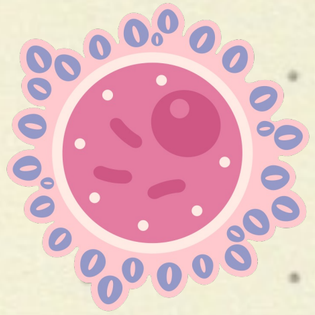
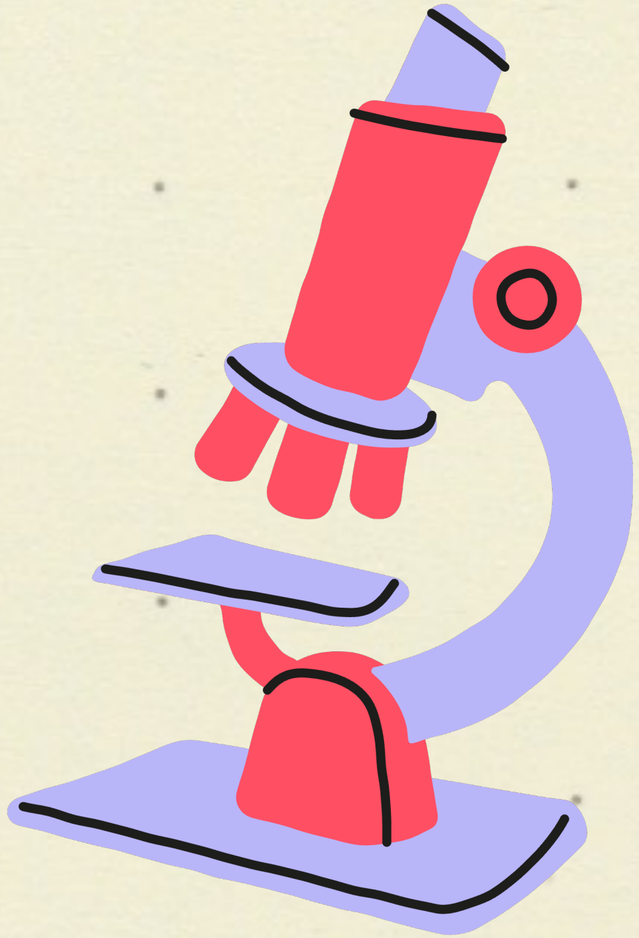
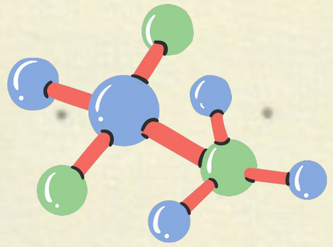
Chapter 30: CLOSTRIDIUM

C is for *Clostridium*







General Overview

Historically:

- Clostridium included all anaerobic, Gram positive, endospore forming bacilli.
- BUT WAIT:
- *C. perfringens & C. ramosum* are weakly spore forming
- *C. tertium & C. histolyticum* are aerotolerant
- *C. ramosum & C. clostridioforme* are Gram negative

Clostridium Now:

- Lots of reorganization based on gene sequencing
- Clinically significant organisms remain in genus *Clostridium*
- Clostridium organisms exist **everywhere**: soil, water, sewage, GI flora of humans and animals
- Most are harmless saprophytes

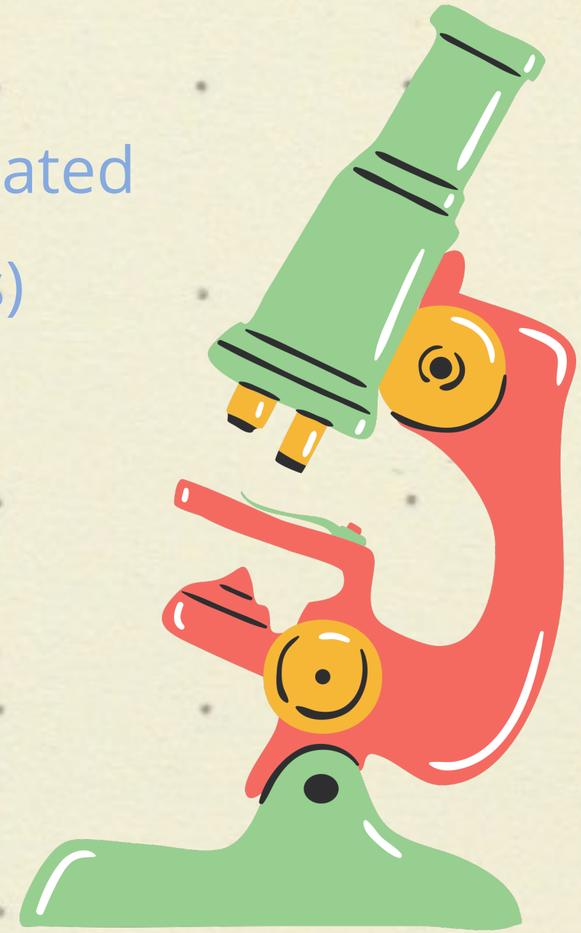
Clinical Overview

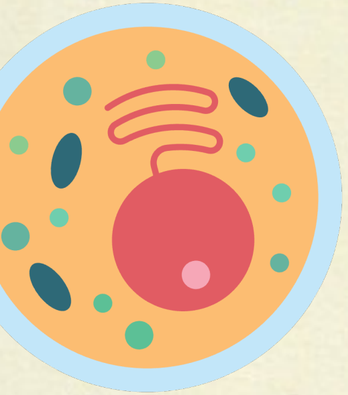
- Diseases include: Food poisoning, diarrhea, colitis, botulism, tetanus, myonecrosis/gas gangrene
- Ability to cause disease is mediated by:
  - **Spore formation (survivability)**
  - **Rapid growth in anaerobic environment**
  - **Toxin production**

01.

Clinically Significant Species:

- Clostridium difficile (Antibiotic-associated diarrhea, pseudomembranous colitis)
- Clostridium perfringens (Soft tissue infections, food poisoning, enteritis necroticans, septicemia)
- Clostridium tetani (Tetanus)
- Clostridium botulinum (Botulism)



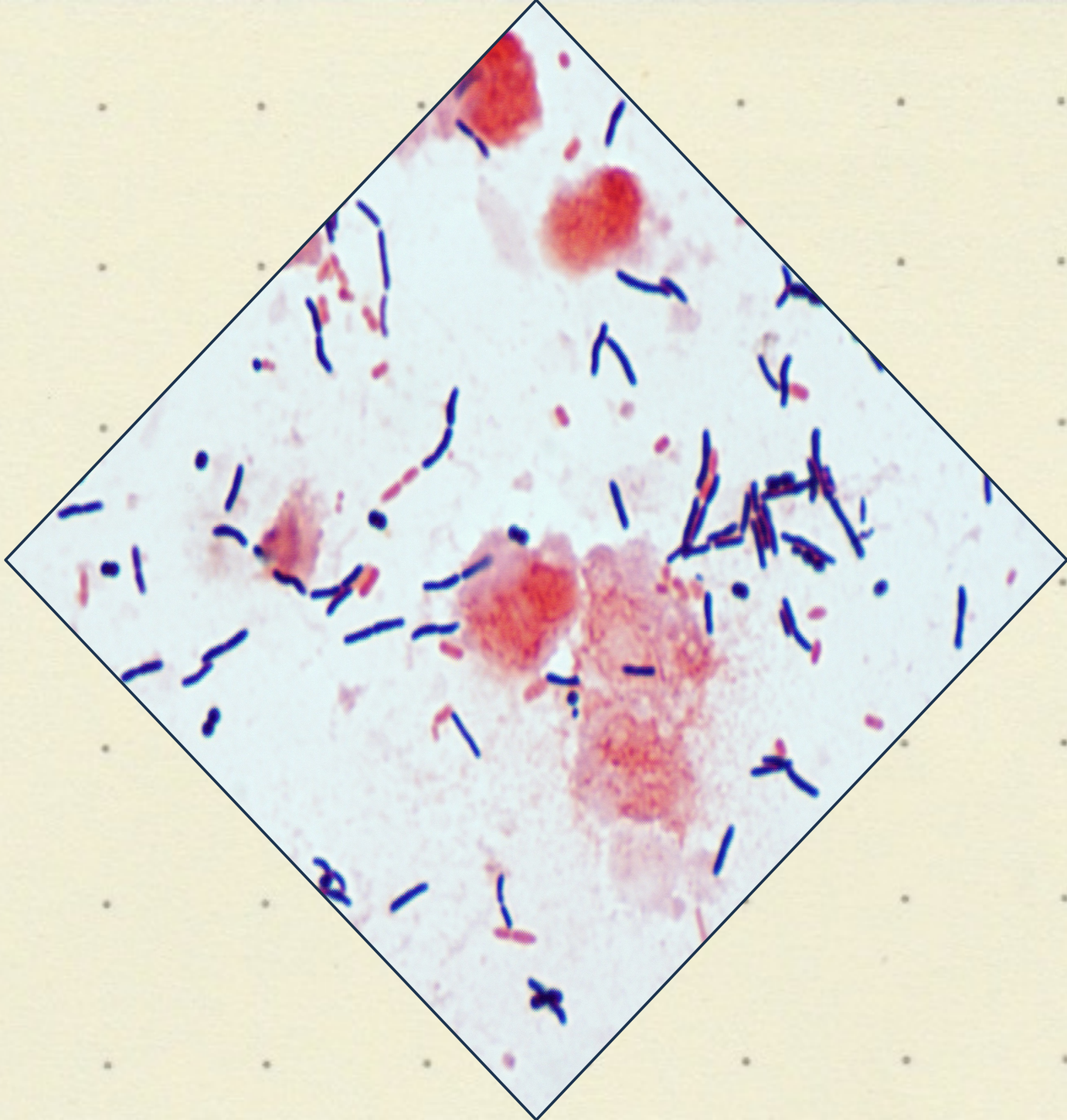


Clostridium difficile

C. difficile

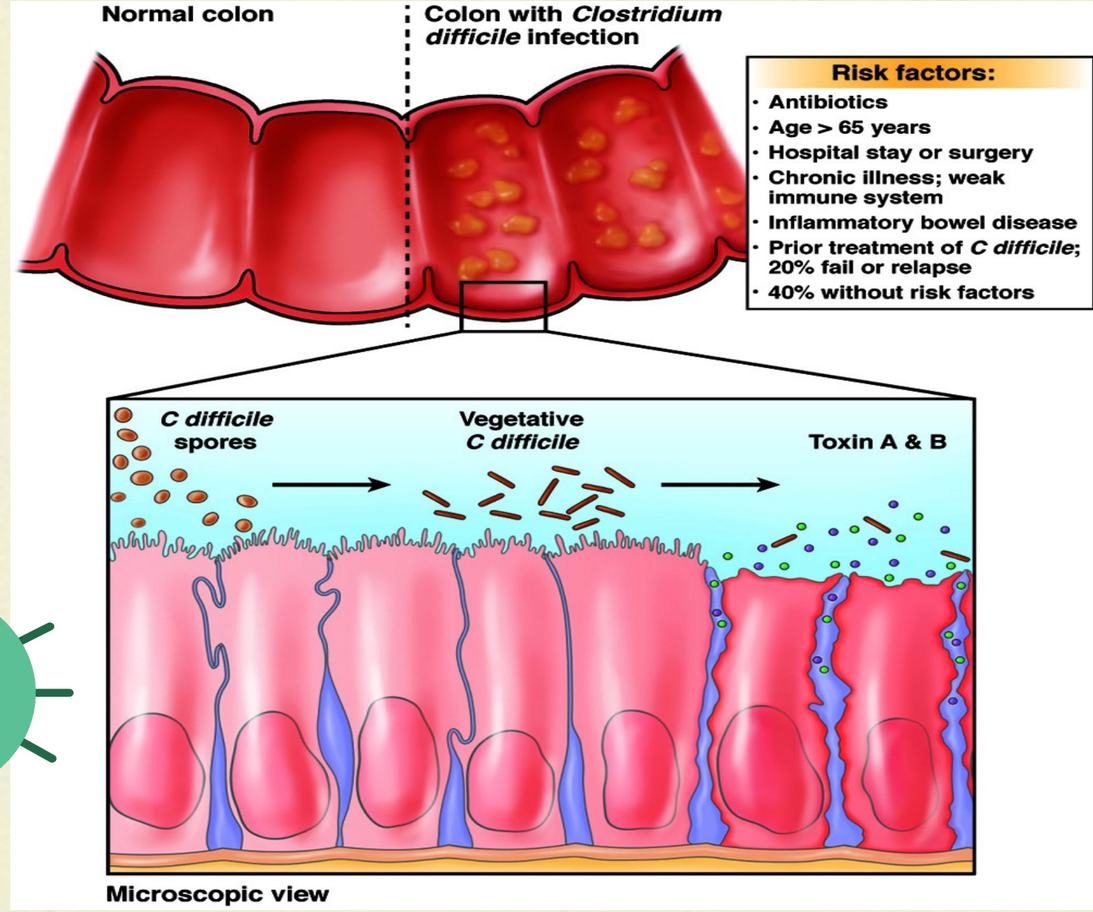
Physiology & Structure

- *difficile* refers to how difficult it is to isolate this organism due to extreme oxygen sensitivity
- Large, anaerobic spore forming rods
- Generally, Gram positive but some variability
- Produces many **volatile fatty acids** that give off characteristic **“barnyard” smell**



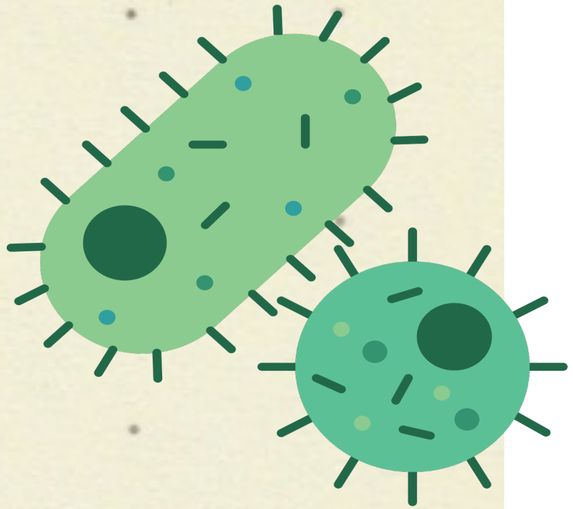
C. difficile

Pathogenesis & Immunity



Toxin Production

- Produce 2 toxins:
  - Enterotoxin (**Toxin A**)
  - Cytotoxin (**Toxin B**)



- **Toxin A** attracts neutrophils to Ileum → Inflammation and increased intestinal permeability (diarrhea)
  - Cytopathic activity e.g. disrupting tight junctions in intestinal lining
- **Toxin B** depolymerizes actin → Destruction of host cell's cytoskeleton

