

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

16.3.2023

# Spore forming gram positive rods

**Dr. Saeed Ur Rahman**

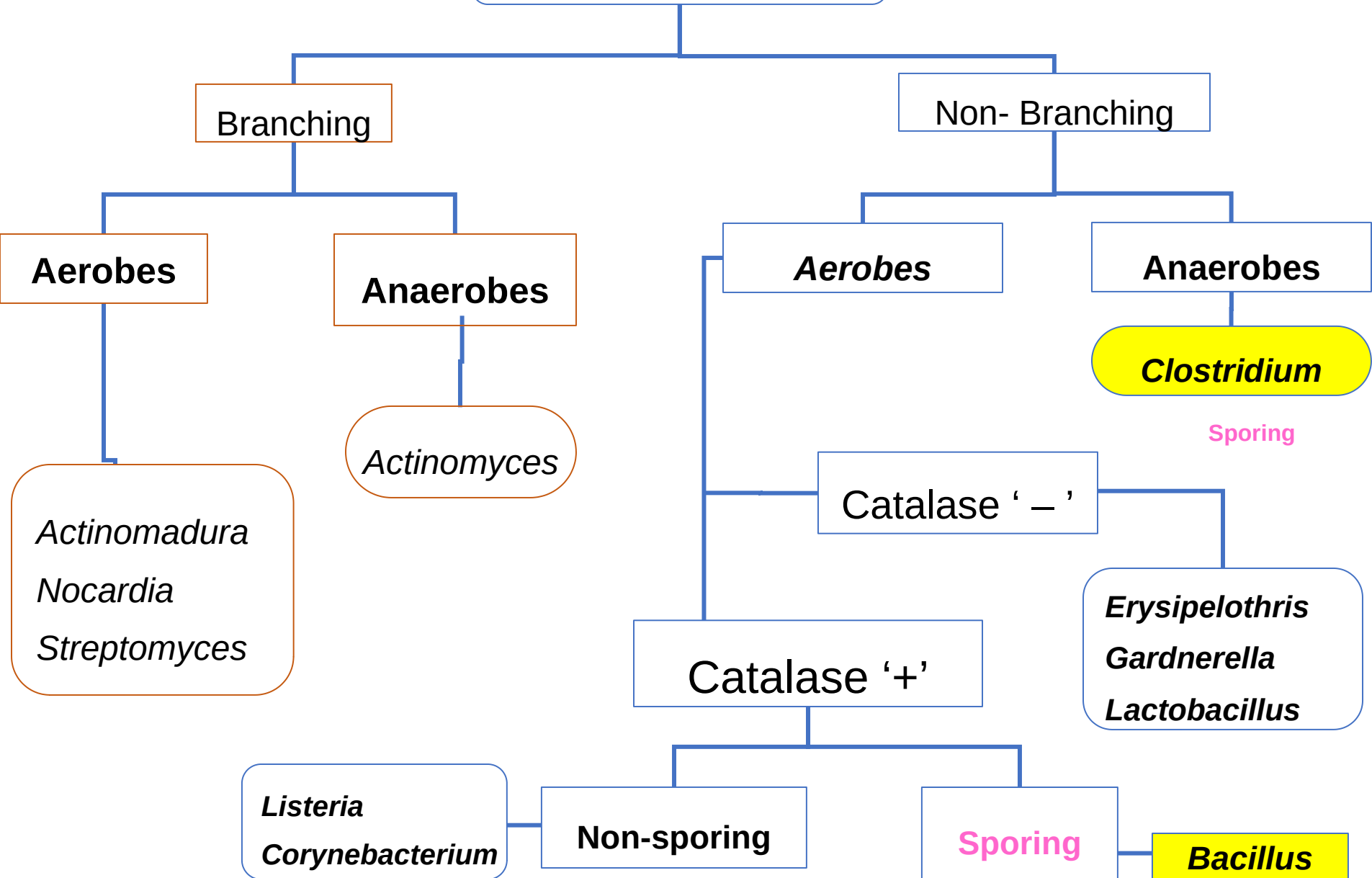
MBBS, MPhil, PhD, CBact, CHPE

# Aims and Objectives

At the end of the session students of 3<sup>rd</sup> Year MBBS should be able to

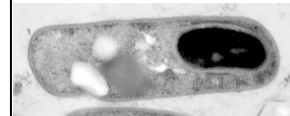
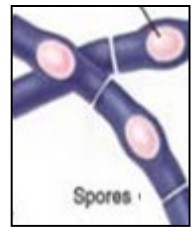
1. Enumerate spore forming GP rods.
2. Describe the important properties, pathophysiology, clinical features and Lab diagnosis of diseases caused by spore forming GPR.

