem in developing countries. The cause is **sepsis of umbilical stump**.

Cl. tetani

Diagnosis:

- Mostly clinical, the bacteria rarely isolated
- **Toxigenicity Test:** For demonstration of toxin production
  - In vitro hemolysis inhibition test: detects tetanolysin
  - In vivo mouse inoculation test: detects tetanospasmin

❑Host immunity:

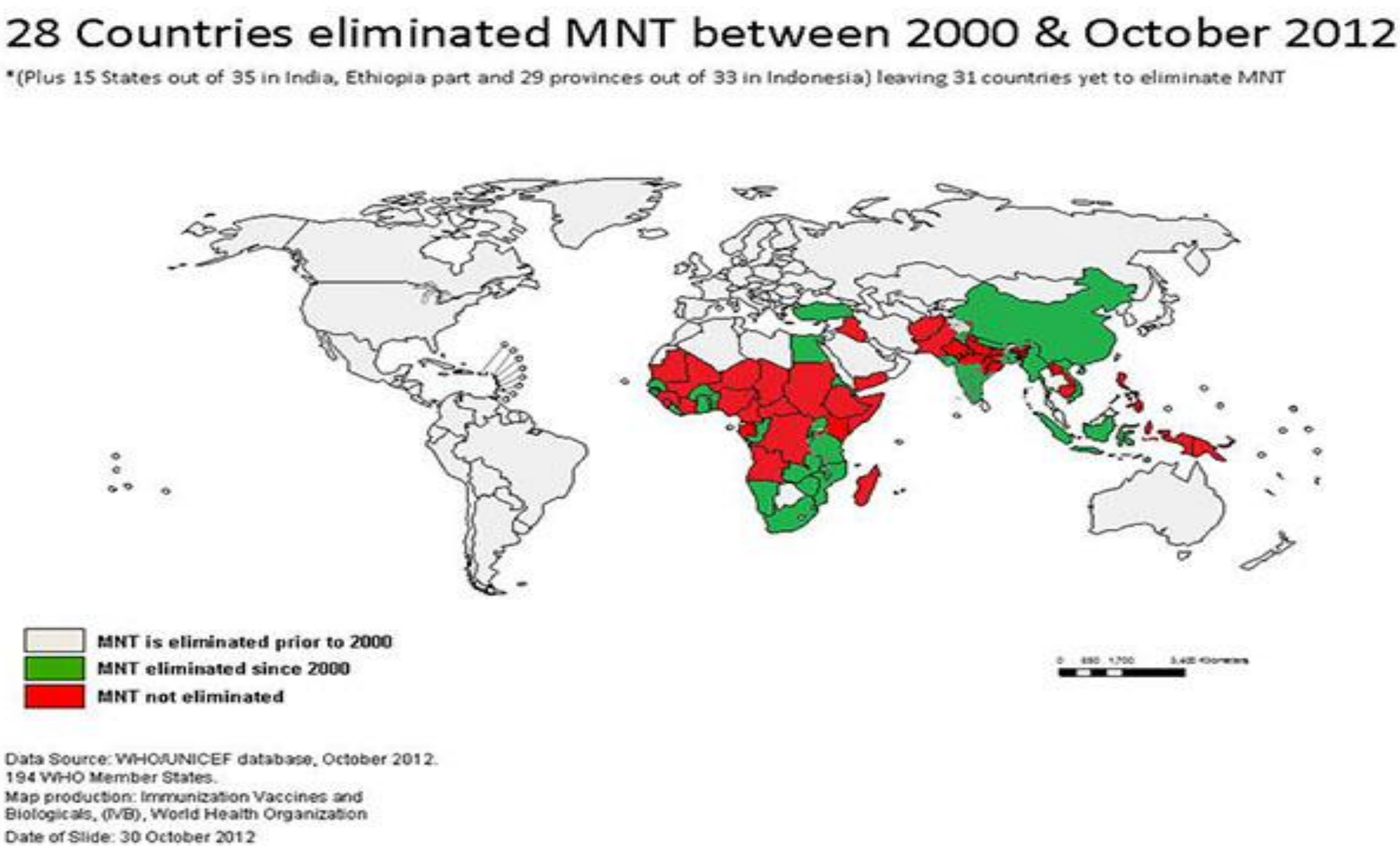
Specific antibodies produced against tetanus toxin are protective. Antibodies specifically combine with free toxin and prevent the action of the toxin.