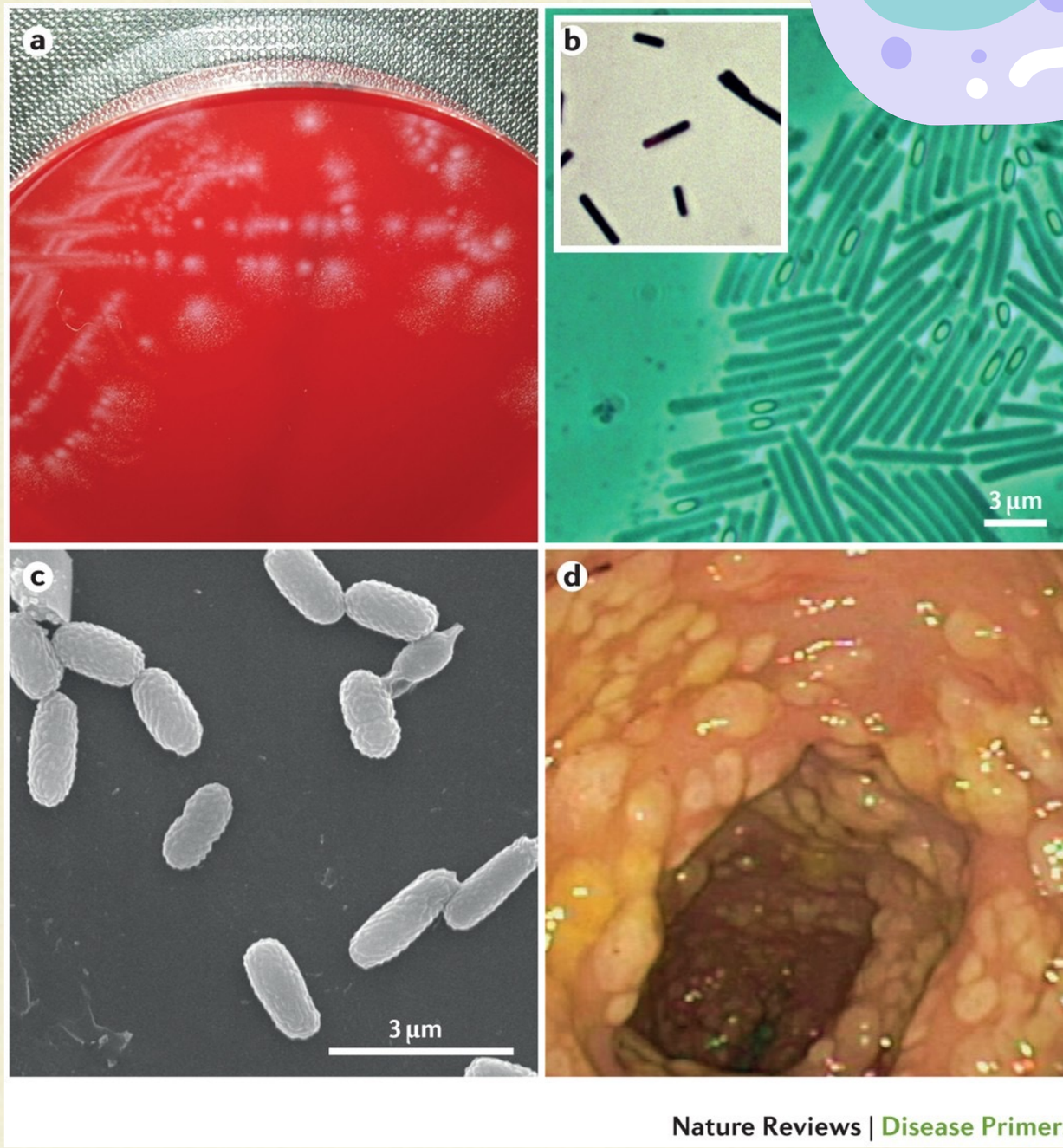
→ Production of toxins alone does not cause disease: Bacteria must adhere!

"surface layer proteins" of *C. difficile* help bind to intestinal epithelium

C. difficile

Epidemiology

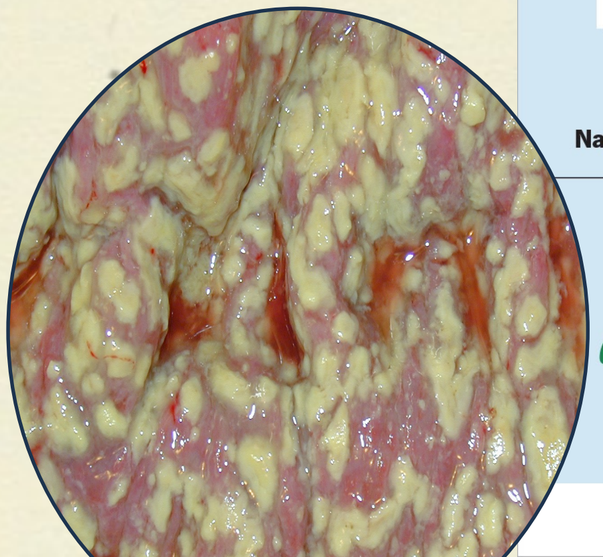
- *C. difficile* can be a normal part of gut flora (<5%)
- Endogenous infections are associated with **antibiotic use** (overgrowth of C. difficile after depletion of competitors)
- Exogenous infections are associated with exposure to spores found in hospital rooms of infected patients
- Highly virulent strain currently causes disease in North America and Europe

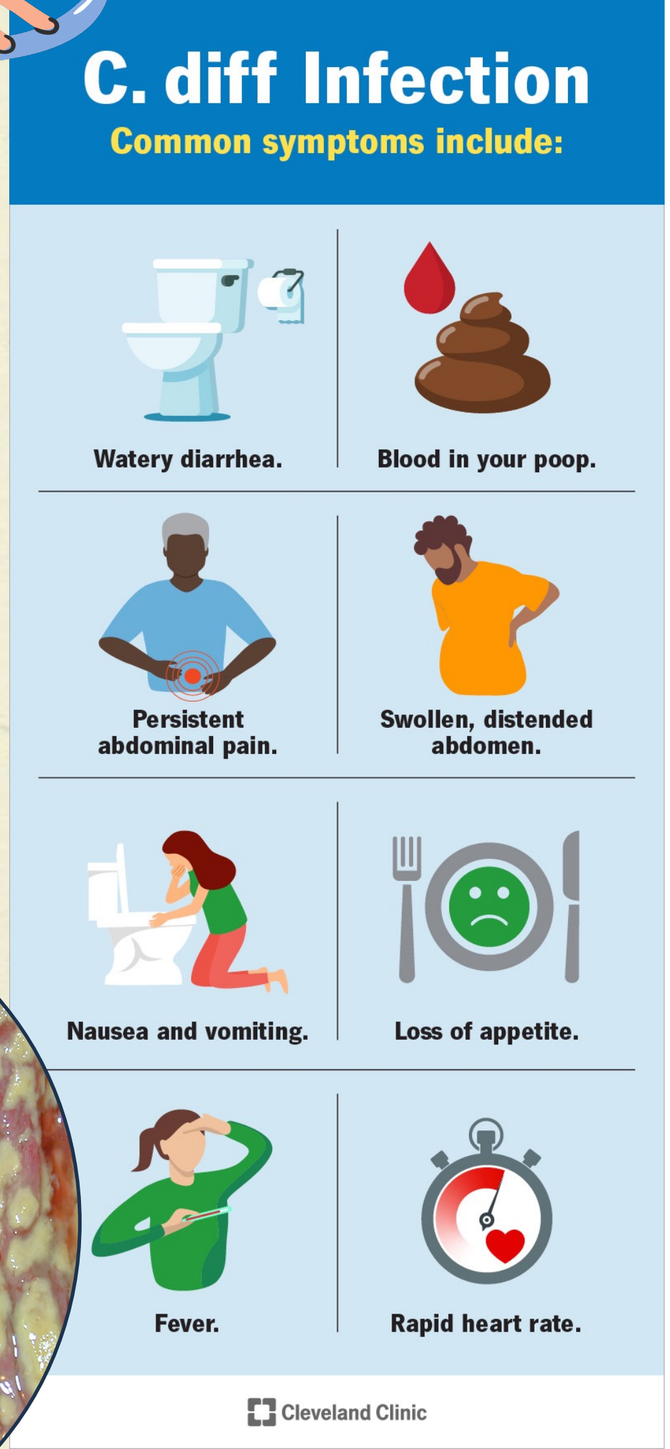


C. difficile

Clinical Diseases

- 2 main forms of disease: antibiotic-associated diarrhea & pseudomembranous colitis
  - Antibiotic-associated diarrhea: acute diarrhea beginning 5-10 days after initiating antibiotic use
  - Pseudomembranous colitis: profuse diarrhea, abdominal cramping, fever, whitish plaques on colonic tissues

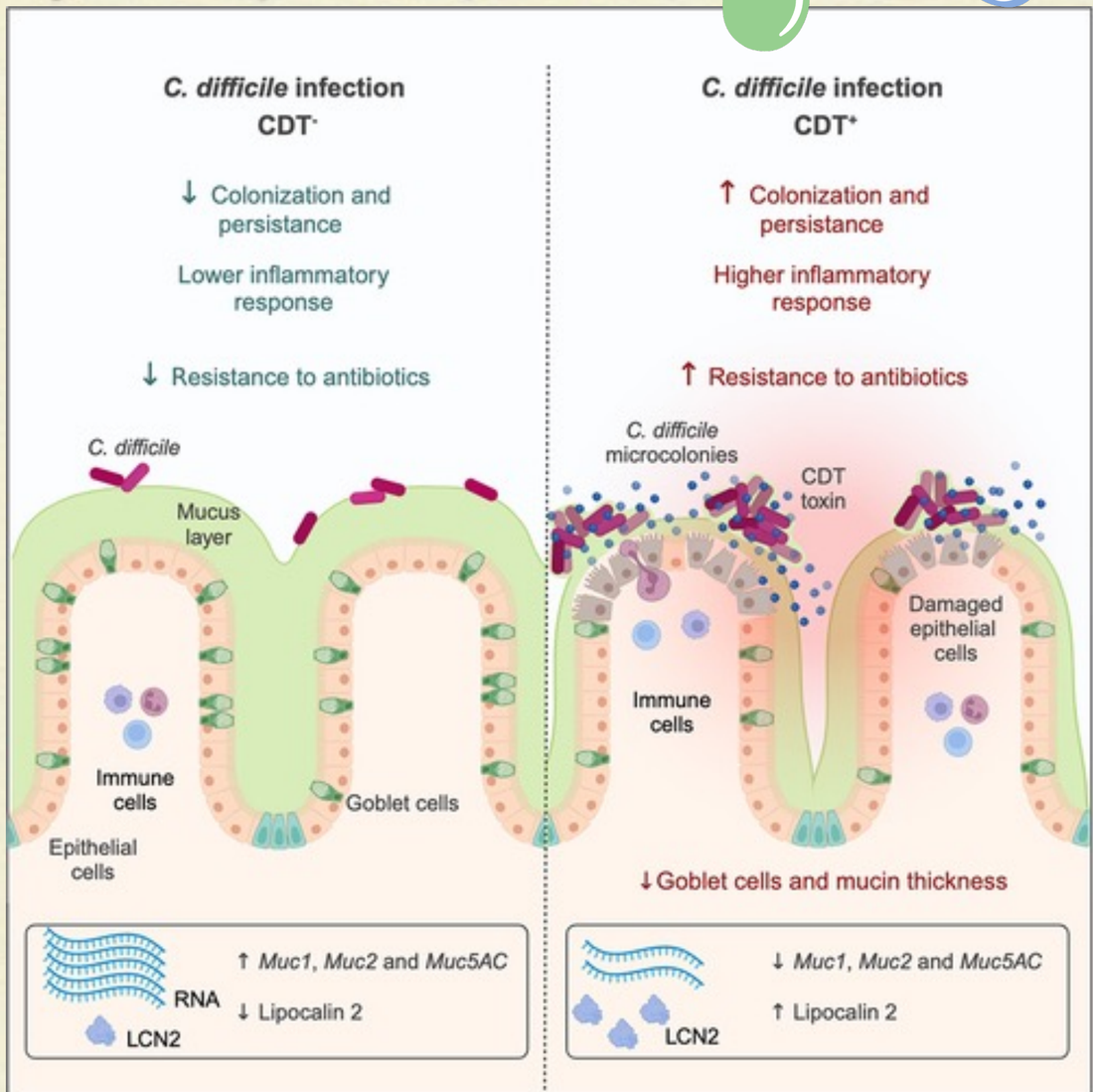




C. difficile

Clinical Diseases

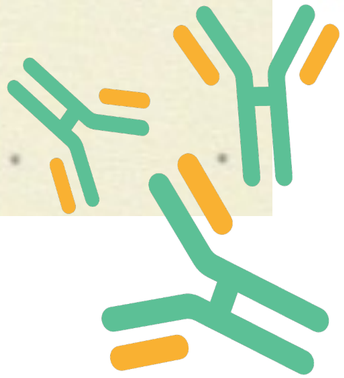
- Since 2003, a highly virulent *C. difficile* strain causes more severe disease with high mortality rate, increased risk of relapse, and complications
  - Locations affected include Canada, US, and Europe
  - Increased toxin production due to nonfunctional gene that regulates toxin production
    - Also produces 3rd toxin: binary toxin
      - Useful in detection and increases adherence of bacteria
  - Resistant to fluoroquinolones (could have contributed to selection for this strain)