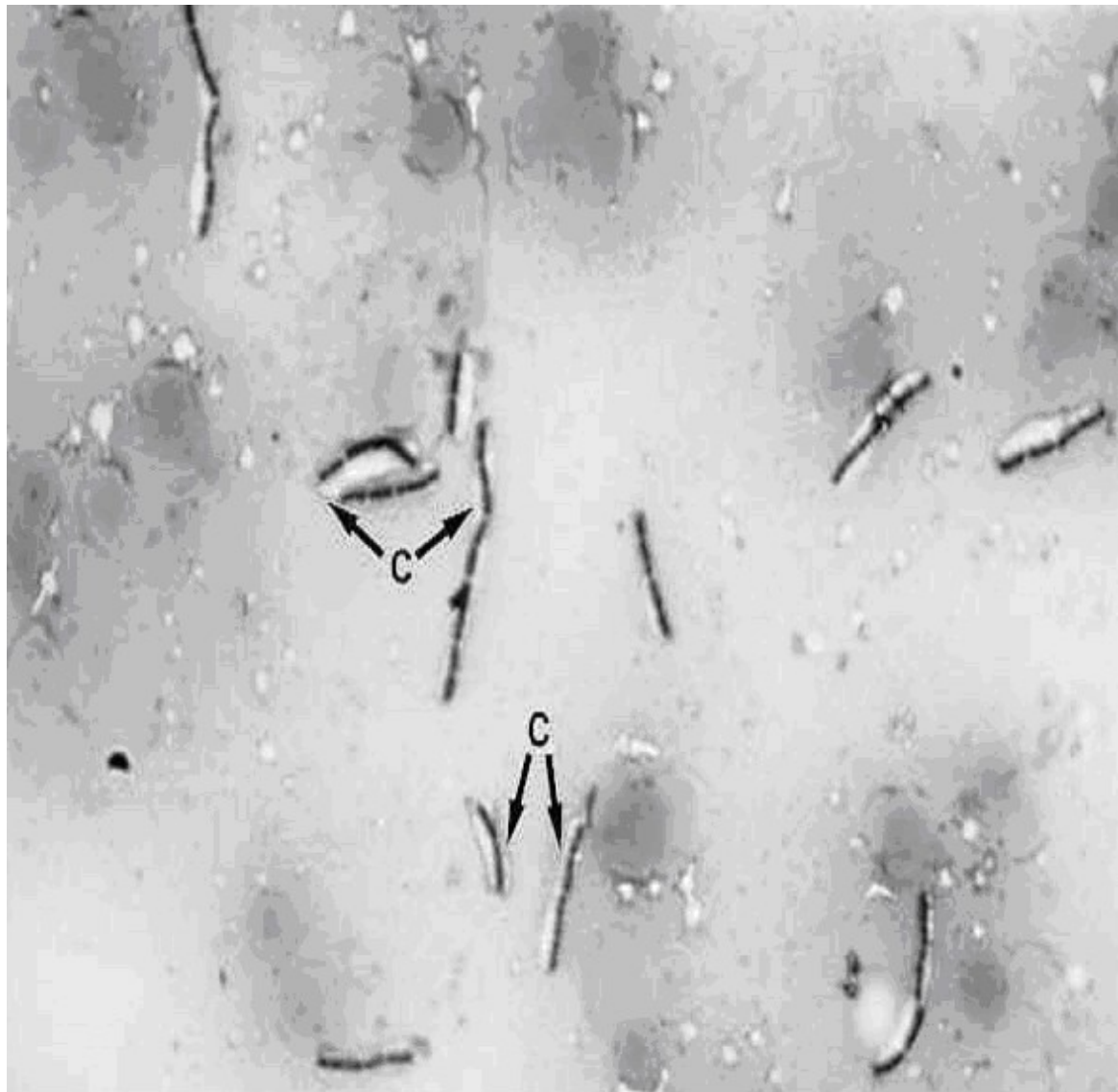
# Gram ' + ' bacilli



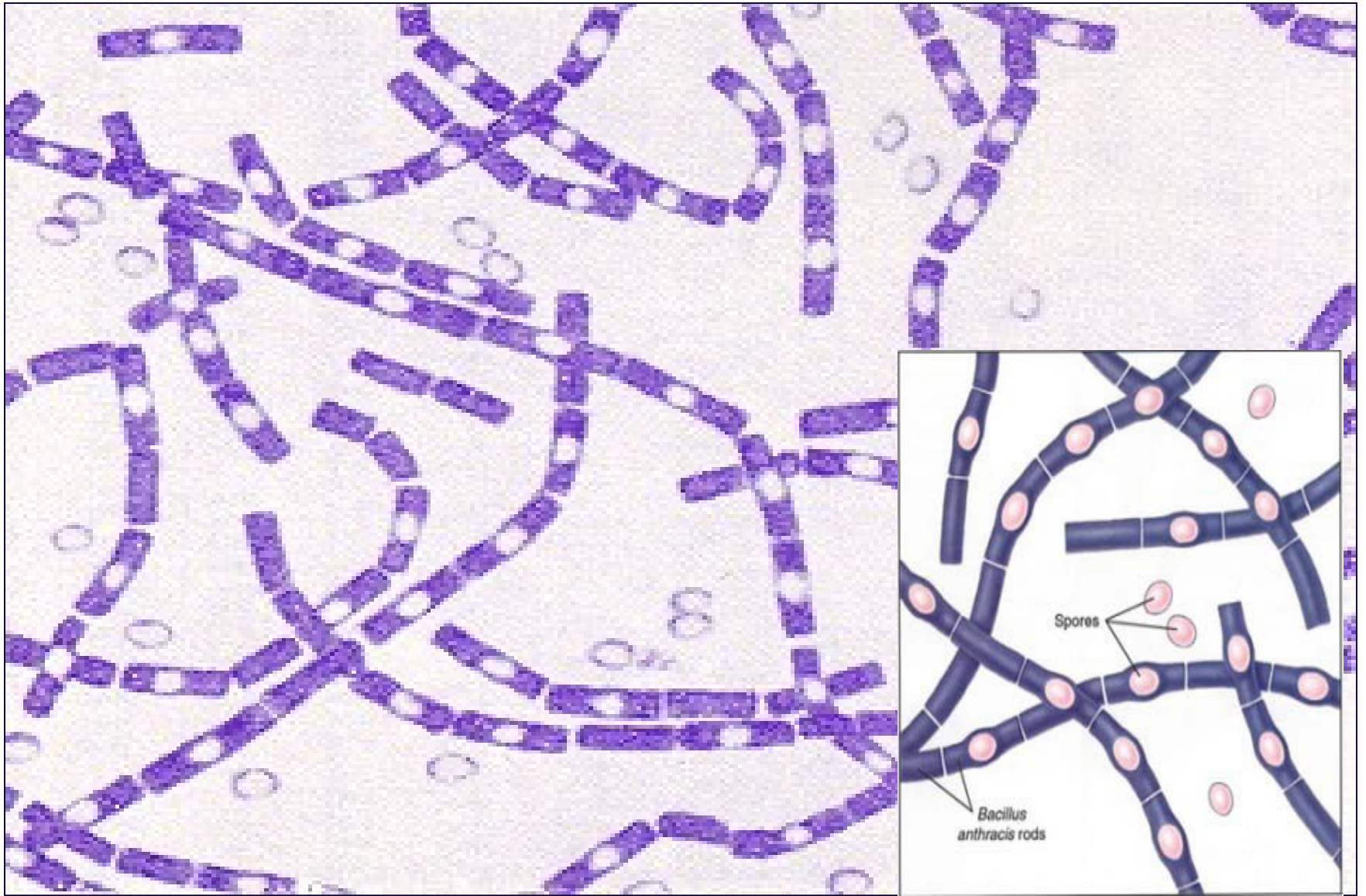
# Bacillus

- 'HIGH RISK' zoonotic pathogen
- Gram positive, spore forming, aerobic, long rods with square ends,
- Frequently in chains and form endospores
- Surrounded by antiphagocytic capsule (composed of D glutamate & other polysaccharides)
- Motile except *B anthracis*.
- deeply staining bacteria.

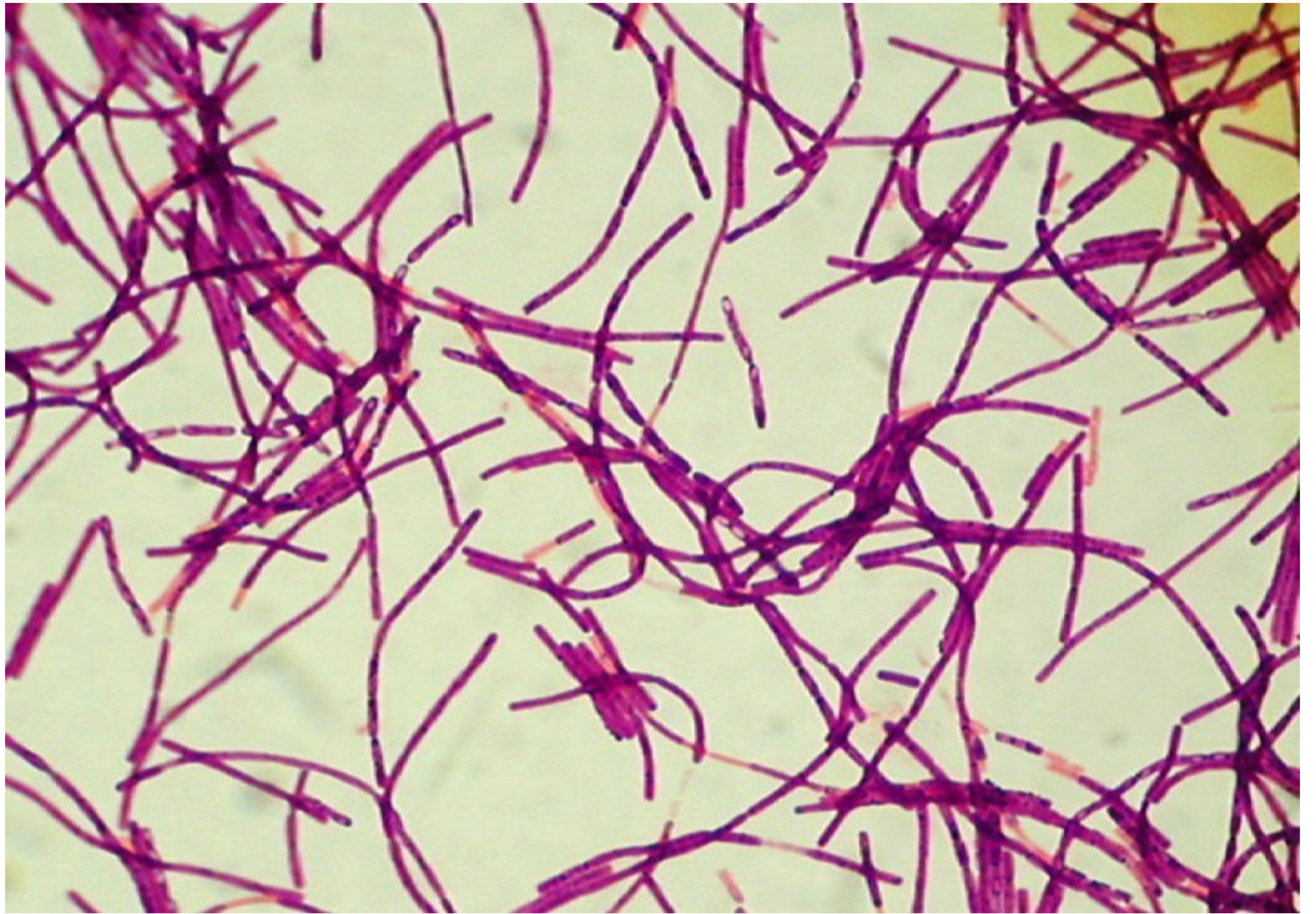




**Stained with M'Fadyean stain (polychrome methylene blue).**  
The capsule (C) is pink around the dark-blue bacilli.  
anthrax bacilli frequently have square ends.