Cl. tetani

❑Prevention and treatment: depends on:

- **Active immunization** with toxoids (during the first year of life, tetanus toxoid is often combined with diphtheria toxoid and acellular pertussis vaccine (**DPT**).
- Proper care of wounds contaminated with soil
- **Passive immunization:** prophylactic use of antitoxin (It neutralizes toxin that has not been fixed to nervous tissues).
- Administration of penicillin.

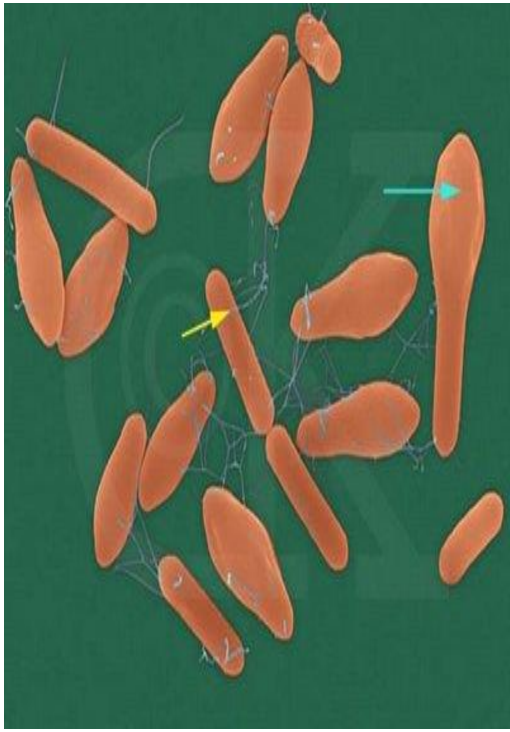
Elimination of maternal & neonatal tetanus



Cl. Botulinum:

The causative agent of botulism (flaccid paralysis).

- **Morphology:**
- Large G+ve rods, possesses subterminal and oval bulging spores.
- The bacillus is motile by the presence of peritrichous flagella,
- The bacteria are noncapsulated.



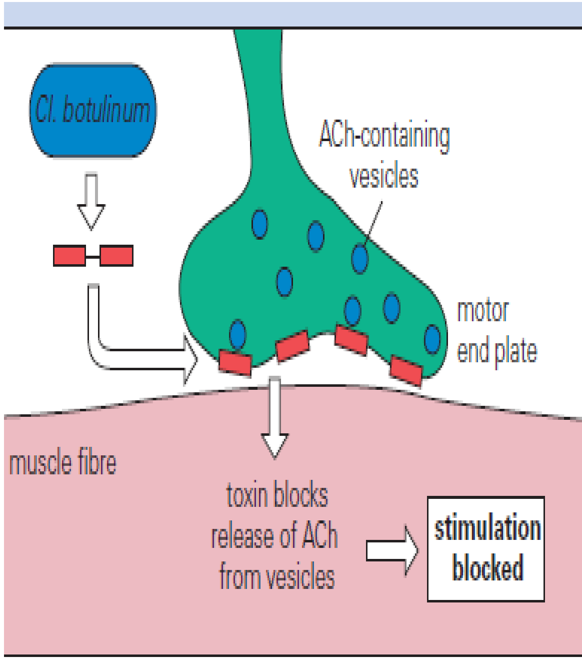
- **Cultural characteristics:**
- Grow on different types of culture media under an aerobic conditions. They produce spores when grown in **alkaline** glucose gelatin media at 20–25°C.
- Produce lipase which form iridescent film on C. botulinum colonies grown on egg yolk agar.
- **Virulence factor:**
- Botulinum toxin: **autolysis,** consists of two subunits A and B.
- There are many types of botulinum toxin form (A–G).
- Bacteriophage coded

Cl. botulinum

- **Pathogenesis:**
  - Botulism is an intoxication resulting from the ingestion of food in which *C botulinum* has grown and produced toxin.
  - Spores of *C botulinum* germinate in infected canned food under anaerobic conditions, vegetative forms grow and produce toxin.
  - The toxin acts by blocking release of acetylcholine at synapses and neuromuscular junctions resulting in lack of muscle contraction and flaccid paralysis.
  - Reversible*

Infant botulism:

- "floppy baby"
- One of the causes of sudden infant death syndrome.
- **Honey** is implicated as a vehicle of the spores.