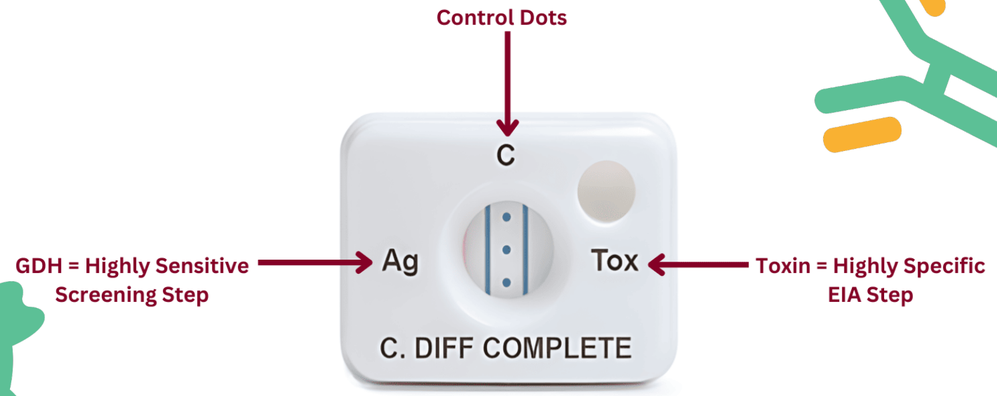


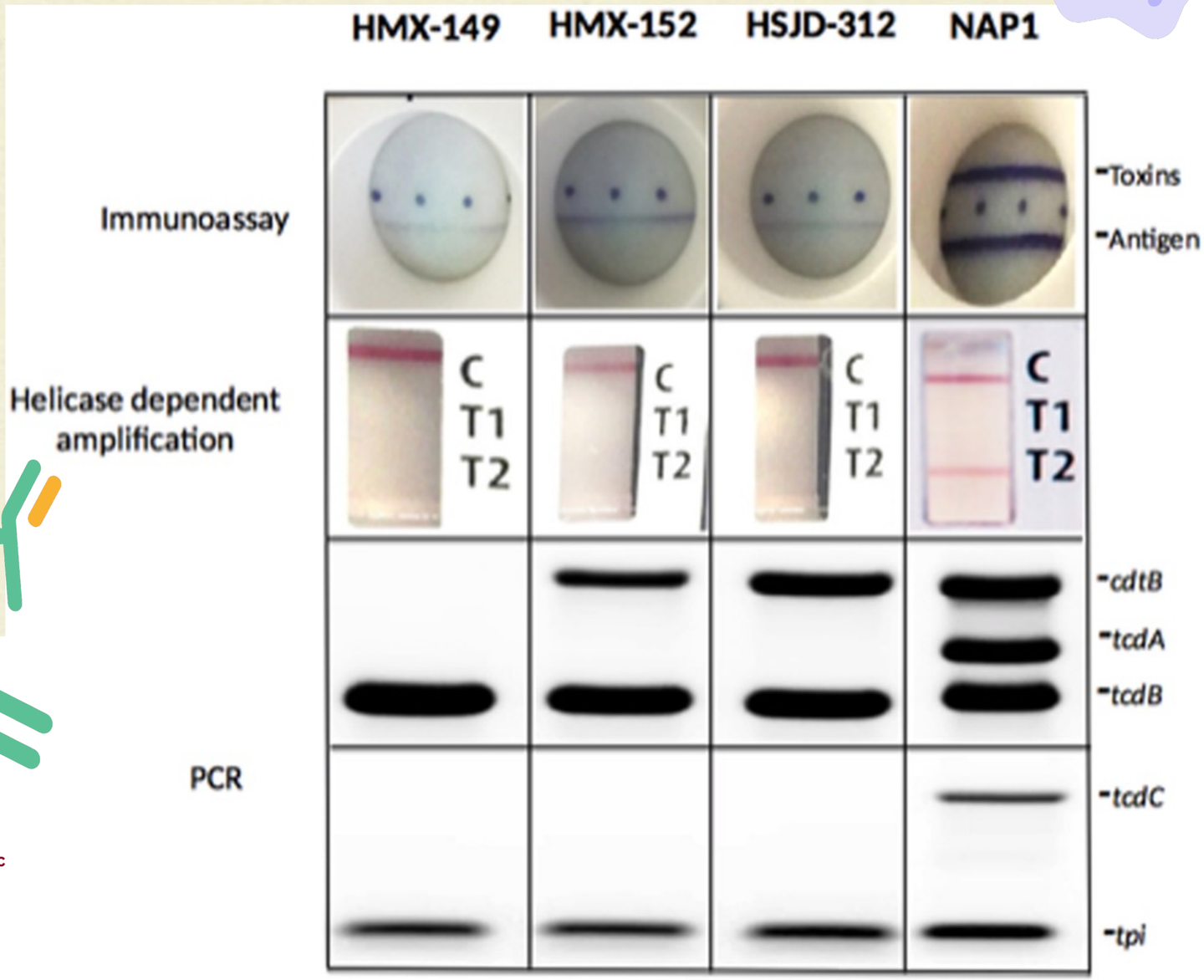
C. difficile

Laboratory Diagnosis

- Diagnosis is confirmed via toxin detection via PCR or immunoassays in patients that fit clinical signs and symptoms







C. difficile

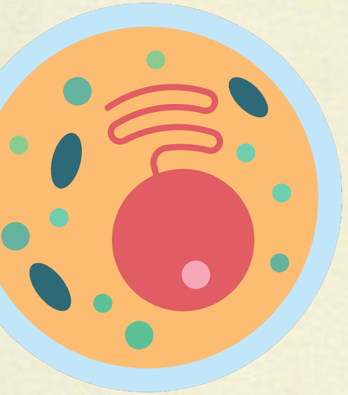
Treatment & Prevention

Treatment:

- Mild disease can be alleviated by discontinuation of antibiotics
- Severe diarrhea/colitis requires more treatment: metronidazole or vancomycin treatment
  - Only kills vegetative bacteria, relapse common as any remaining spores can germinate (**20-30% reoccurrence**)
  - Can require multiple rounds of antibiotic treatment
- Fecal transplant has high success rate

Prevention:

- Disinfection and sanitization procedures particularly in hospital settings
- Spores are difficult to eliminate
- No FDA approved vaccine
  - However, research exists on vaccines targeting intestinal mucosa directly (rectal administration) (Thomas et. al., 2026)

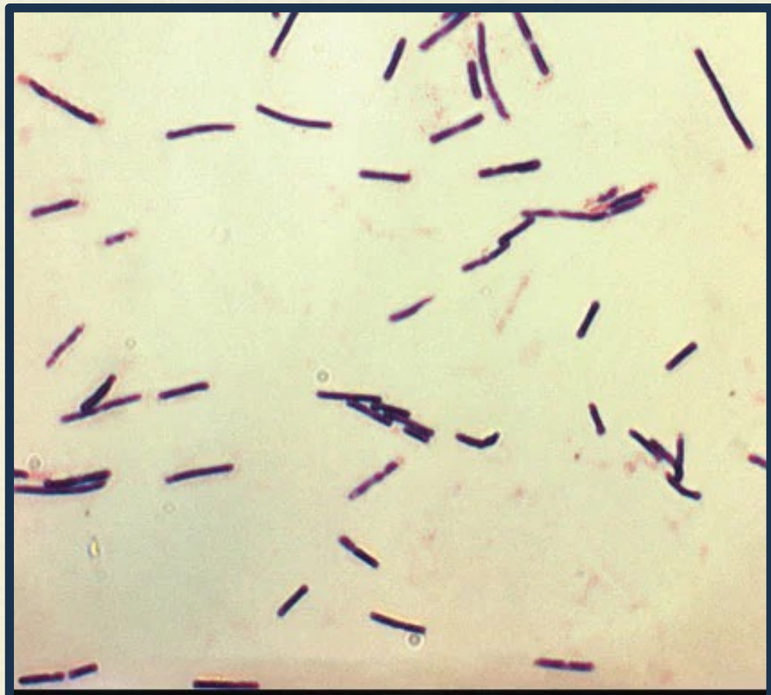


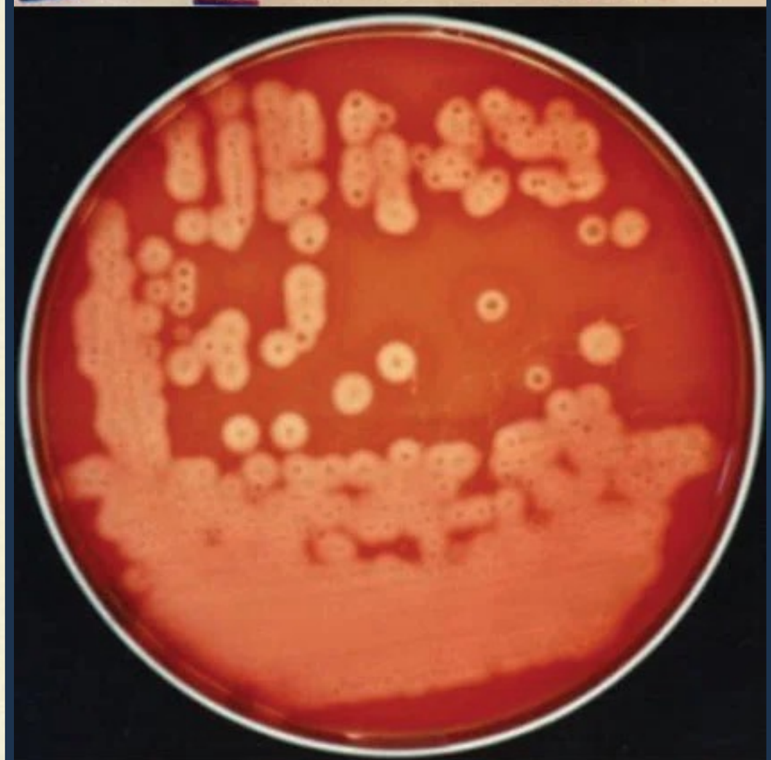
Clostridium perfringens

C. perfringens

Physiology & Structure

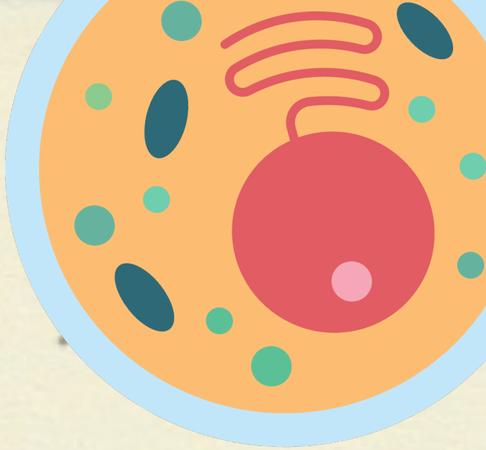
- *perfringens* refers to “breaking through” as this microbe is highly invasive in tissues
- Large, anaerobic, rectangular shaped, Gram-positive rods (some appear Gram-negative)
- **Weakly spore forming**
- β-hemolysis on blood agar (complete hemolysis)
  - Rapid, spreading growth on media
- Five types based on “major lethal toxins” (A-E)





C. perfringens

Pathogenesis & Immunity

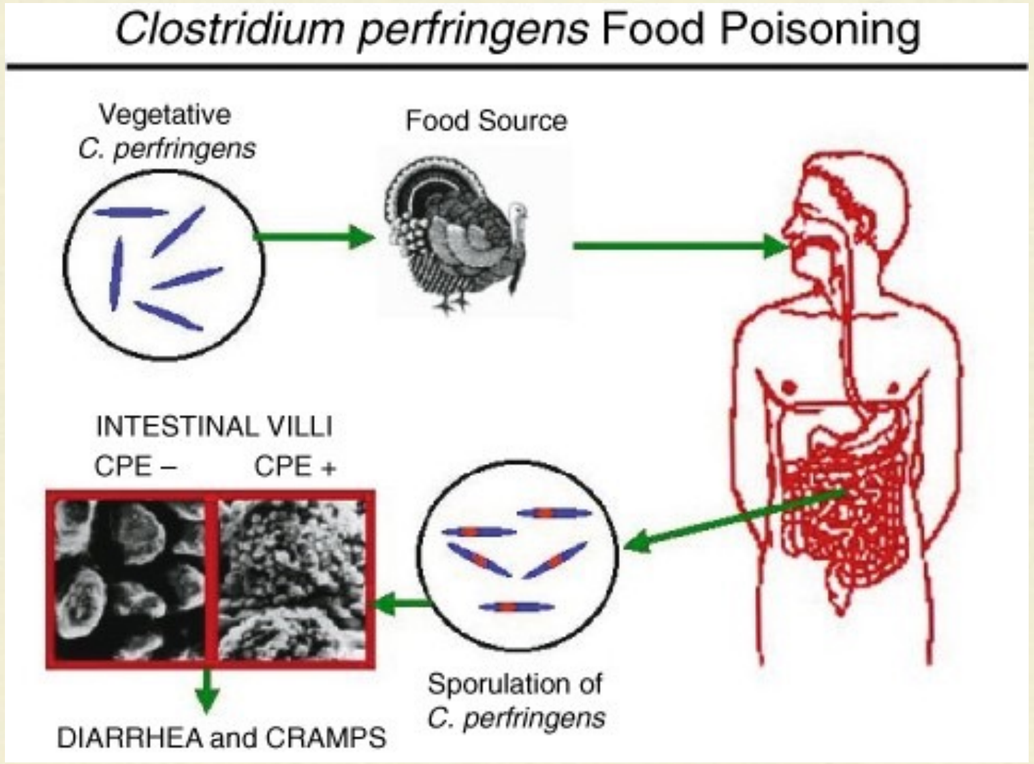


- **TOXINS, TOXINS, and more TOXINS**
  - **ALPHA TOXIN**
    - Produced by all 5 types
    - Phospholipase C that lyses RBCs, platelets, WBCs, endothelial cells
    - Results in massive hemolysis, bleeding, vascular permeability, tissue destruction, liver toxicity, myocardial dysfunction
  - **BETA TOXIN**
    - Causes intestinal stasis, loss of mucosa, formation of necrotic lesions on mucosa, necrotizing enteritis, trypsin inactivates toxin
  - **EPSILON TOXIN**
    - Protoxin activated by trypsin that increases vascular permeability of GI wall
  - **IOTA TOXIN**
    - Produced by type E *C. perfringens,* increases vascular permeability and is necrotic
  - **ENTEROTOXIN**
    - Produced by type A strains, trypsin enhances activity, produced during sporulation, binds to receptors on brush border in ileum, alters membrane permeability leading to fluid and ion loss, **superantigen** activity

C. perfringens

Epidemiology

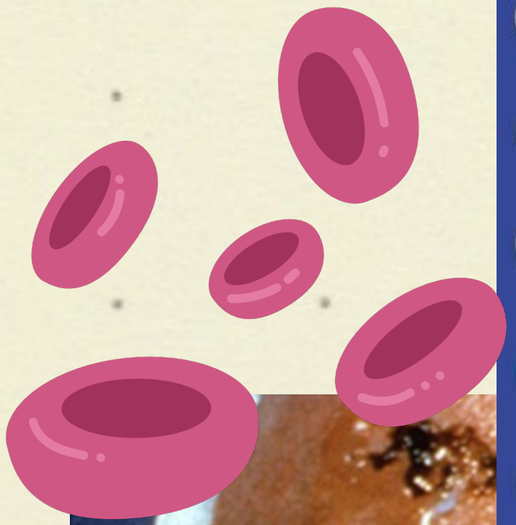
- **Type A** is the most common agent in disease associated with *C. perfringens*
  - Type A strains inhabit GI tracts of humans and animals
  - It is the only strain that can survive in soil
  - It and its spores exist in soil and water contaminated with feces
- Types B-E colonize the GI tract of animals (occasionally humans too)
- Disease is associated with ingesting spores or bacteria or open wounds coming into contact with contaminated materials