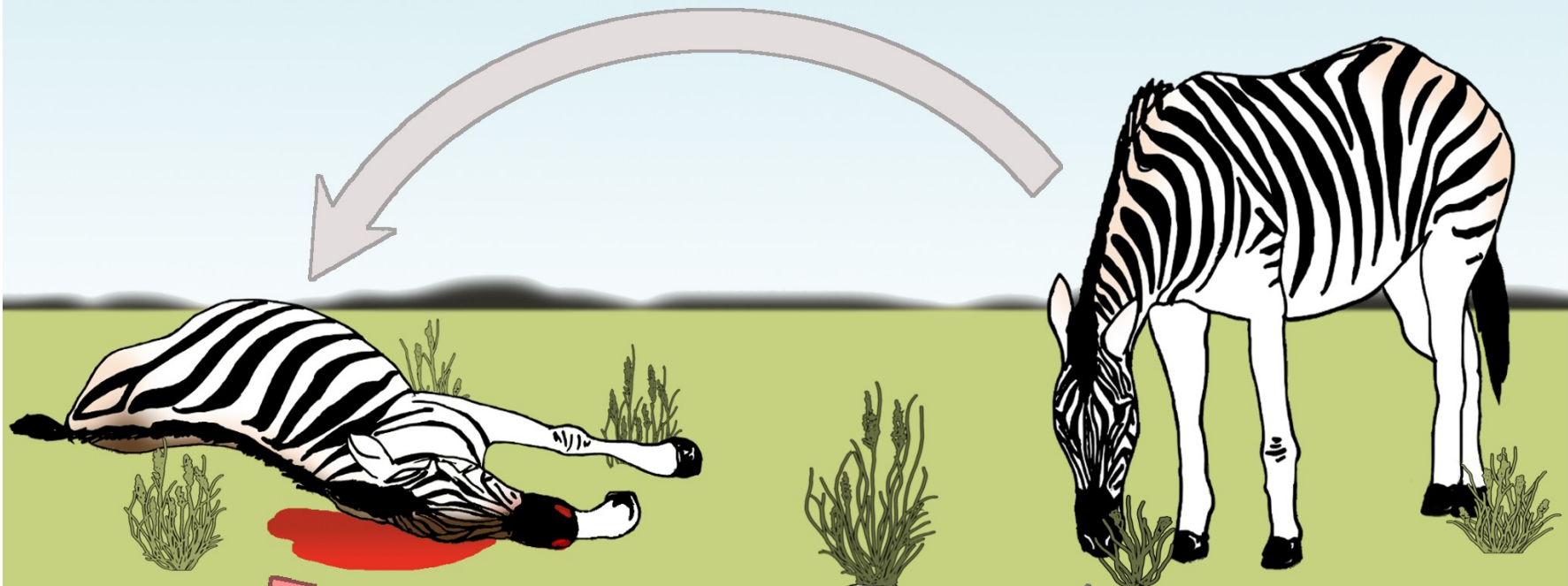








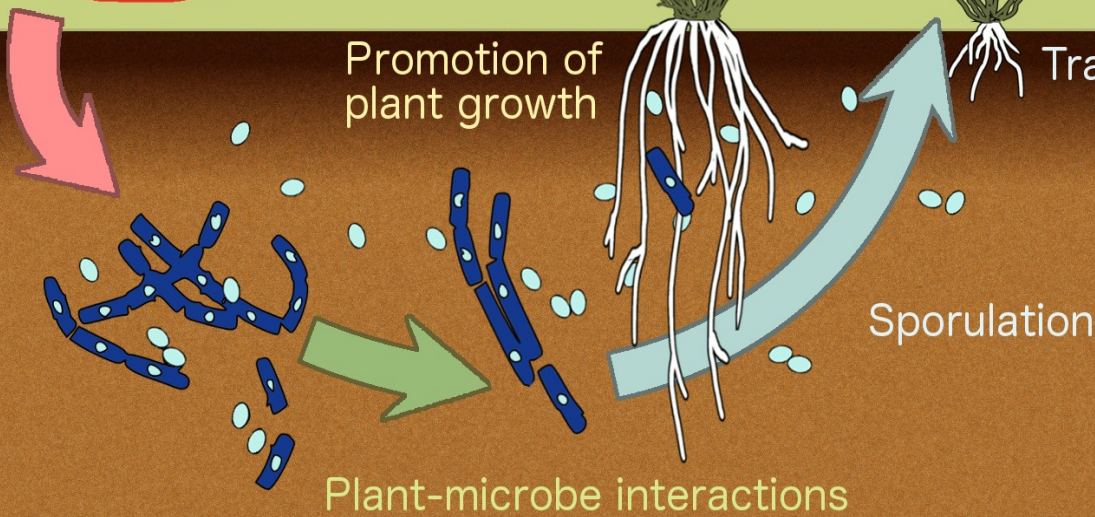
Disease and host death



Inoculation of soil

Promotion of plant growth

Transmission to host



Plant-microbe interactions

- Physiologically Bacillus can live in wide range of natural environment
- Spores are resistant to heat, cold, radiation, desiccation and disinfectants.
- Oxygen is necessary for sporulation
- Large majority of Bacillus species are harmless saprophytes.
- *Bacillus anthracis* is common in animals but **rare** in humans.
- A number of other species, in particular *B cereus*, are **occasional** pathogens of **humans and livestock**.

# Important Pathogenic Bacillus



## ***B. anthracis***

causes anthrax; it is common in animals but rare in humans

## ***B. cereus***

causes food poisoning (& occasional pathogens of humans and livestock).

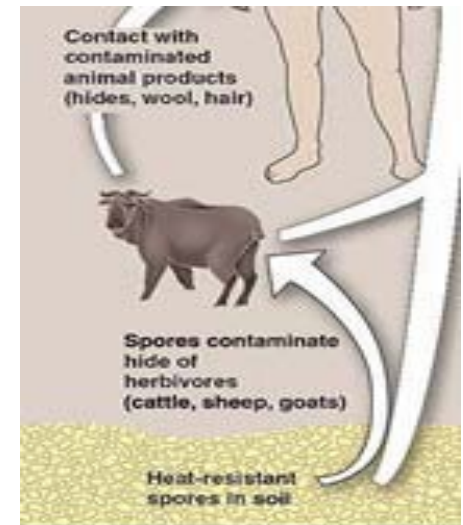
- **Bacillus** species produce enzymes, antibiotics, and other **metabolites** (used in many medical, pharmaceutical, agricultural, and industrial processes) .
- **Bacitracin** and **Polymixin** are two well-known antibiotics obtained from *Bacillus* species.
- Koch established his famous postulates in 1876 on anthrax studies.
- first bacterial vaccines developed against anthrax by Pasteur (in 1881).

# Epidemiology of *Bacillus anthracis*

- Rare in US (17 cases reported by CDC in 1974 – 1990)
- Enzootic in certain countries (e.g. Iran, Turkey, Pakistan, Sudan)

## Three well defined cycles

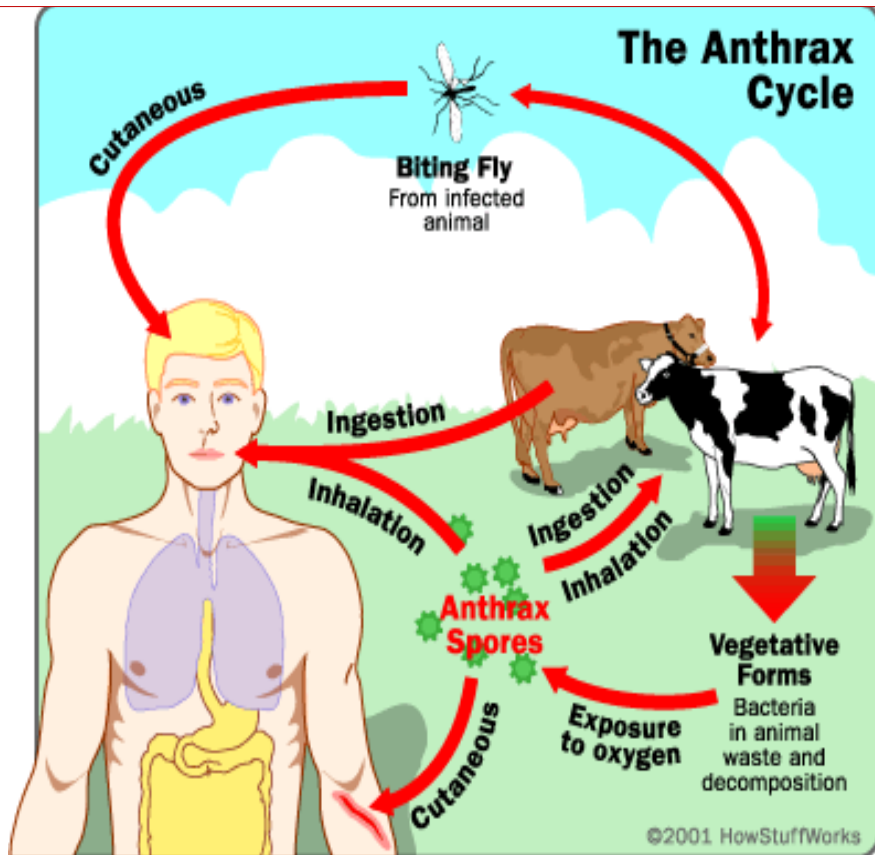
- Survival of spores in the soil
- Animal infections
- Infection in human
- People in contact with infected animals or animal products are at risk for anthrax.





# Transmission

*B. anthracis* spores may remain viable for many years in contaminated pastures, or in bones, wool, hair, hides, or other materials of infected herbivorous animals.