- **Clinical findings:**
  - Symptoms begin 18–24 hours after ingestion of the toxic food
  - visual disturbances and blurred vision.
  - inability to swallow, and speech difficulty.
  - death occurs to respiratory paralysis or cardiac arrest. The mortality rate is high. Recovered patients do not develop serum antitoxin.
- **Treatment:**

(a) Respiratory supportive therapy.

(b) neutralizing unbound toxin by specific antitoxins.

(c) stopping toxin production by use of antibiotics (Metronidazole is the current antimicrobial drug of choice with penicillin).

- **Therapeutic uses:** As BT produces flaccid paralysis it can be used therapeutically for the treatment of spasmodic conditions, such as strabismus, blepharospasm and myoclonus

Cl. perfringens

Cl. perfringens: Causes gas gangrene, myonecrosis, and food poisoning.

❑ Morphology:

- Large G+ve rods, occur as single, in chains, or in bundles.
- Capsulated
- **Non-motile**, however, the bacteria multiply rapidly, giving a characteristic spreading colony appearance on the media, resembling the growth of motile clostridia.
- They possess central or sub-terminal spores (rarely seen).

❑ Cultural characteristics:

- **Robertson cooked meat:** turn the meat pink then black.
- **Milk media**: "**stormy clot**" phenomenon (anaerobic)
- **Blood agar**: produce **double zone of hemolysis**; a narrow zone of β- hemolytic due to theta-toxin and a much wider zone of incomplete α- hemolysis due to alpha-toxin of the bacteria.
- Some strains of C. perfringens can grow optimally at 45°C with a generation time reduced to 10 minutes

❑ Virulence factors:

❖ Toxins:

- **Alpha toxin (lecithinase):** breaks down Lecithin. It is responsible for toxemia and increases vascular permeability of blood vessels, thereby causing massive hemolysis and bleeding, tissue destruction, and myocardial dysfunction.
- **Theta toxin** has hemolytic, cytolytic & necrotizing effect.
- **Enterotoxin:** heat-labile toxin can induce diarrhea within 6-18 hrs.
- DNase, hyaluronidase, collagenase

❖ Enzymes:

- **Neuraminidase**.
- Fibrinolysin "**bursting factor**", responsible for typical muscle lesions observed in gas gangrene.
- histamine "**circulating factor**" increases adrenaline sensitivity of the capillary membrane and also inhibits phagocytosis.

- **Reservoir:**

- Soil and human colon

- **Transmission:**

- Food borne and traumatic implantation.

- **Pathogenesis:**

- **Gas gangrene (clostridial myonecrosis):** is a mixed infection (Toxogenic & proteolytic clostridia with various cocci & G negative rods).
- The spores reach tissues either through contamination of area with soil or feces or from the intestinal tract.
- The spores germinate, the vegetative cells multiply & ferment carbohydrates in tissues producing gas.
- Production of Alpha toxin, disrupts membranes, damaging RBCs, platelates, WBCs, endothelial cells → massive hemolysis, tissue destruction.
- Spread of infection due to secretion of necrotizing toxin & hyaluronidase.
- Extension of tissue necrosis increased the bacterial growth result in hemolytic anemia, severe toxemia & death.
- **Food poisoning:** enterotoxin produced in intestines disrupts ion transport → watery diarrhea, cramps (similar to E. coli); resolution within 24 hrs.

Clinical findings:

- From contaminated wounds (Fractures, postpartum uterus) the infection spread in 1-3 days to produce:
- crepitation in the subcutaneous tissue & muscles.
- foul-smelling discharge.
- Necrosis, Fever, toxemia, shock & death.
- From reheated meat dishes:
- I. C. 8-24 hrs. self limiting, watery diarrhea.