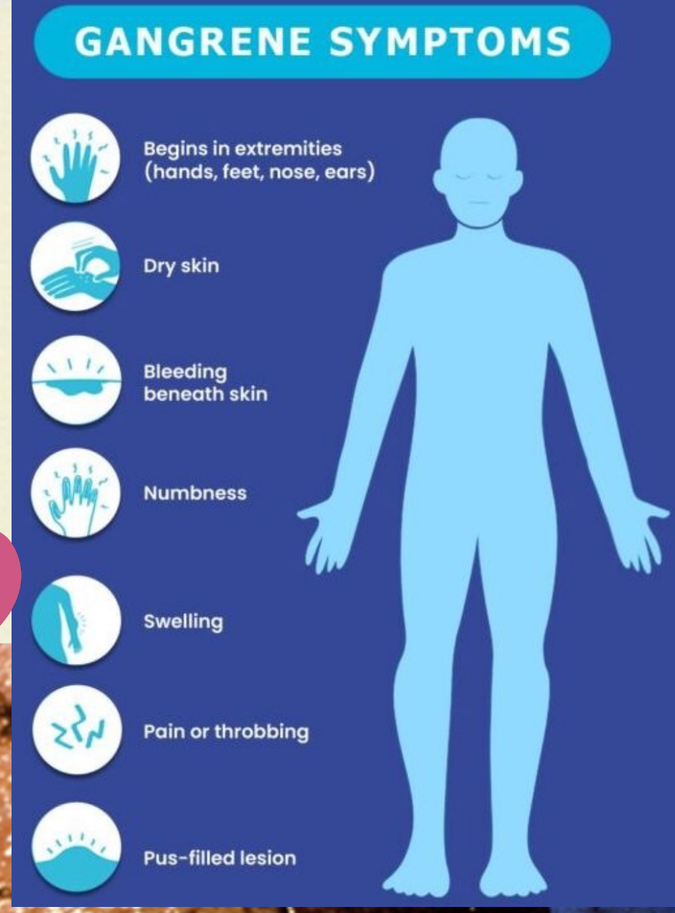


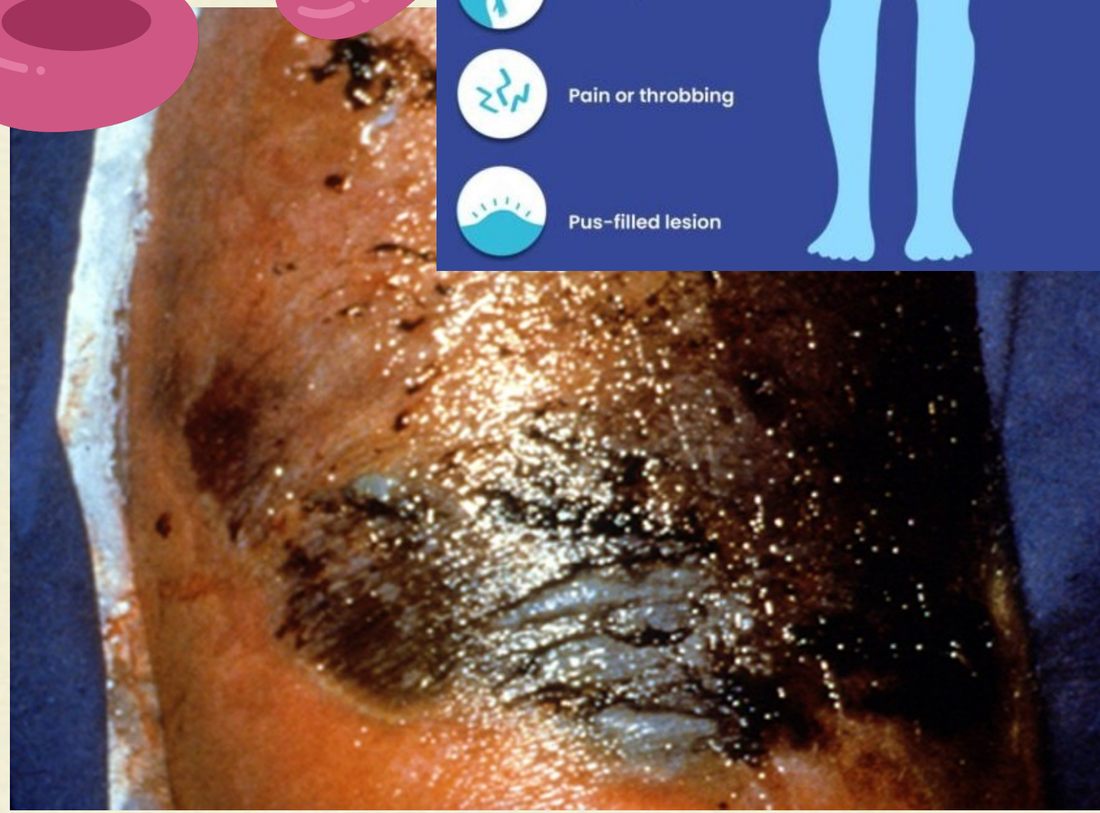
C. perfringens

Clinical Diseases – Soft Tissue Infections

- Soft tissue infections include cellulitis, fasciitis, suppurative myositis, and **myonecrosis with gas formation (gas gangrene)**
- Clostridial myonecrosis is a life-threatening disease
  - Symptom onset is about 1 week after introduction of clostridia via trauma or surgery
  - Symptoms include intense pain, rapid and extensive muscle necrosis, shock, and renal failure
    - Toxins cause extensive hemolysis and bleeding
  - Death occurs after about 2 days of symptom onset
  - Signs include devitalized necrotic tissues, abundant, rectangular Gram-positive rods in wound exudate, gas production in wound is due to rapid bacterial division

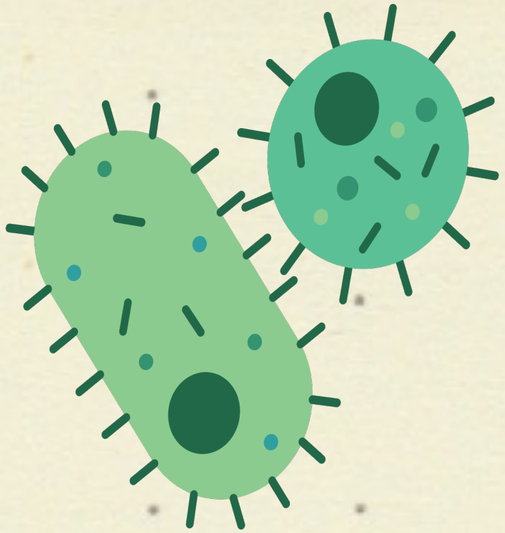






C. perfringens

Clinical Diseases – Clostridial Food Poisoning



- **Has three main characteristics**

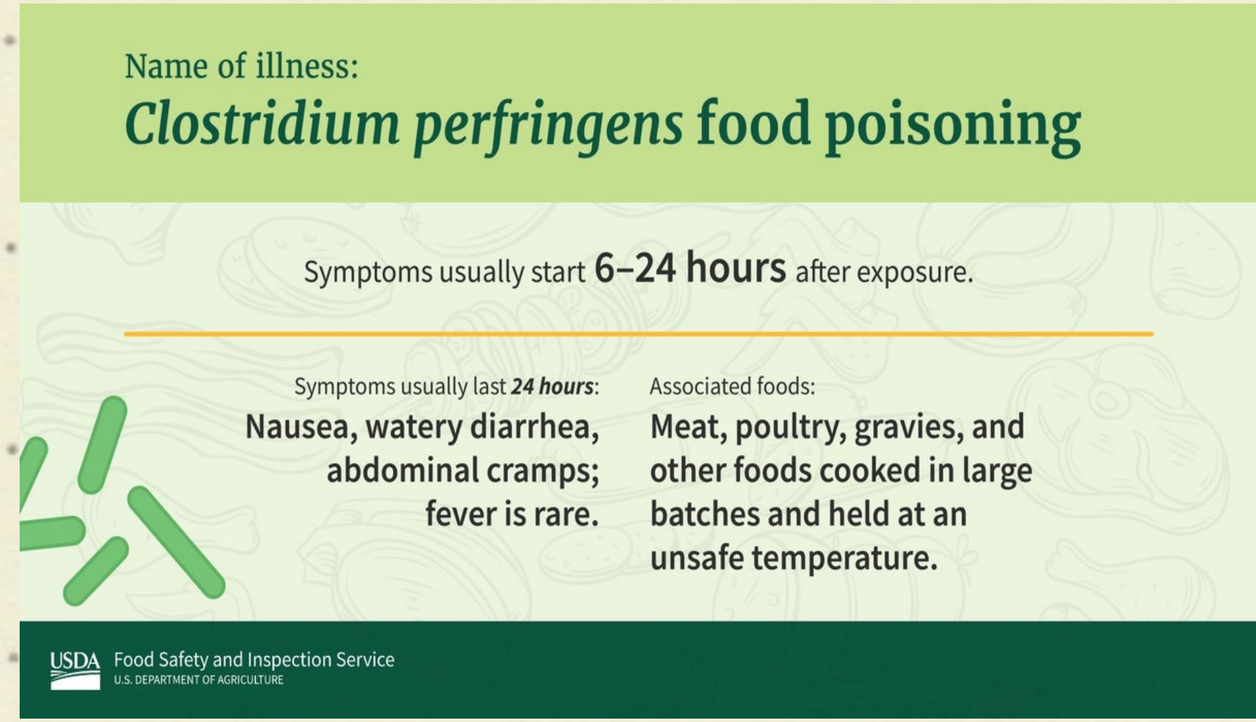
    1) Short incubation period of 8 to 12 hours

    2) Clinically presents as abdominal cramping, watery diarrhea, **NO fever**, nausea, and vomiting

    3) Typically lasts less than 24 hours

- Route of infection is typically ingesting meat products contaminated with high dose of **Type A** strains

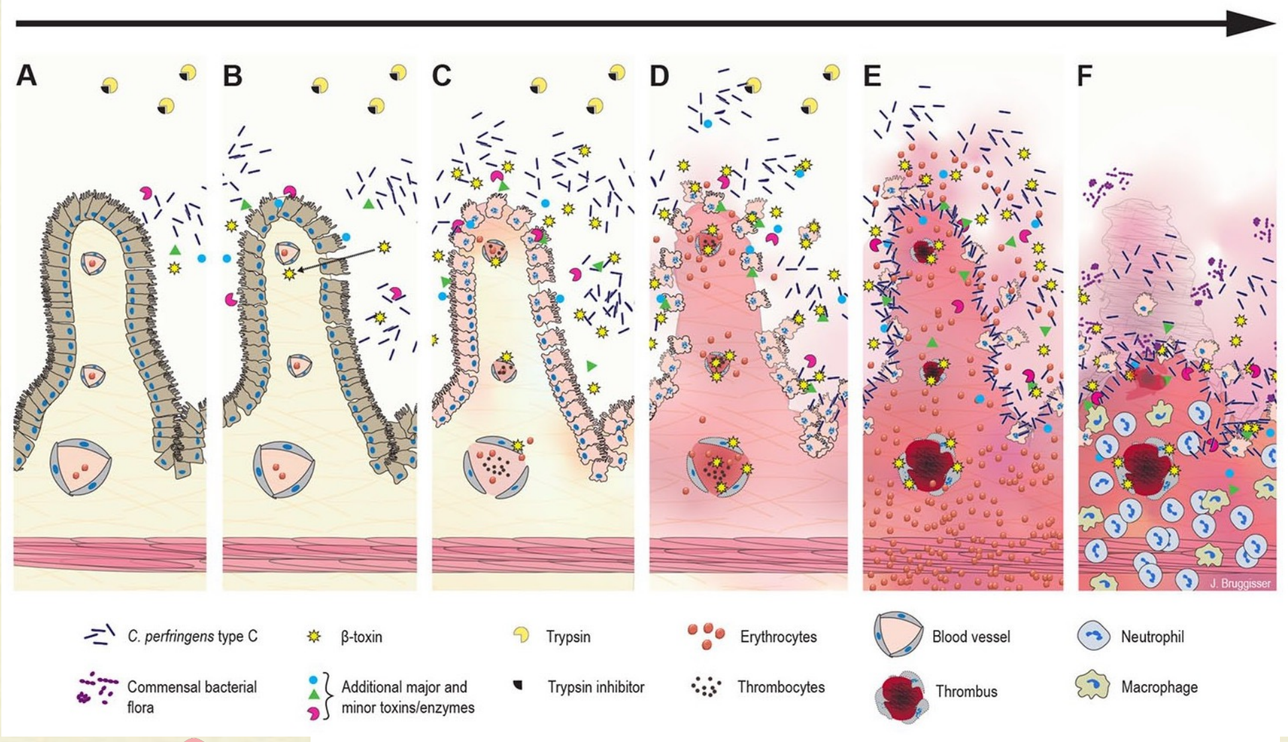
- Optimal growth temperature is 46°C, prevention can include rapid refrigeration or reheating above 74 °C

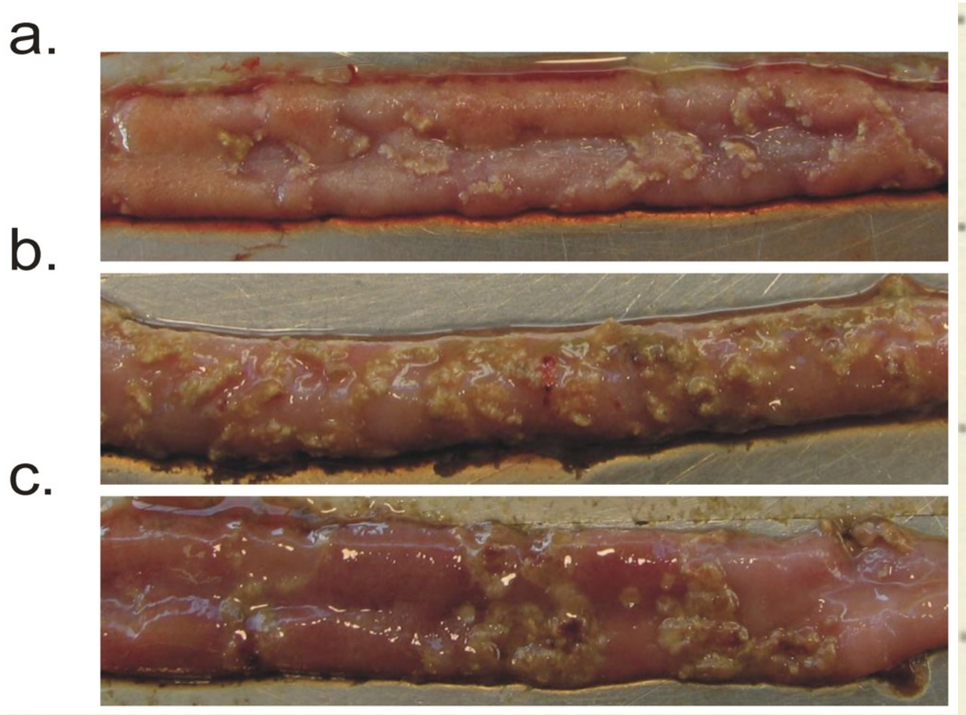


C. perfringens

Clinical Diseases – Necrotizing Enteritis

- Caused by **Beta toxin** produced by **Type C** strains
- Characteristics include necrosis of the jejunum, acute abdominal pain, vomiting, bloody diarrhea, ulceration of small intestine, perforation of intestinal wall, peritonitis and shock
  - Mortality approached 50%
- Disease is rare, occurs most commonly in Papua New Guinea
- Sweat potatoes contain heat resistant trypsin inhibitor that protects Beta toxin from inactivation
- Many *C. perfringens* can be isolated from blood cultures, but more than half are clinically insignificant. Patients with septicemia from myonecrosis or necrotizing enteritis present with massive hemolysis and septic shock