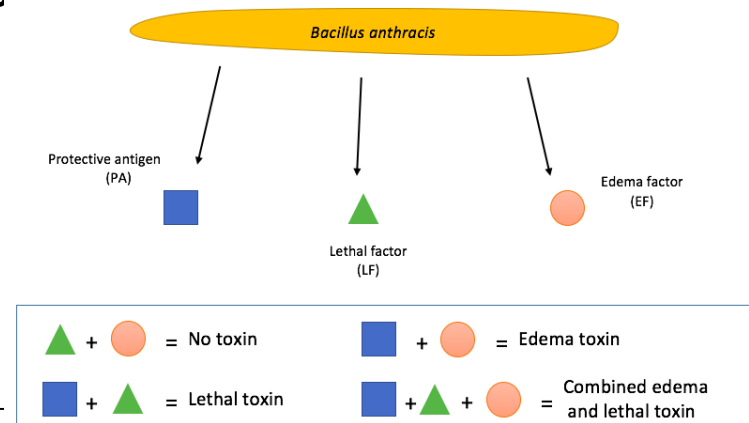
# Pathogenesis

The pathogenicity of *B anthracis* depends on two virulence factors:

- polypeptide capsule, which protects it from phagocytosis and
- a toxin.

This toxin consists of three proteins:

- protective antigen (PA) (82.7 kDa),
- lethal factor (LF) (90.2 kDa), and
- edema factor (EF) (88.9 kDa).



# Clinical Manifestations

- The clinical forms include
  - **Cutaneous anthrax** (eschar with edema), acquired from handling infected material (this accounts for more than 95% of cases);
  - **Enteric anthrax**, from eating infected meat, causes severe bloody gastroenteritis;
  - **pulmonary anthrax**, from inhaling spore-laden dust (wool sorter's disease).
  - **Meningoencephalitis**, usually a complication of septicemia.

# Pathogenesis

## Cutaneous

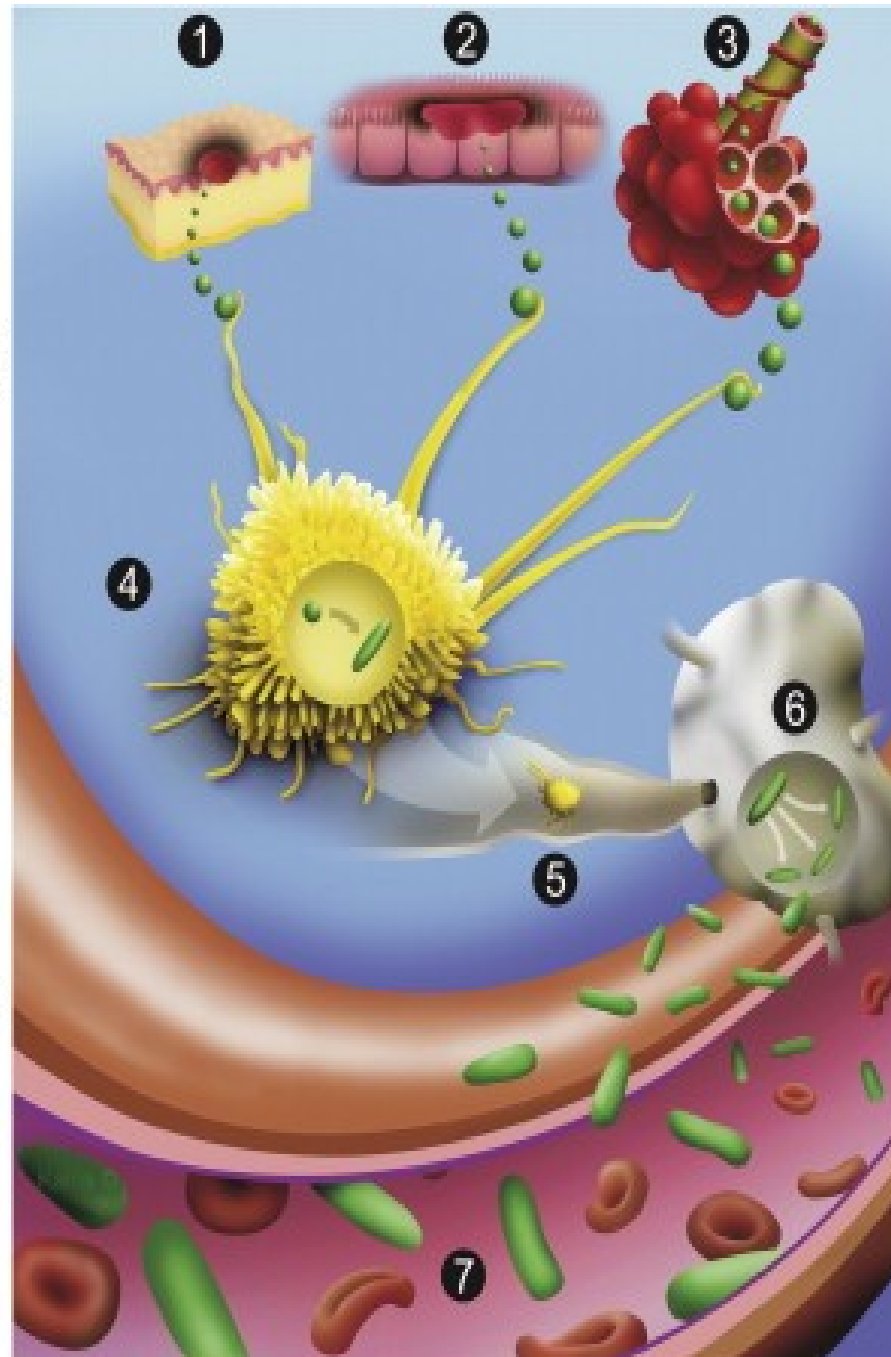
Spores gain access to subepidermal structures in the host through an abrasion of the skin, followed by uptake via resident macrophages.

## Gastrointestinal

Spore uptake by phagocytes occurs after ingestion of contaminated food. Germination follows soon after entry in host immune cells.

## Inhalational

Alveolar macrophages take up inhaled spores from the alveoli and respiratory tract. A small fraction of spores will evade destruction in the phagolysosome. Lung lesions are not found after inhalational anthrax.



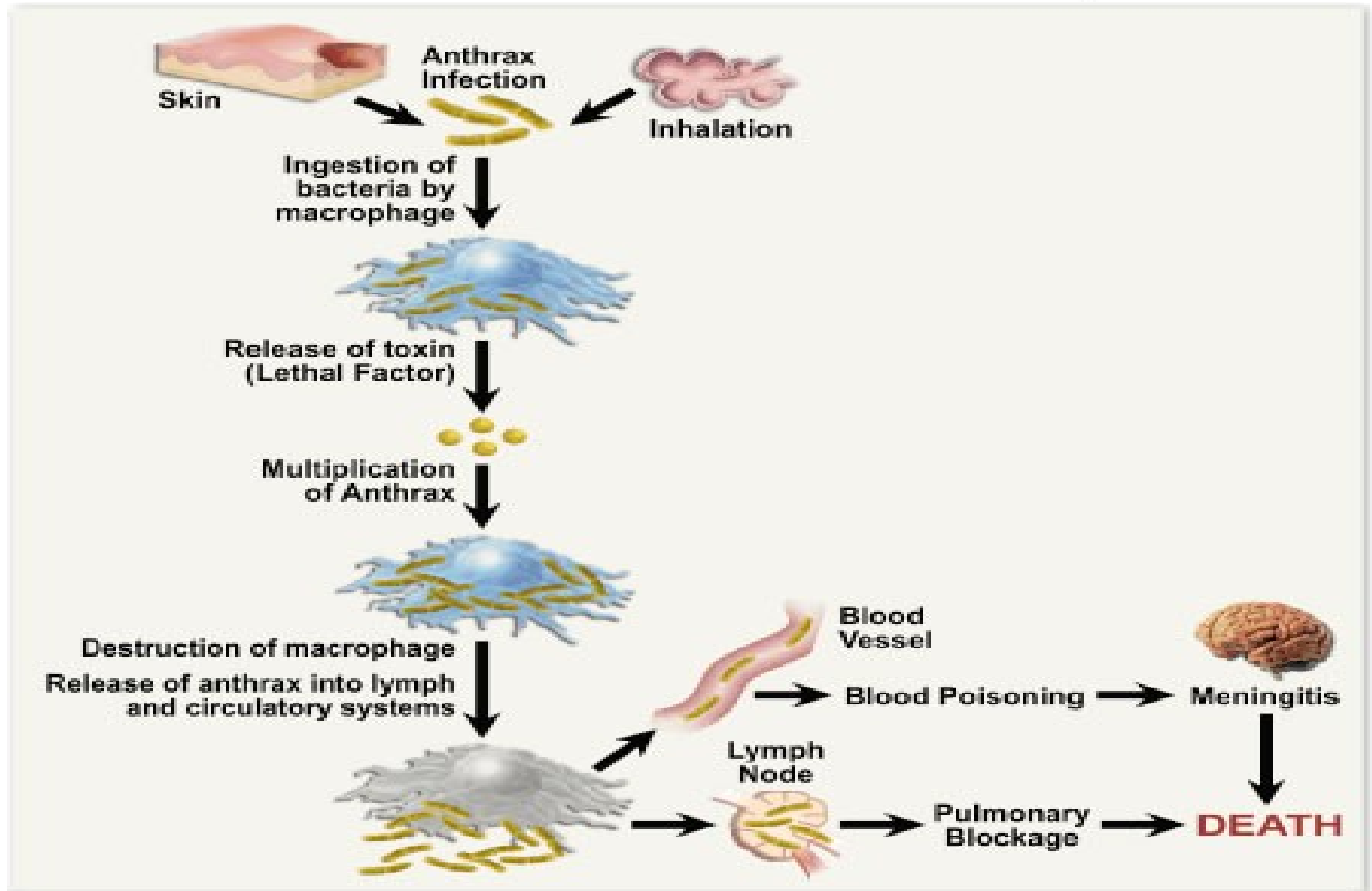
4 In each case, spore germination into mature *B. anthracis* bacilli takes place in the macrophages at the primary site of infection. In the case of inhalational anthrax, germination occurs later upon arrival at the local lymph node.