- **Treatment:**

- **Removal of damaged tissue.**
- **Metronidazole** is the antibiotic of choice.
- Prophylactic use of the antibiotic in association with surgery is effective. Antibiotic prophylaxis using broad-spectrum antibiotics, such as gentamicin, amoxicillin, and metronidazole, is effective, since occurrence of mixed infections with aerobic and anaerobic bacteria is frequent.
- Antibiotic therapy is not recommended for the treatment of C. perfringens food poisoning.

***Cl. difficile*: causes pseudomembranous colitis**

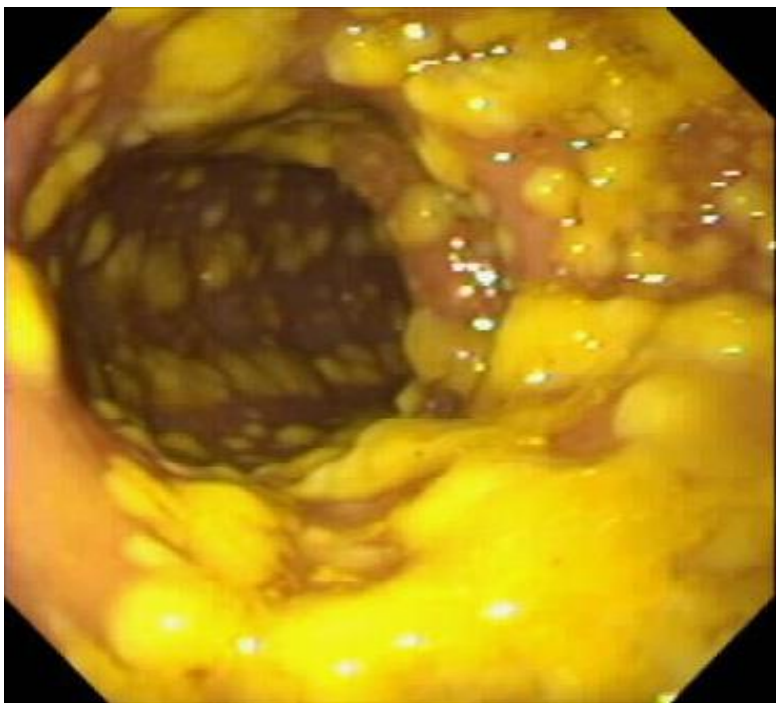
- **Reservoir:** human colon
- **Transmission:** endogenous.
- **Pathogenesis:**