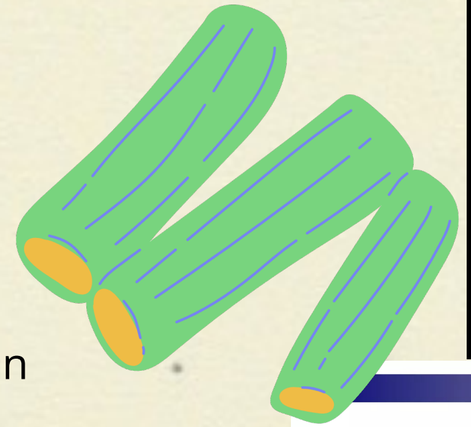


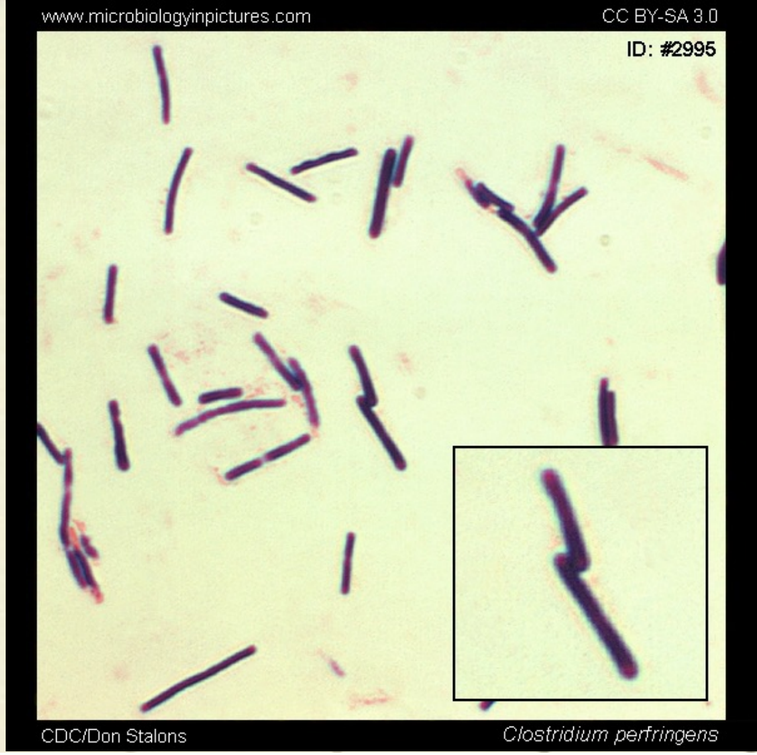


C. perfringens

Laboratory Diagnosis

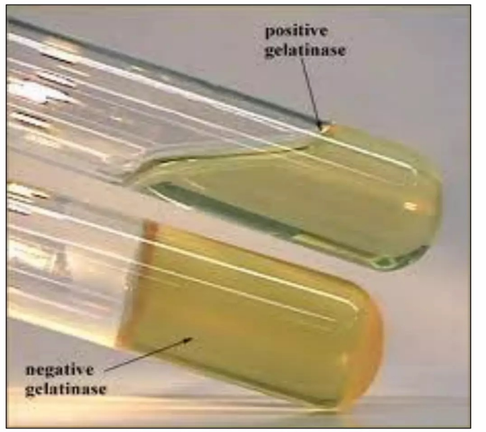
- Soft-tissue diseases must be treated **immediately**
  - Lab is confirmational not diagnostic
  - Characteristic rectangular shape helps ID bacteria in wound exudates
  - *C. perfringens* can divide every 8-10 minutes; cultures can be detected on appropriate media within hours
- Food poisoning caused by *C. perfringens* is documented by recovery of 10^5 organisms/gram food or 10^6 organisms/gram of feces collected within 1 day of disease onset
  - Immunoassay have also been developed for enterotoxin
  - Clinical diagnosis so culture or immunoassay not common





BIOCHEMICAL TESTS

- **Gelatin liquefaction test:** ***C. perfringens*** produces proteolytic enzyme (**gelatinase**) that liquefy gelatin.



C. perfringens

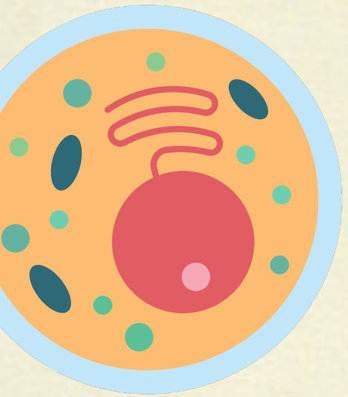
Treatment & Prevention

Treatment:

- Soft-tissue infections treated with **surgical debridement** and **high-does penicillin therapy**
  - Mortality remains 40-100%
- Food poisoning is managed by **oral hydration**
  - Severe cases involve IV fluids and electrolytes
  - Antibiotics not recommended

Prevention:

- Preventing exposure is unrealistic (ubiquitous organism)
- Preventions involved proper wound care and prophylactic antibiotic use
  - Organism produces disease when introduced to devitalized, anaerobic environment
- No approved human vaccine

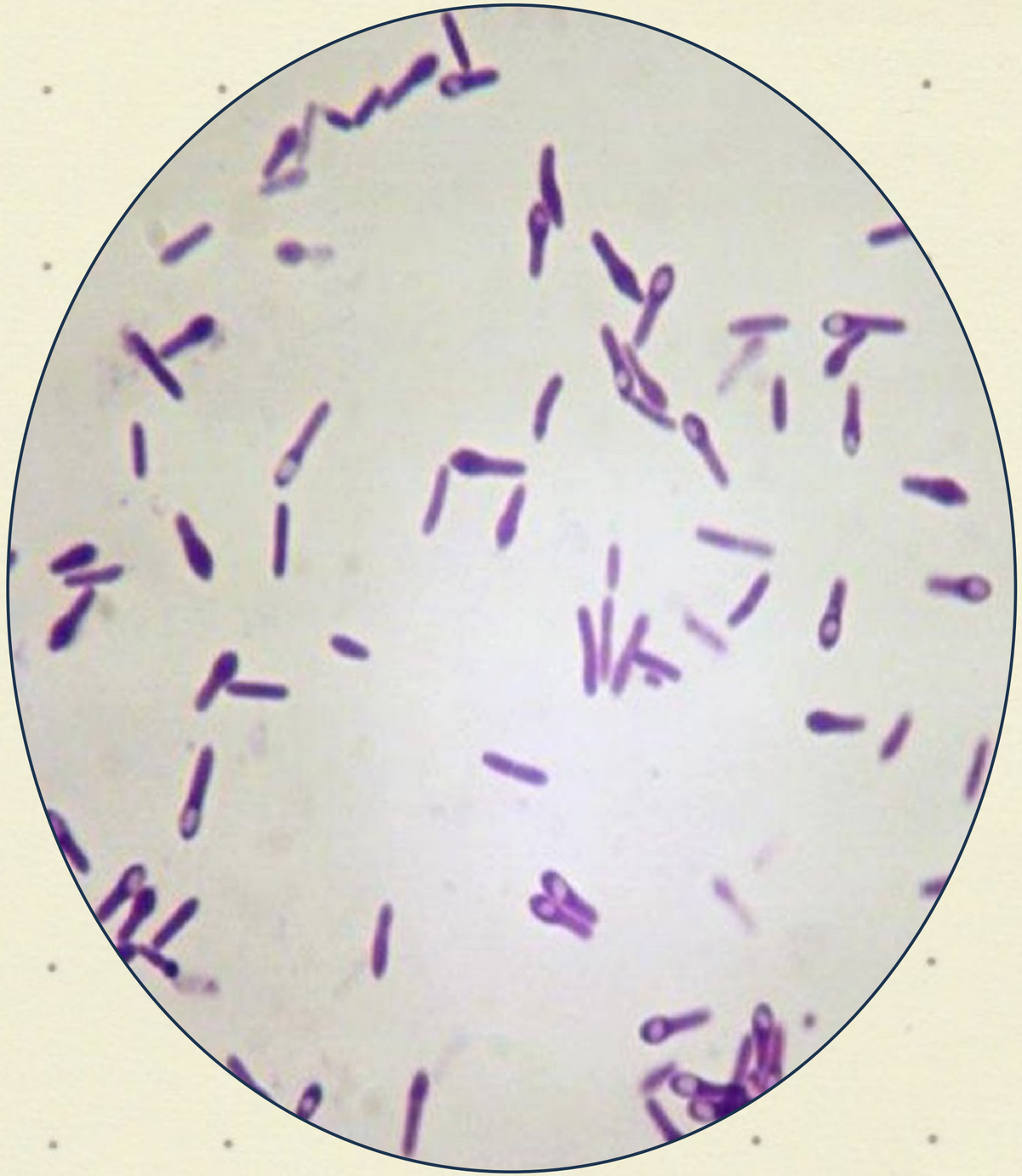


Clostridium tetani

C. tetani

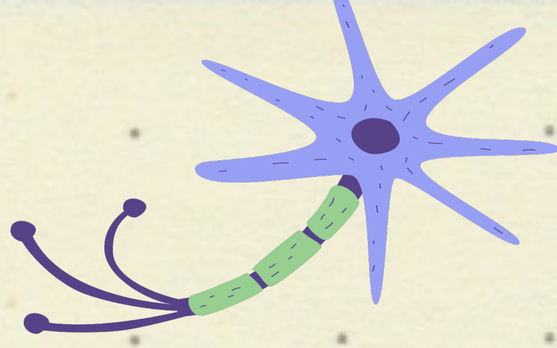
Physiology & Structure

- The name *tetani* is related to tension
- Large, motile, Gram positive, and spore forming rods
- Spores form on terminal end of rod – "**drumstick**" appearance
- Difficult to culture (**obligate anaerobe)**
  - forms film over agar not discrete colonies
- Proteolytic but **nonfermenting** (of carbohydrates)



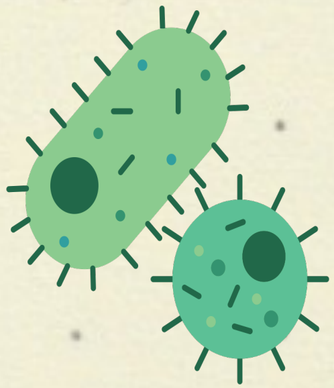
C. tetani

Pathogenesis & Immunity



Infection Route

- Introduction of the spores is typically through open wounds exposed to dirt, feces, or through puncture wounds `
- Growth is optimum in dead tissues



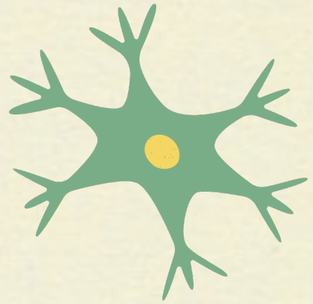
Toxins!

- *C. tetani* produces 2 toxins:
  - Oxygen-labile hemolysin **Tetanolysin**
  - Heat-labile neurotoxin **Tetanospasmin**
    - The plasmid that encodes **Tetanospasmin** for is nonconjugative

Tetanospasmin

- **Tetanolysin** is *not* clinically significant as O_2 inhibits it
- **Tetanospasmin** is an A-B toxin that is synthesized as a single polypeptide
  - It is cleaved into light (A) and heavy (B) chains when released from the bacteria
  - Disulfide bridge holds chains together

C. tetani



Tetanospasmin Mechanism

1.

B subunit binds to specific **sialic acid receptors** on **motor neurons** and is **endocytosed**

Just a note, this is VERY different to phagocytosis

2.

Intact toxin within endosomal vesicle moves to soma/body of the neuron utilizing the retrograde axonal transport system

Once at soma, toxin is released to cytoplasm when endosomal vesicle is degraded

3.

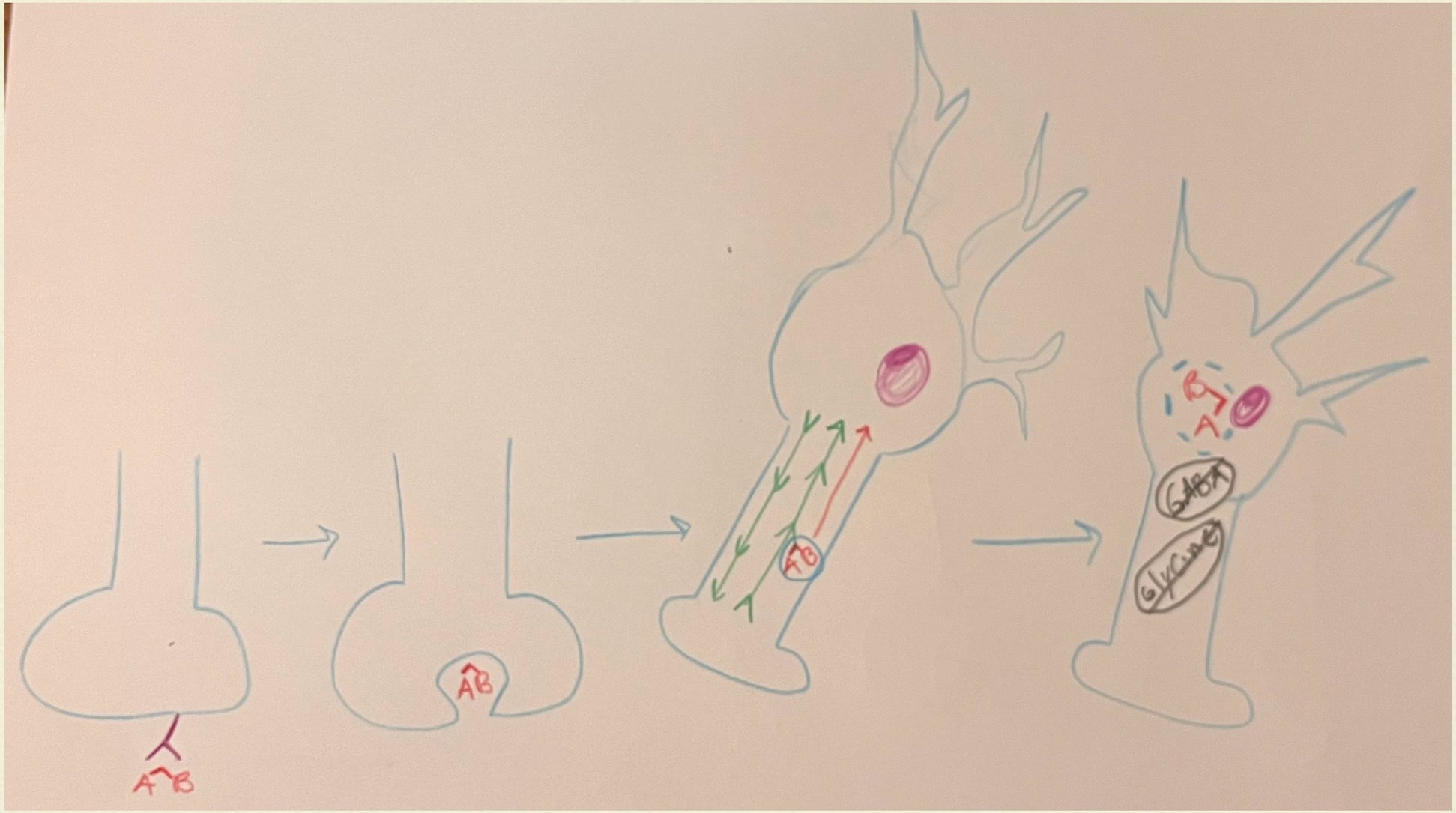
A subunit of toxin is a **zinc endopeptidase** which cleaves proteins involved in releasing vesicles of **inhibitory** neurotransmitters **GABA** and **glycine**