5 After spore uptake into phagolysosomes by tissue macrophages, the bacilli are transported via lymphatic channels to local and regional lymph nodes.

6 Final germination takes place in the lymph nodes draining the primary site of infection. Through an unclear mechanism, mature bacilli escape from macrophages and multiply systemically.

7 Bacilli spread through the circulatory system, causing septicemia and infection of other target organs.

# 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.



# Cutaneous anthrax



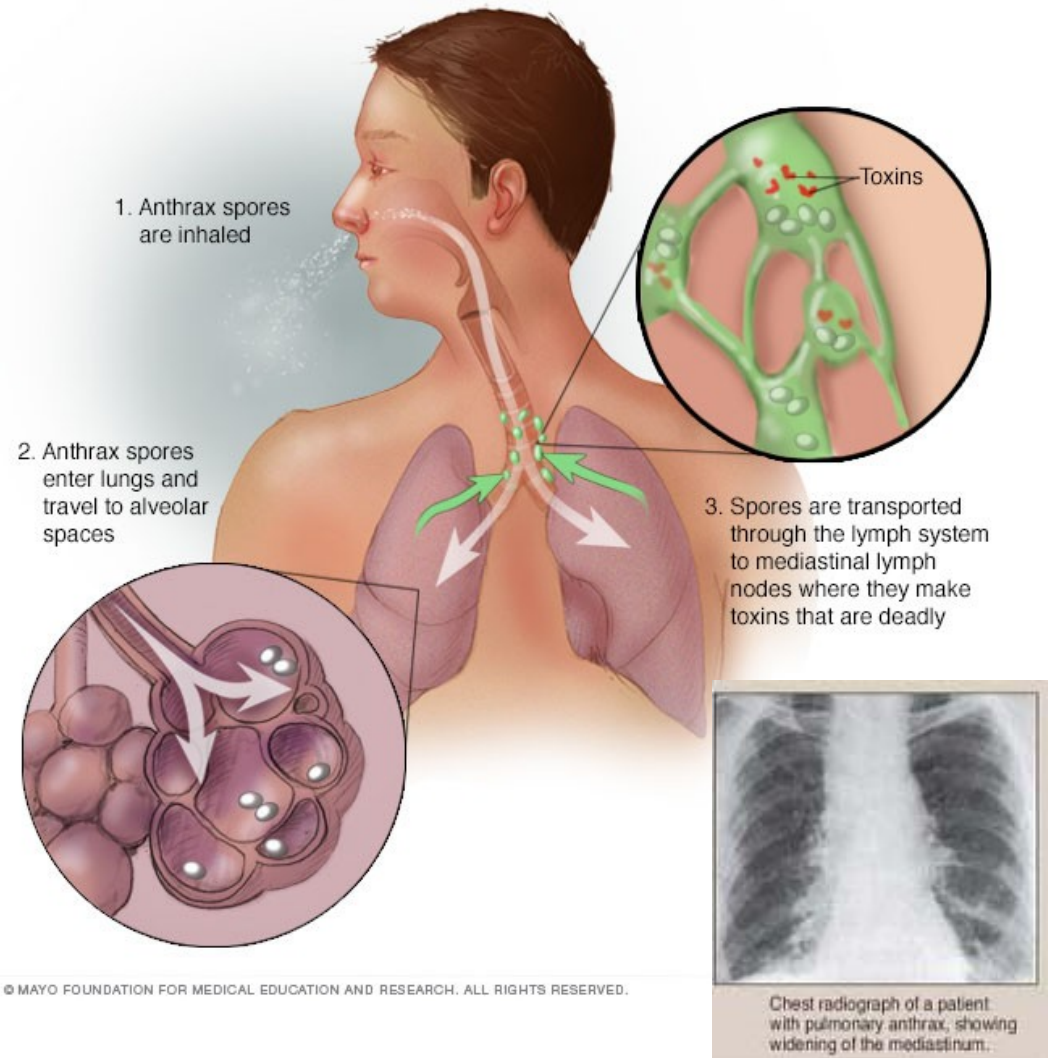
- occurs through contamination of a cut or abrasion,
- although in some countries biting flies may also transmit the disease.
- After incubation period of 2 – 3 days, a small pimple or papule appears at the inoculation site.
- A surrounding ring of vesicles develops.
- Approximately 20% of untreated cases of cutaneous anthrax progress to fatal septicemia.



# Pulmonary anthrax

Woolsorter's disease

- The inhaled spores are transported by alveolar macrophages to the mediastinal lymph nodes, and then initiate systemic disease.



**Intestinal anthrax** occurs by ingesting infected meat.

- Severe gastroenteritis including fever abdominal pain and bloody diarrhea. Septicemia often develops.

➡ Gastrointestinal and pulmonary anthrax are both more dangerous than the cutaneous form because they are usually identified too late when the treatment is ineffective.

# *B. cereus*

- *B. cereus* is sometimes associated with bacteremia/septicemia, endocarditis, meningitis, and infections of wounds, ears, eyes, respiratory tract, urinary tract, and gastrointestinal tract.
- Bacillus cereus causes two distinct food poisoning syndromes:
  - a rapid-onset **emetic syndrome** characterized by nausea and vomiting, and
  - a slower-onset **diarrheal syndrome**.

# *Bacillus Food Poisoning*

- The **diarrheal type** (diarrhea and abdominal pain occurring 8 to 16 hours after consumption of the contaminated food).
- It is associated with a variety of foods, including meat and vegetable dishes, sauces, pastas, desserts, and dairy products.

- In **emetic disease**, nausea and vomiting begin 1 to 5 hours after taking the contaminated food.
- **Boiled rice** that is held for prolonged periods at ambient temperature and then quick-fried before serving is the usual offender,
- **dairy products** or other foods are occasionally responsible.



- The principal virulence factors are a **necrotizing enterotoxin** and a **potent hemolysin (cereolysin)**.
- Emetic food poisoning probably results from the release of **emetic factors** from specific foods by bacterial enzymes.

# Lab Diagnosis

- **Fluid aspirates** from cutaneous lesions and **visualizing GPR**
- **Culture** of aspirate.
- **Microscopic examination** of fresh stained smears of fluid from under the eschar, CSF, lymph node or spleen aspirates [polychrome methylene blue staining (M'Fadyean's stain)] shows characteristic square-ended, blue-black bacilli surrounded by a pink capsule & central oval spore.
- when indicated, do culture of **sputum, CSF, and blood**
- In bioterrorism attack, rapid diagnosis is confirmed **by PCR, and direct fluorescent antibody test.**

- Bacillus anthracis is **susceptible to penicillin** and almost all other broad-spectrum antibiotics.
- In uncomplicated anthrax cases, give
  - Penicillin V (500 mg ) every 6 hours for 5 days, or
  - Procaine penicillin (600 mg (1 million units) I/M every 12 to 24 hours for 5 days.
  - **Drug of choice is ciprofloxacin and doxycycline**
  - In severe cases, Penicillin G (1,200 mg (2 million units) should be administered intravenously every 6 hours, reverting to the intramuscular regime of 600 mg every 12 to 24 hours once recovery starts.

- In **pulmonary anthrax**, continuous-drip administration is advisable.
- Tetracyclines, chloramphenicol, gentamicin, or erythromycin may be used if the patient has penicillin sensitivity.
- The fluoroquinolone, ciprofloxacin, would be expected to be effective in human anthrax.

# Prevention

1. Ciprofloxacin or Doxycycline as prophylactic drugs.
2. Vaccination (6 doses over 18 months and annual booster in high risk people) and (18 – 65 yrs).
3. Active immunization “Anthrax vaccine” (adsorbed) (AVA) (attenuated) or BioThrax™
4. Incineration of dead infected animal.