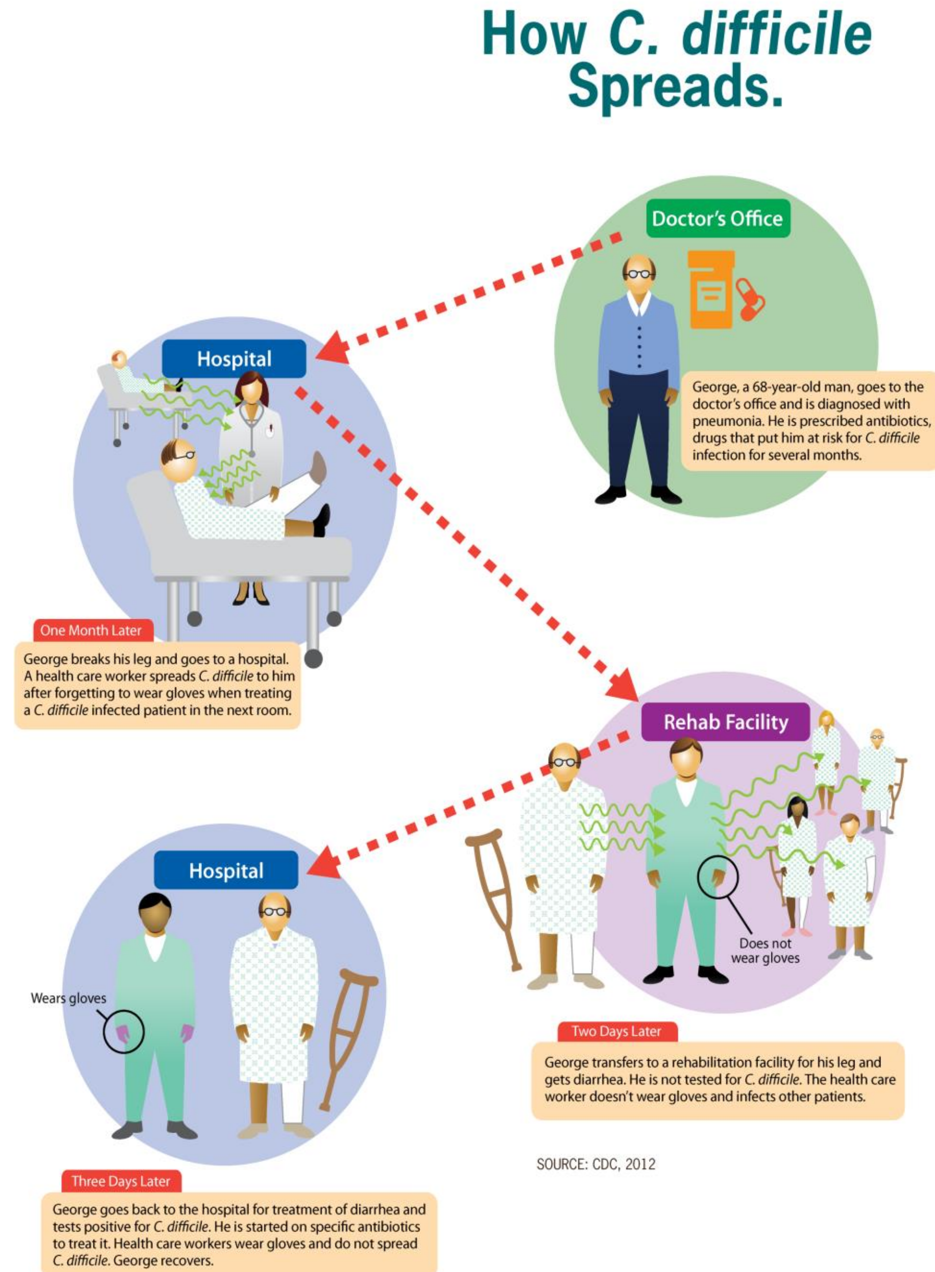
- *C. difficile* colonization occurs by the ingestion of the spores. Hence, outbreaks of *C. difficile* diarrhea may occur in hospitals where contamination with spores is more common.
- Normal flora of the gastrointestinal tract resists colonization and overgrowth with *C. difficile*
- *C. difficile* produces two antigenically distinct toxins: toxin A and toxin B.
- Toxin A: enterotoxin damaging mucosa leading to fluid increase; granulocyte attraction.
- Toxin B: cytotoxin, cytopathic.
- Administration of antibiotics result in proliferation of drug-resistant *Cl. diffcile* causing watery or bloody diarrhea, sometimes with abdominal crumps, leukocytosis & fever.
- Pseudomembrane (yellow plaques on colon)
- The most common antibiotics that associated with PMC are ampicillin & clindamycin.

- **Diagnosis:** stool exam for toxin production (screening; ELISA or cyto test)

❖ **Antibiotic associated diarrhea:**

The administration of antibiotics frequently leads to a mild to moderate form of diarrhea termed AAD. The condition is less severe than PMC. About 25% of AAD is caused by *Cl. difficile*.





Case study:

An 18-year-old college student came to a hospital with a complaint of facial spasm. He has been unable to eat for 2 days due to severe pain in his jaw. Examination revealed trismus and risus sardonicus. The student gave a history of playing football for his college team, during which he had sustained a minor knee injury with abrasions 6 days earlier. He did not visit any doctor earlier or receive any toxoid for the injury. He did not remember of receiving any booster vaccination with tetanus toxoid in last many years. A clinical diagnosis of tetanus was made.

- What laboratory tests should be performed to confirm the diagnosis?
- Should treatment wait until laboratory results are available?
- Describe the pathogenesis of tetanus.
- What are the vaccines available against tetanus in humans?

References:

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- Parija. (2012). Textbook of Microbiology and Immunology. 2nd ed. Elsevier. ISBN: 978-81-312-2810-4


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