Without inhibitory signals, **spastic paralysis** develops

Toxin binding is **irreversible**

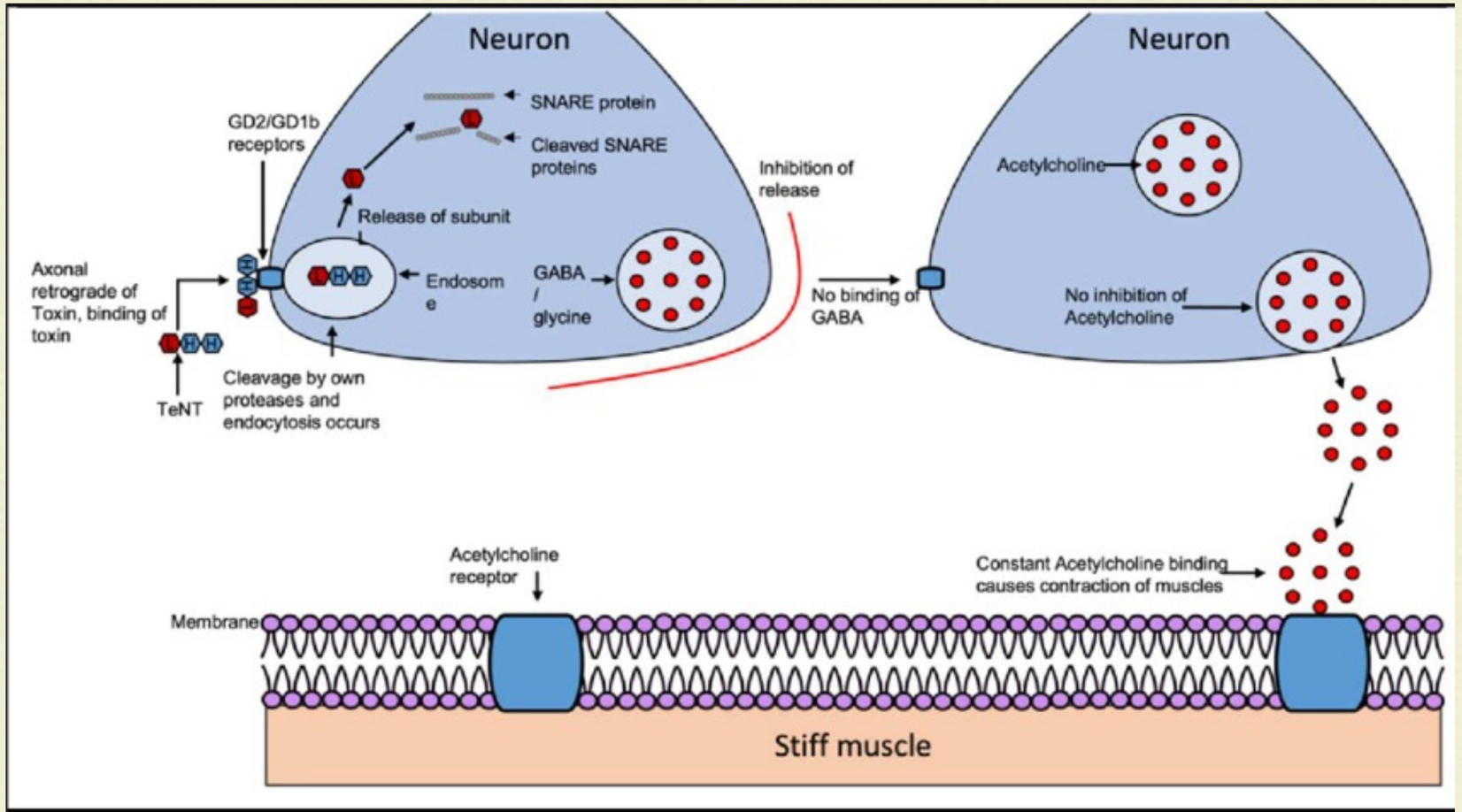
C. tetani

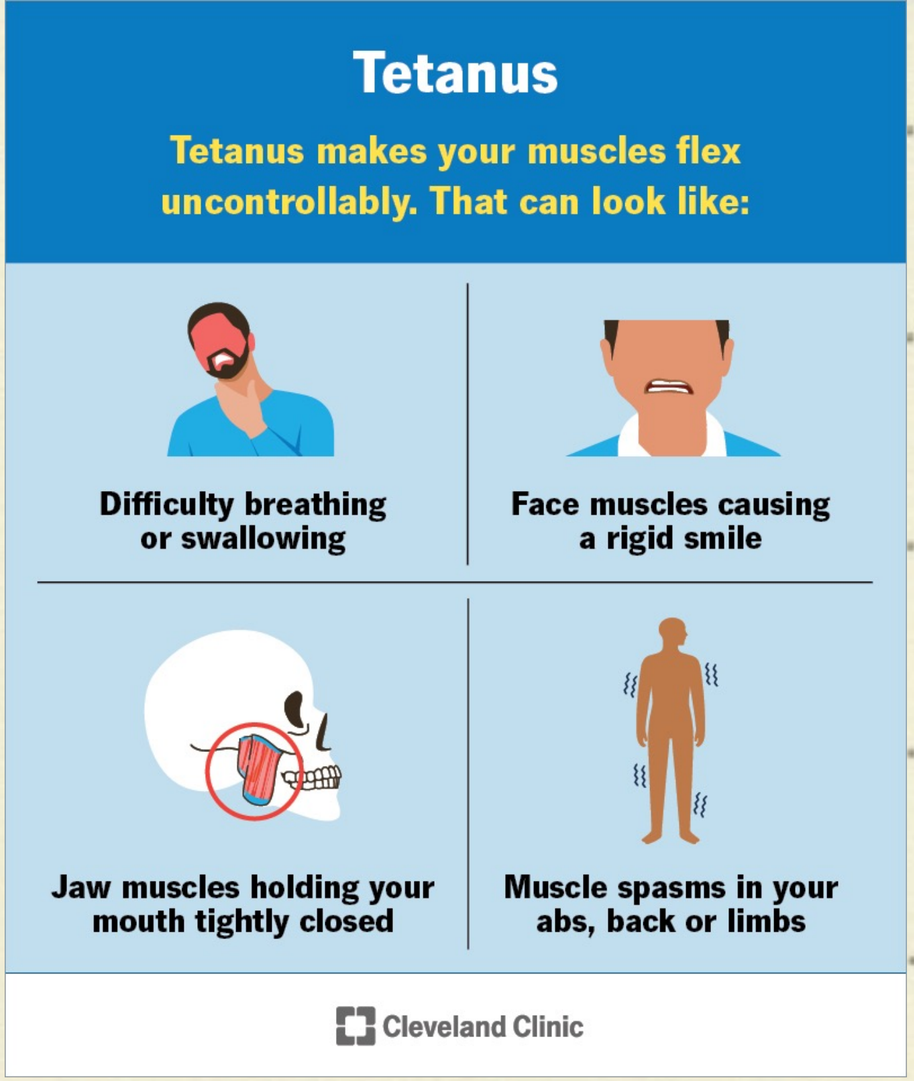
Tetanospasmin Mechanism



C. tetani

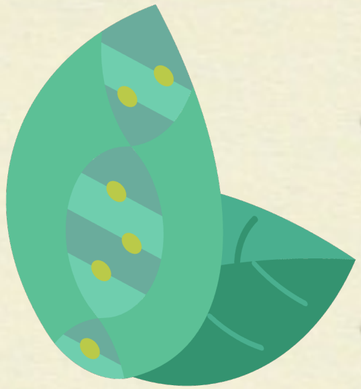
Tetanospasmin Mechanism





C. tetani

Epidemiology



X.

- *C. tetani* is **ubiquitous**, lives in soil & GI tracts of humans and animals
- It is highly sensitive to oxygen toxicity, but **spores** in environment have longevity

Y.

- Due to **vaccination**, tetanus is rare in the US.
- Cases are primarily in elderly people with waning immunity

Z.

- Tetanus is more common in parts of the worlds without as much access to vaccination
- Cases in these areas are primarily **neonates**

C. tetani

Clinical Diseases

Generalized Tetanus

- ❑ Most common form
- ❑ Incubation = distance of wound to CNS
- ❑ Symptoms include:
  - ❑ Lockjaw & 'sardonic smile'
  - ❑ Drooling, sweating
  - ❑ Muscle spasms

Localized Tetanus

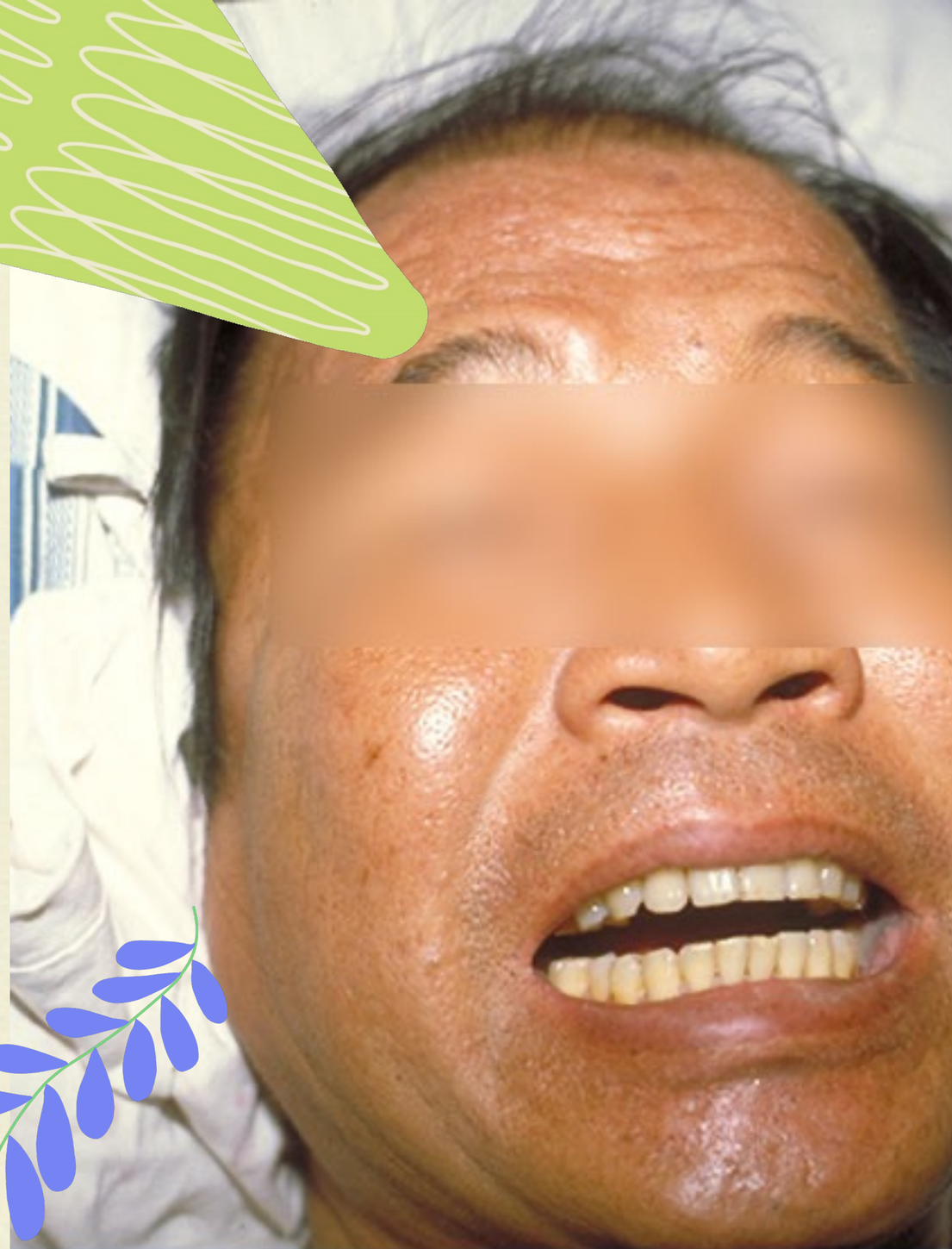
- ❑ Infection is only in musculature of infection site
- ❑ Prognosis is better than other forms

Cephalic Tetanus

- ❑ Variant of localized tetanus
- ❑ The head is the primary site of infection
- ❑ Prognosis is worse than localized tetanus

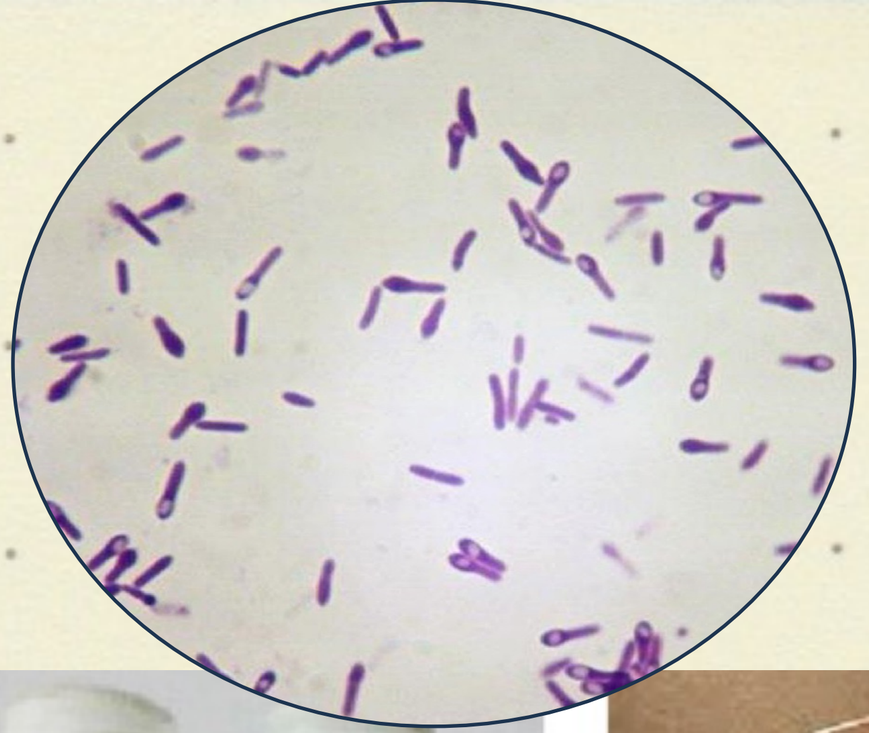
Neonatal Tetanus

- ❑ Infection begins at umbilical stump and progresses to generalized tetanus
- ❑ Mortality rate is >90%