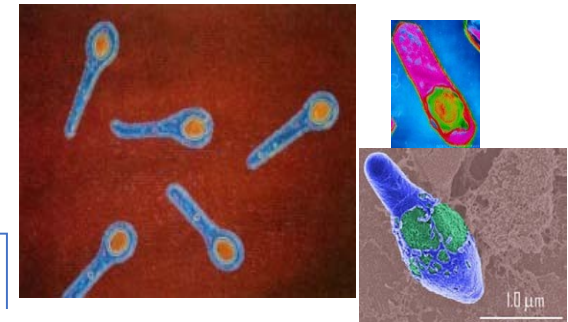
(Genus)

# Clostridium

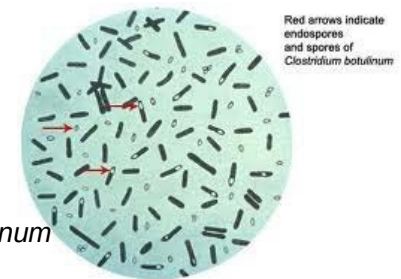
- Anaerobic, Gram-positive, motile rods,
- Form endo-spores.
- Part of intestinal flora of human & mammals
- Synthesize the most potent **exotoxins**

- Significant human pathogens

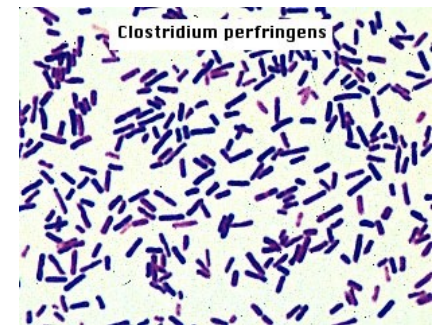
1. ***C. perfringens***: myonecrosis & food poisoning
2. ***C. botulinum***: botulism
3. ***C. tetani***: tetanus (lockjaw)
4. ***C. difficile***: antibiotic associated pseudomembranous colitis



*C. tetani*



*C. botulinum*



*C. difficile*

# *C. perfringens* (*C. welchii*)

- Vegetative (non-spore) form is the normal flora of vagina & gastrointestinal (GI) tract.
- **Spores** are present in soil.
- **When infect** tissue, can cause
  - anaerobic cellulitis, &
  - myonecrosis (gas gangrene).
- Some strains also cause **food poisoning**.



cellulitis



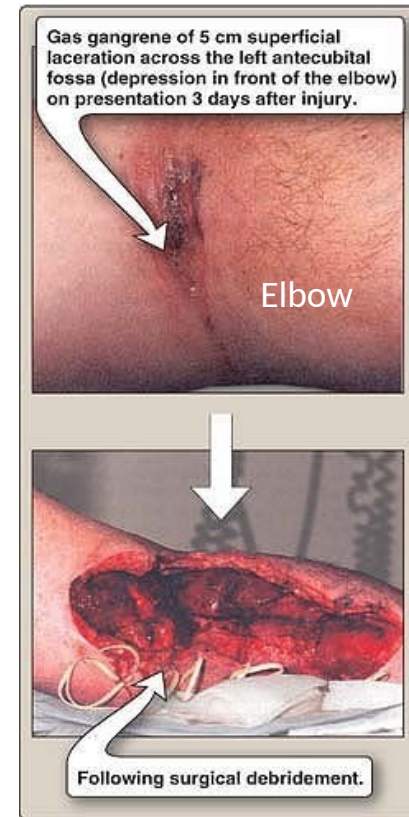
myonecrosis



# Epidemiology & Pathogenesis

## Myonecrosis (gas gangrene):

- Spores infect **tissue** by traumatic contamination with infected soil / **endogenous transfer** from the intestinal tract.
  - ➔ predisposing factors are compound fractures & accidents
  - ➔ Toxins produce extensive cell necrosis.
  - ➔ Excreted enzymes help in dissemination of infection.
  - ➔ Fermentation of tissue carbohydrates → gaseous accumulation in subcutaneous spaces [crinkling sensation on palpation (crepitation); [gas gangrene]
  - ➔ Copious exudates with foul smelling
  - ➔ Exotoxins move from damaged tissue to other organs → & exert systemic effects □ shock, renal failure, and intravascular hemolysis.
  - ➔ If untreated □ **Fatal within days**



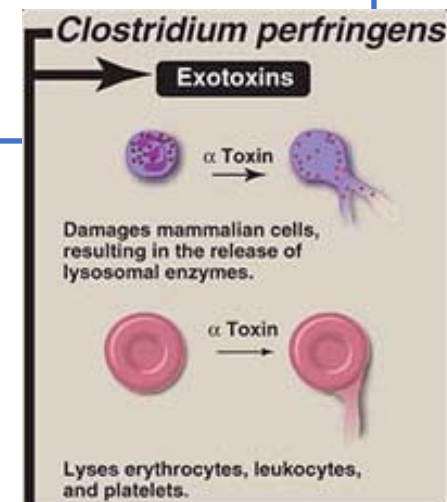
# Pathogenesis

*C. perfringens* secretes variety of **exotoxins**, enterotoxins, & hydrolytic enzymes that facilitate the disease process.

## Exotoxins

- >12 exotoxins.
- Most important is alpha toxin; *Lecithinase* (phospholipase C) □ Degrades lecithin in mammalian cell membranes, causing lysis of endothelial cells □ destruction of erythrocytes, leukocytes & platelets,
- Other exotoxins cause hemolytic, cytotoxic & necrotic effects.

- *C. perfringens* are grouped A – E on the basis of their spectrum of exotoxins.
- Type A strains produce both alpha toxin & enterotoxin, responsible for most human clostridial infections.



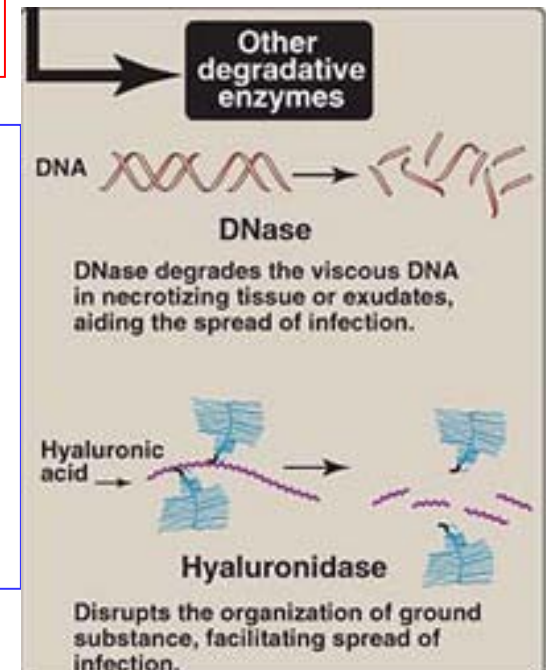
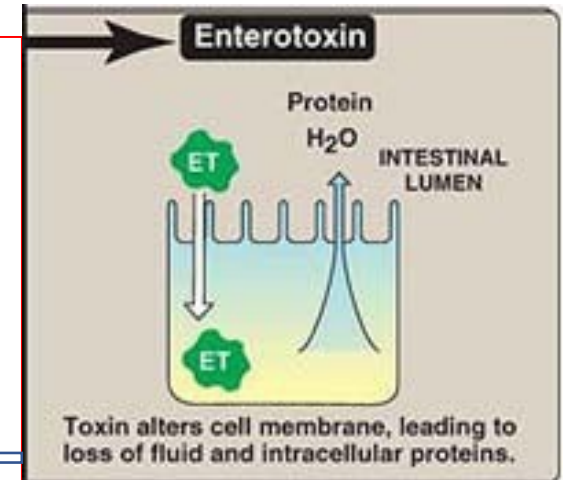
## Enterotoxin:

- Acts on the lower portion of small intestine by binding to epithelial cell membrane
  - Causes loss of fluid & intracellular proteins.

- The heat resistant spores remain viable for >1-hr at 100°C; □ food- poisoning.

## Degradative enzymes

- Many hydrolytic enzymes, including proteases, DNases, hyaluronidase, and collagenases **liquefy tissue** & **promote spread of infection**.

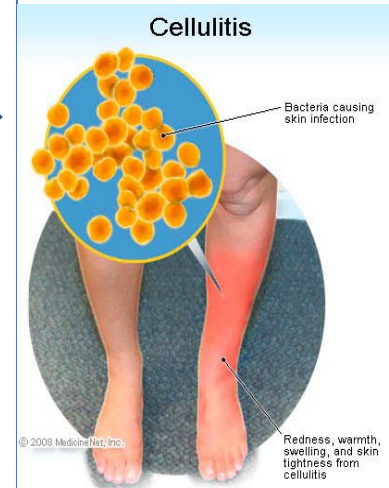




## ***C. Perfringens* causes**

- **Anaerobic cellulitis of fascia (fasciitis):**

- Does not involve invasion of muscle tissue.
- Rapid spread of infection.

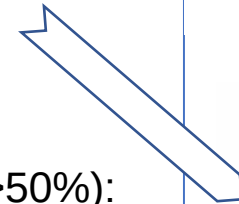


- **Food poisoning:**

- Onset of nausea, abdominal cramps, & diarrhea: 8 –18 hrs after eating contaminated food. No fever; vomiting is rare; self-limited, with recovery within 1-2 days.

- **Enteritis necroticans:**

- Outbreaks of a necrotizing bowel disease with high mortality (>50%): usually sporadic cases are reported.