C. tetani

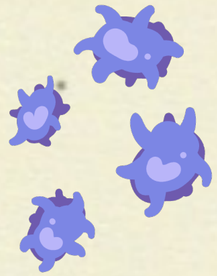
Laboratory Diagnosis

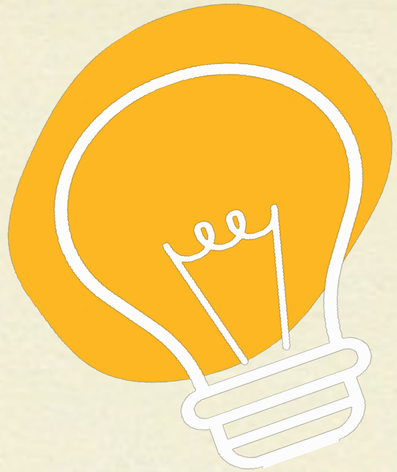


- Tetanus is primarily diagnosed by **clinical presentation** and here's why:
  - *C. tetani* is difficult to culture (obligate anaerobe)
  - Successful culturing occurs about 30% of the time
  - Tetanus toxin binds irreversibly in motor neurons so detecting the toxin is extremely difficult (no PCR)
  - If culturing is successful, toxin production can be confirmed through an antitoxin neutralization test in mice

C. tetani

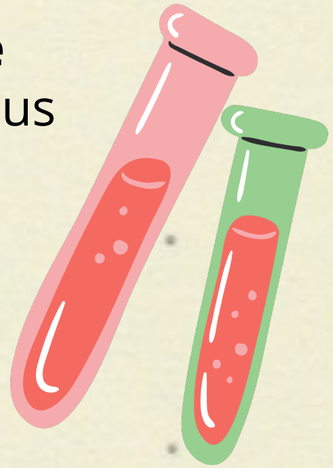
Treatment & Prevention





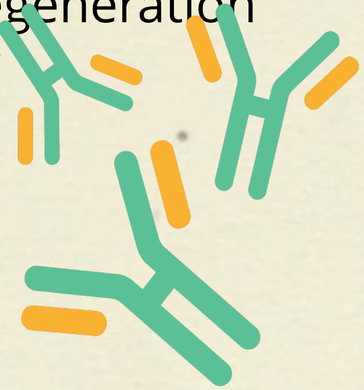
Treatment:

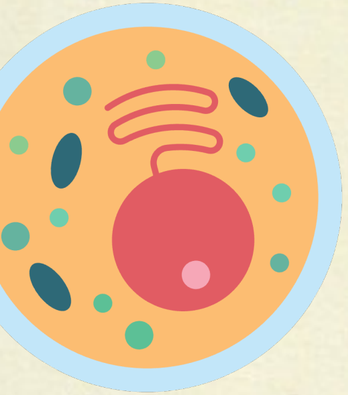
- ❑ **Debridement** of primary wound
- ❑ Kill bacteria & reduce toxin production: **metronidazole** (penicillin works too but may also inhibit GABA)
- ❑ Neutralize unbound toxin: **passive immunization** using human tetanus immunoglobulin



Prevention:

- ❑ Infection does not confer immunity!
- ❑ Vaccine is made with **tetanus toxoid**
- ❑ 3 doses of vaccination followed by boosters every 10 years (Tdap)
- ❑ Recovery from infection involved regeneration of nerve endings/synaptic plasticity

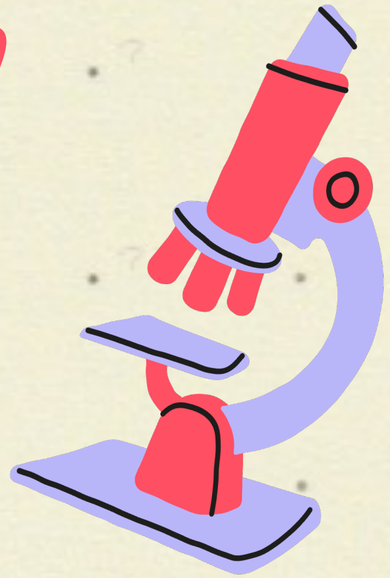




Clostridium botulinum

C. botulinum

Physiology & Structure



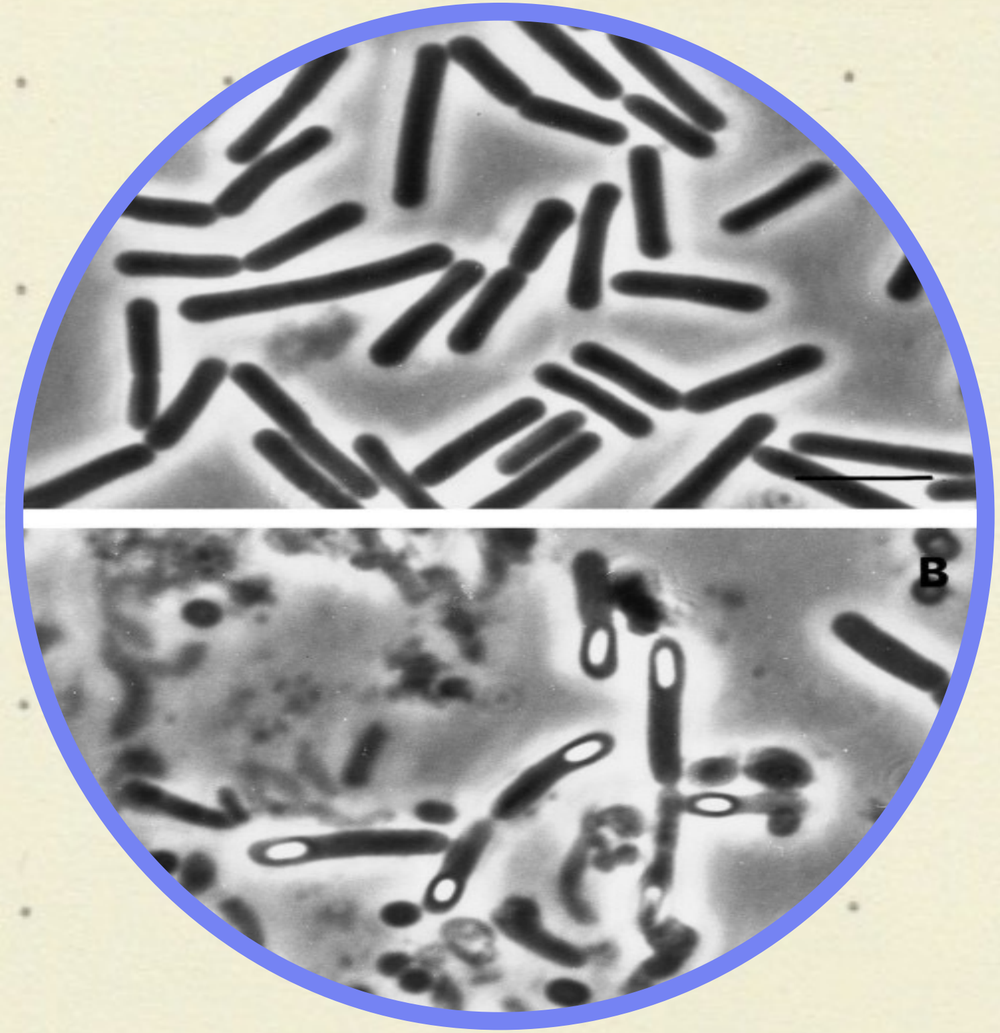
- The name *botulinum* is derived from *botulus* which means sausage
  - First outbreak was from sausages

- There are 4 species that cause botulism
  - Historically they were all classified as *C. botulinum*

- All **are large, fastidious, spore-forming, anaerobic rods**

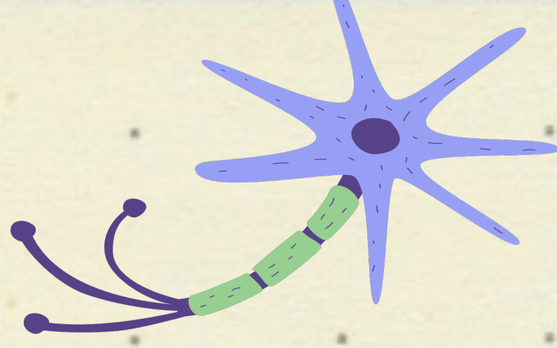
- There are 7 antigenically distinct toxins that cause botulism (A-G)
  - A, B, E, and F associated with human disease

- *C. butyricum*, *C. baratii*, & *C. argentinense* produce toxins E, F, G respectively



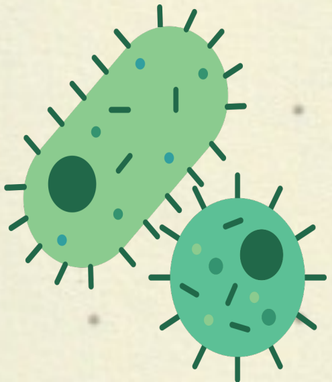
C. botulinum

Pathogenesis & Immunity



More Toxins!

- A-B toxin similar to *C. tetani*
  - A chain also a **zinc-endopeptidase**
- Unlike *C. tetani,* botulism toxin is packed with nontoxic proteins
  - Protects neurotoxin as it passes through GI tract

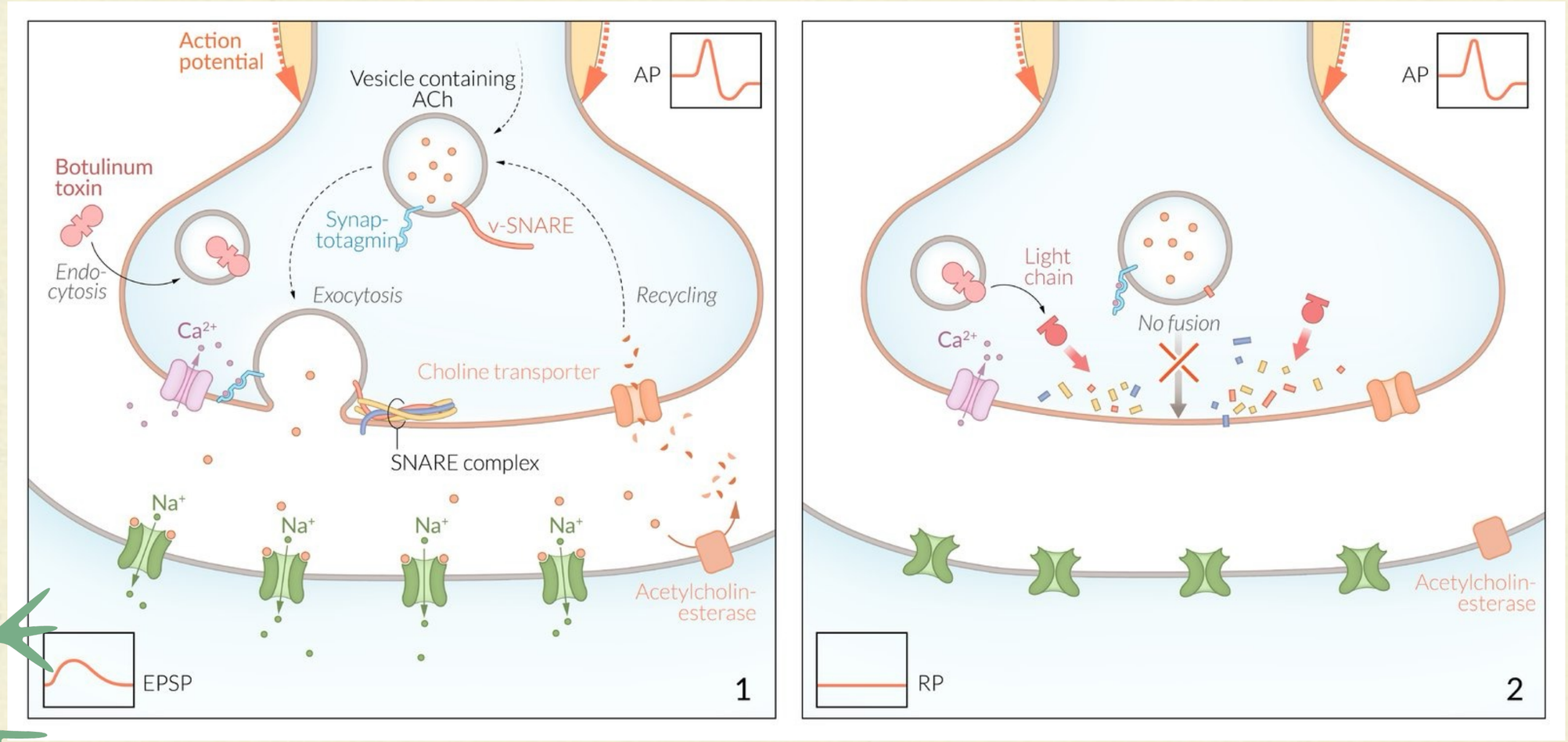


Mechanism

- Botulism toxin binds to sialic acid receptors on motor neurons
  - Crucially, these are different receptors from tetanospasmin
- Toxin remains in the neuromuscular junction and inactivates proteins involved in releasing acetylcholine
- Acetylcholine excites muscles to contract. Without this neurotransmitter, **flaccid paralysis** results.

C. botulinum

Mechanism



C. botulinum

Epidemiology

A.

- Also **ubiquitous** in soil and water – disease is still uncommon in US
- Type A toxin = alkaline soil of Mississippi river
- Eastern US (rich, organic soil) = type B
- Wet soil = Type E

B.

- Four forms of botulism:
  - **Foodborne botulism** (uncommon)
  - **Infant botulism** (most common)
  - **Wound botulism** (rare)
  - **Inhalation botulism** (biowarfare/bioterrorism)

C.