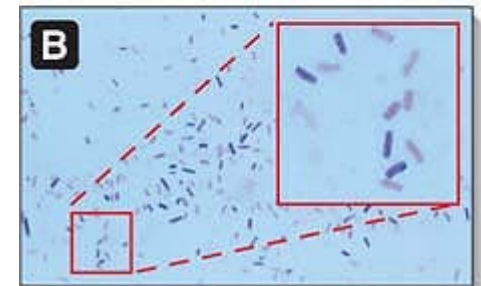
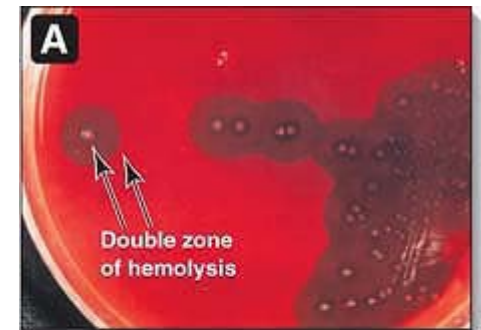
- **Clostridial endometritis:**

- Complication of incomplete abortion, or the use of inadequately sterilized instruments.
- Gangrenous infection of uterine tissue is followed by toxemia & bacteremia.

# Laboratory identification &

## Diagnosis:

- Myonecrosis / cellulitis is diagnosed largely on clinical impression.
- food poisoning, the organism can be cultured from suspected food & patient's feces.
- **Anaerobic culture on blood agar** shows rapid growth, producing colonies with a unique double zone of hemolysis.



# Treatment and prevention:

- **Gas gangrene** requires immediate **debridement** & thorough removal of foreign material & devitalized tissue
- exposure of the wound to **O<sub>2</sub>** [hyperbaric oxygen chambers]
- If debridement is unable to control spread of gangrene, **amputation** is mandatory.
- Administration of **antibiotics** in high dose.
  - *C. perfringens* is sensitive to penicillin



## Prevention:

- surgical toilet of contaminated wounds
- Penicillin as prophylaxis



# *C. botulinum*

## Epidemiology:

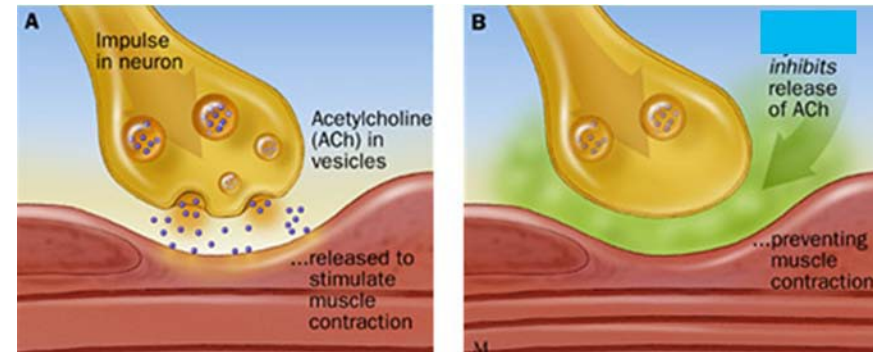
- Soil & water
- Spores contaminate vegetables, meat & fish.
  - Under appropriate conditions (anaerobic environment at neutral/alkaline pH ) spores germinate & produce toxin in the food.
    - Outbreaks frequently occur in families.
    - Usually found in improperly sterilized (canned) food.
- Contact with bacteria is not necessary. □ disease can be a pure intoxication.



# Clostridium botulinum

## Pathogenesis:

- Botulinum toxins are A – G, but **human disease by types A, B, or E.**
- Toxins inhibit release of Acetyl choline causing failure of neuro-transmission that cause **flacid paralysis.**

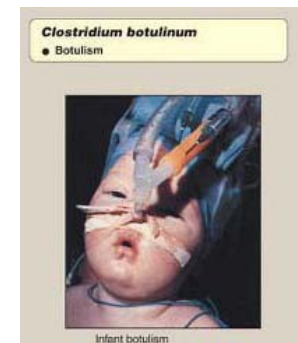


## Clinical significance:

- Classic botulism is a food poisoning
- Patient has difficulties in visual focusing, swallowing & other cranial nerve dysfunctions
- Usually 12-36 hrs after ingestion of toxin-containing food,
- No fever/sign of sepsis.
- Progressive paralysis of muscle,
- Mortality is 15%; due to respiratory paralysis .
- Recovery takes several weeks.

## Infant botulism:

- Most common form of botulism / & a cause of floppy baby syndrome.
- Microbes colonizes large bowel of infants of 3 -24 weeks of age,
  - **Neurotoxin** (produced) is slowly absorbed:
  - early signs: constipation, feeding problems, lethargy, and poor muscle tone.
  - Certain milk formula supplements, such as honey contaminated with *C. botulinum* spores, may transmit the organism.
- normally there is recovery but can be a cause of **sudden infant death syndrome**.



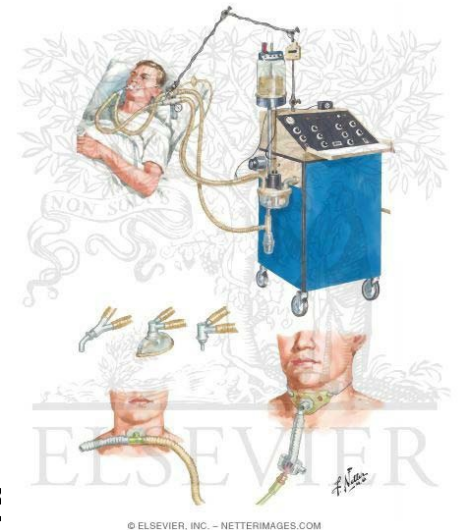
## Laboratory identification

- Anaerobic culture, & identification of Toxin in serum, stool, & food.

## Treatment & prevention:

- **Antitoxin** [neutralizes unbound botulinum toxin];
- should be administered as soon as possible.
  - **Mechanical ventilation**, may be required.
- Wound botulism [rare infection], can be treated with **penicillin**.
- The toxin is inactivated at boiling temperatures but **autoclave** kills the spores

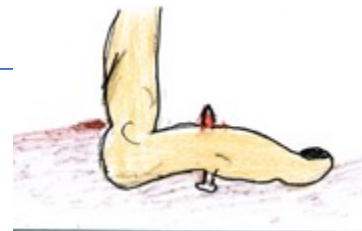
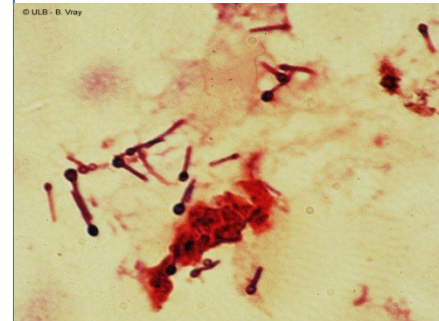
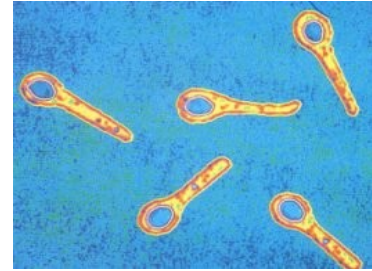
## Mechanical ventilation



# *C. tetani*

## Epidemiology

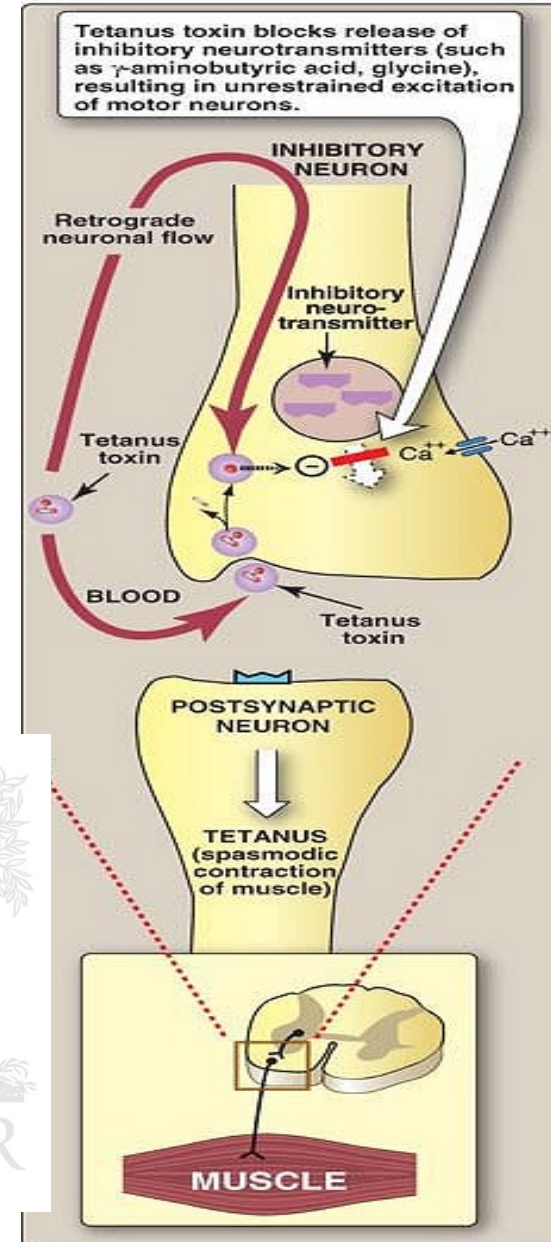
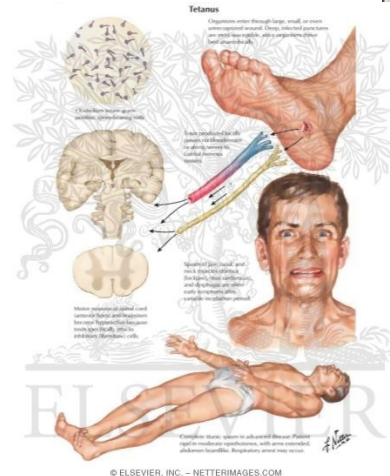
- Spores: found in the intestine of animals, soil & gardens (when the manure is used as fertilizer).
- Tetanus usually follow
  - puncture wound [e.g. splinter], foreign bodies trauma.
  - Severe burns/ surgery & illegal drugs



Use a sterilized pin and tweezers to remove the splinter

## Pathogenesis:

- **Tetanus toxin [tetanospasmin]** is a potent toxin.
- passes **along nerves** to **CNS**, acts on anterior horn cells and **interferes** 'inhibition' of motor impulses by blocking post synaptic release of inhibitory neurotransmitters.
- There is severe prolonged muscle spasms





## Clinical significance:

- Incubation period: 4 days □ many weeks.
- Spastic paralysis.
- Jaw muscles are affected and patient cannot open mouth (trismus, or lockjaw) at first.
- other voluntary muscles are also involved
- any external stimulus (eg, noise / bright light) precipitates painful spasm, & sometimes convulsions.
- Paralysis of chest muscles □ respiratory failure □ **death** (15 – 60 % cases).



present with **lock jaw and trismus, risus sardonicus, opisthotonus, generalized spasm, convulsion and respiratory failure**

## Laboratory diagnosis:

- is based on clinical findings.
- **Treatment must be initiated immediately**

### Treatment:

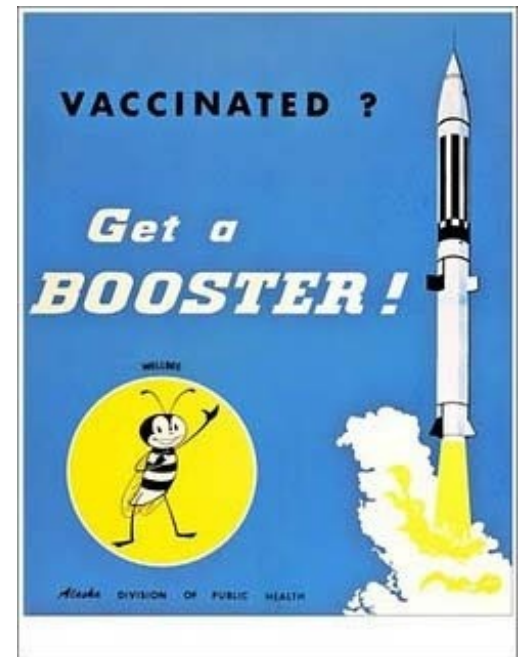
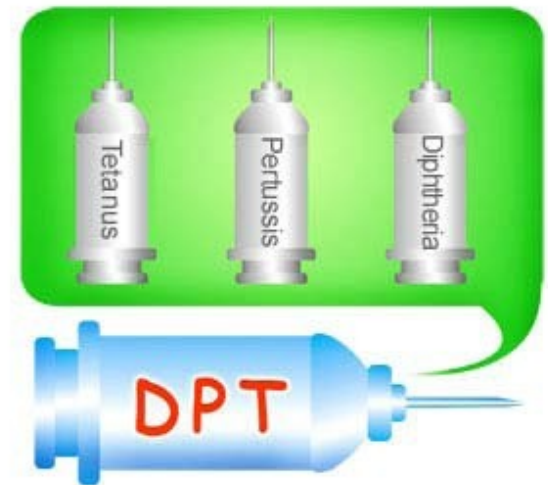
- **Prompt administration of antitoxin** to neutralize any unbound toxin □ Human hyperimmunoglobulin (tetanus immune globulin) is preferred but horse antitoxin can be used.
- *C tetanii* is sensitive to penicillin.





## Prevention:

- **Active immunization with tetanus toxoid** (formalin-inactivated toxin).
- Children are given triple vaccine - (DPT) by 3 injections at 6 – 8 weeks intervals.
- Circulating antibody levels gradually decline, & many older individuals lose protection. It requires lifelong **booster immunizations every 10 years.**
- **Tetanus immunoglobulin** gives immediate passive immunity to injured victims (who are not immunized).



## Management

- Specific treatment: **tetanus antitoxin** (human tetanus immunoglobulin) & Penicillin
- **Wound cleaning** & removal of dead tissues
- Symptomatic & supportive treatment

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- **Clean wound:**

- if patient is **fully immune**: give booster toxoid
- in **non-immune** patients 3 injections of tetanus toxoid at 6 – 8 weeks interval.

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- **Contaminated wound:**

- **fully immunized**....human tetanus globulin (ATS)+ booster tetanus **toxoid**.
- **non-immunized**.... human tetanus immunoglobulin + full course of immunization i.e. 3 injections of tetanus toxoid at 6-8 weeks intervals.

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## Prevention

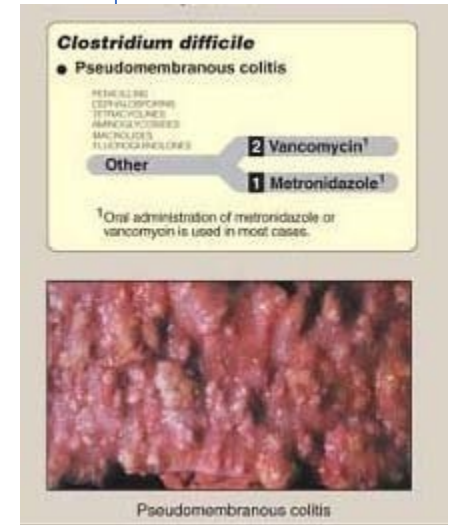
- Vaccination with tetanus **toxoid**
- Active immunization with **(DPT)** with 3 injections of tetanus toxoid at 6 – 8 weeks intervals.

# *C. difficile*

- 25% of antibiotic-associated diarrheas (AAD) in hospitalized patients is *C difficile*
- Almost all cases of life-threatening pseudomembranous colitis.

## Pathogenesis:

- Minor component of normal flora of large intestine.
- Antimicrobial treatment suppresses more predominant species that allows *C. difficile* to proliferate.
  - Pathogenic strains produce toxins.
    - Toxin A: enterotoxin
    - Toxin B: cytotoxin.



- Clinical significance

- **Virtually all antimicrobial drugs predispose to clostridial AAD** [antibiotic-associated diarrheas ] & colitis.
- Most commonly: clindamycin, ampicillin, & cephalosporins.
- Mild diarrhea, varying degrees of inflammation of the large intestine, or fulminant pseudomembranous colitis.
- **The pseudomembranous exudate is best observed by endoscopy.**

Laboratory identification:

- Anaerobic culture of stools
- **More rapid & useful tests:**
  - demonstrate toxin production in stool extracts.
  - enzyme immunoassays (ELISA) for exotoxin A and B

- Treatment:

- Discontinue the drug & provide fluid replacement .

**Frequently associated**

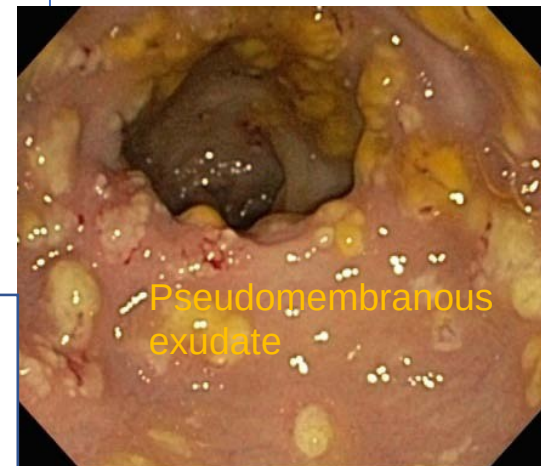
Ampicillin  
Amoxicillin  
Cephalosporins  
Clindamycin

**Occasionally associated**

Penicillins other than ampicillin  
Sulfonamides  
Erythromycin  
Trimethoprim  
Quinolones

**Rarely or never associated**

Parenteral aminoglycoside  
Tetracyclines  
Chloramphenicol  
Metronidazole  
Vancomycin



Thank you