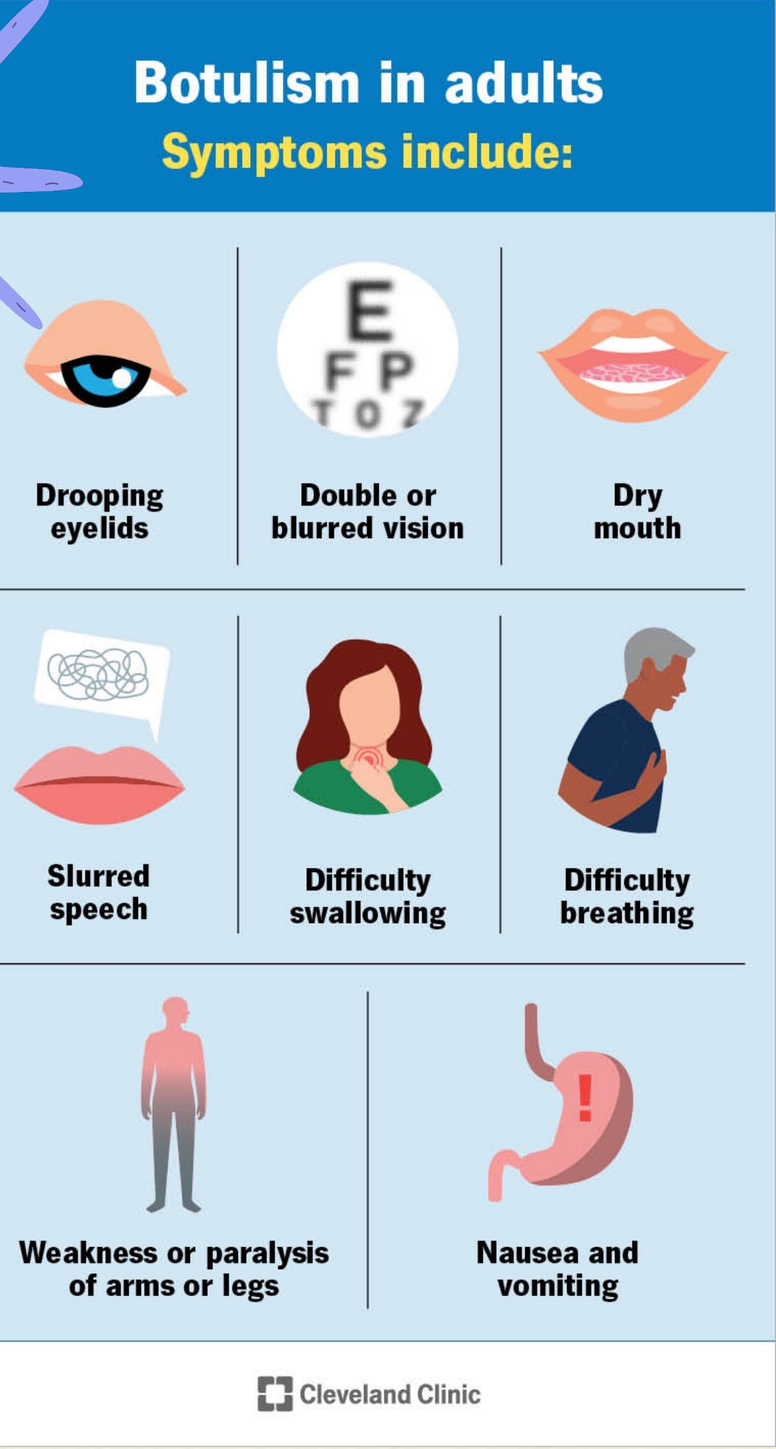
- **Foodborne botulism** is associated with home-canning (A and B types) and preserved fish (type E)
  - May not have any signs of spoilage
- **Infant botulism** caused by contaminated foods (honey, milk formula), soil, or dust

C. botulinum

Clinical Diseases

Foodborne Botulism

- ❑ Dizzy feeling 1-3 days after exposure
- ❑ Initial signs: blurred vision, fixed dilated pupils, dry mouth, constipation, abdominal pain
- ❑ Progressive disease includes bilateral descending muscle weakness and flaccid paralysis
- ❑ Death is largely due to respiratory paralysis
- ❑ Sensation is not affected, just ability to contract muscles
- ❑ Recovery can be months to years as toxin binding is irreversible

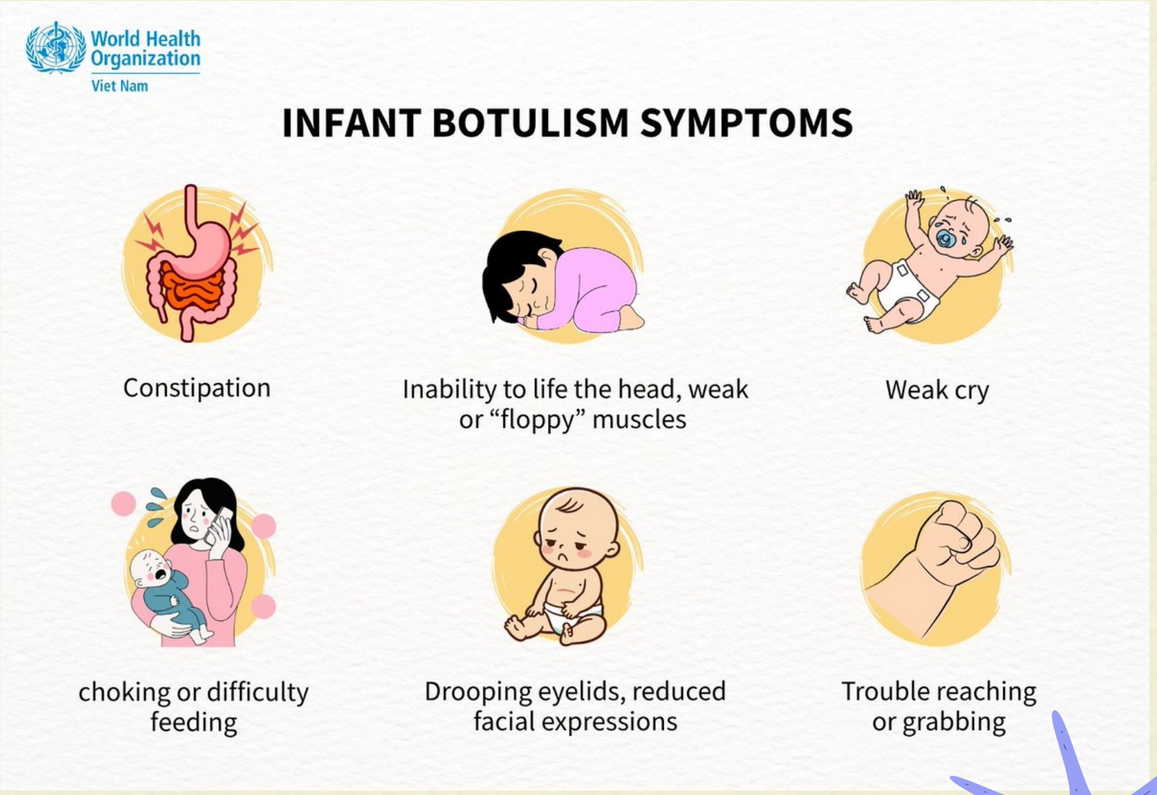


C. botulinum

Clinical Diseases

Infant Botulism

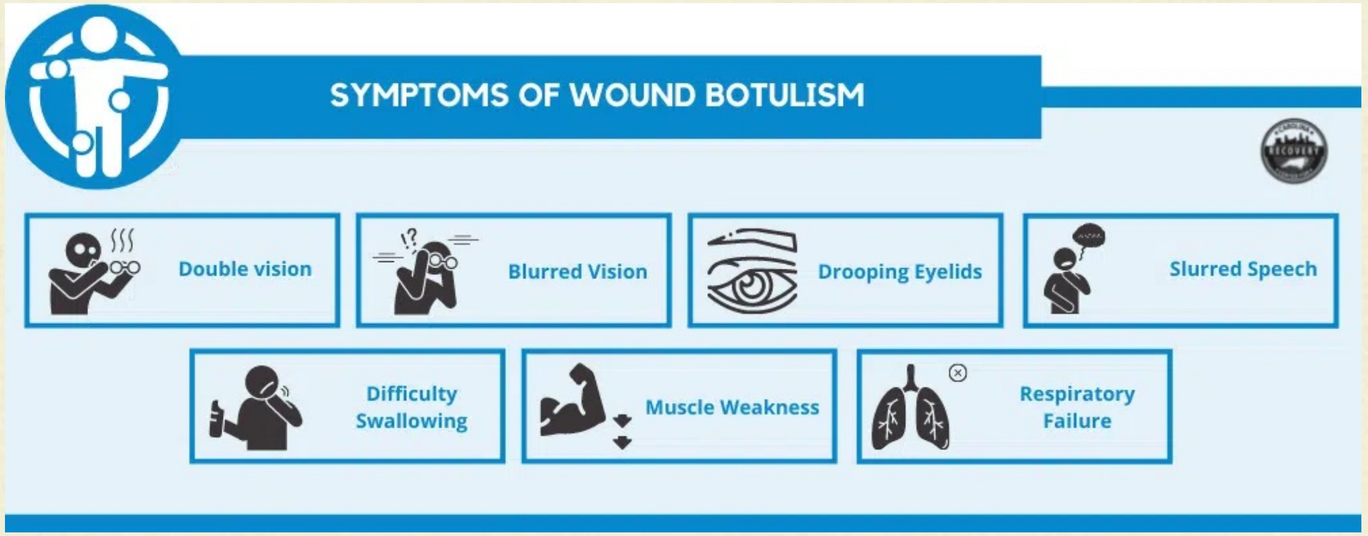
- First recognized in 1976
- Caused by consumption of contaminated foods/environment
  - BUT neurotoxin is produced **in vivo.** *C. botulinum* can colonize GI tract in absence of competitive microbes
- Infants under 1 year are most affected
- Symptoms are nonspecific at first: constipation, weak cry, “failure to thrive”
- Progressive disease includes flaccid paralysis and respiratory arrest.
- THANKFULLY mortality rate is very low (1-2%)



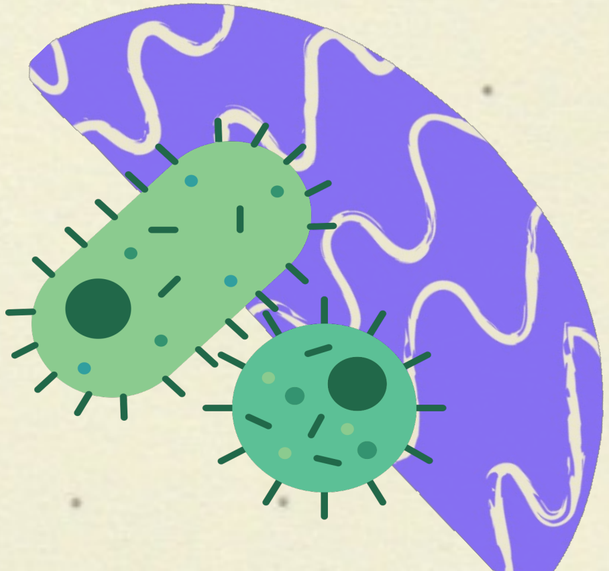
C. botulinum

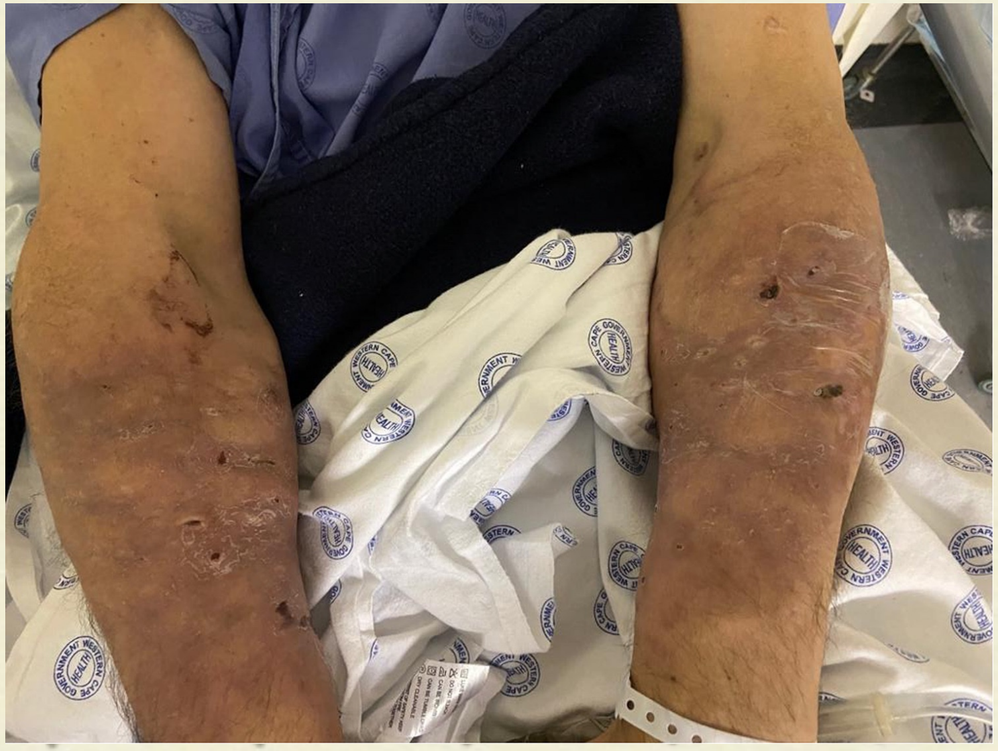
Clinical Diseases

Wound Botulism



- Caused by toxin production in wounds
- Can be associated with intravenous drug use
- Just foodborne but with longer incubation period (4+ days longer)
- GI symptoms less prominent

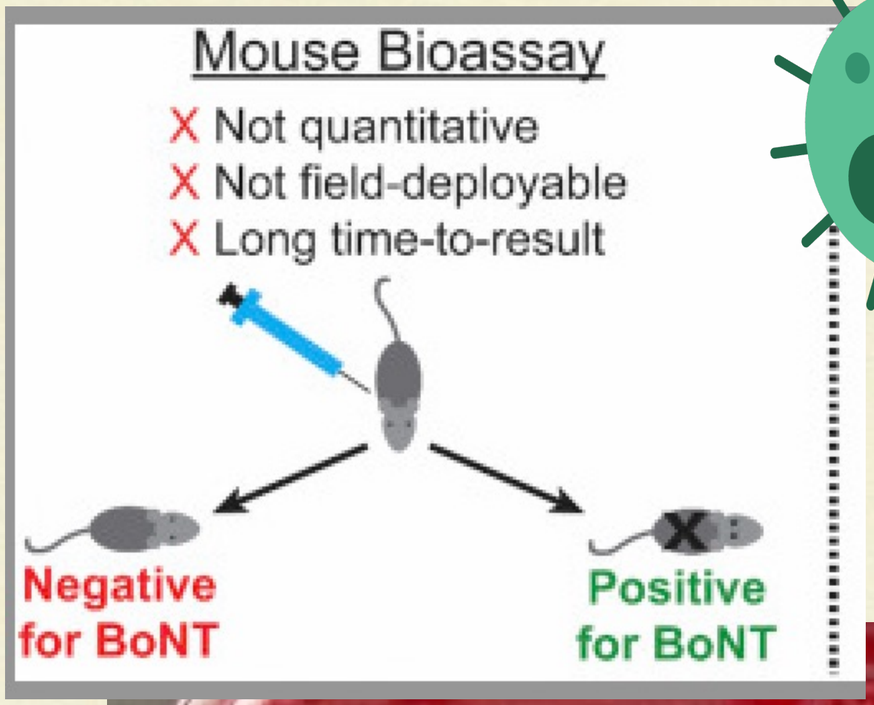


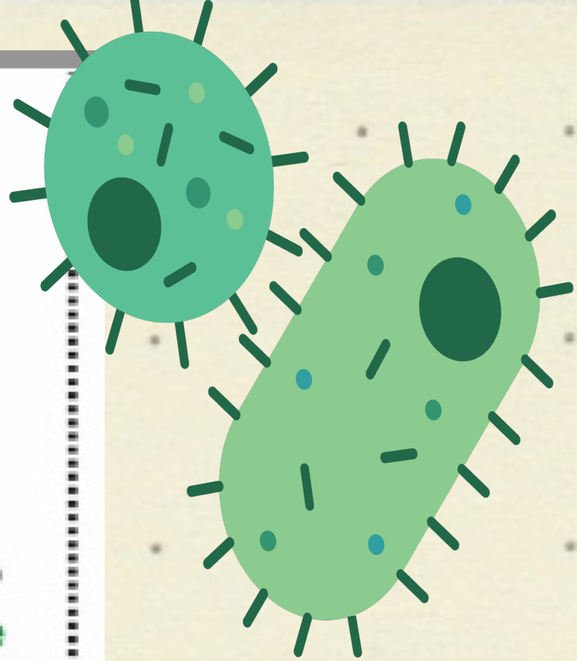


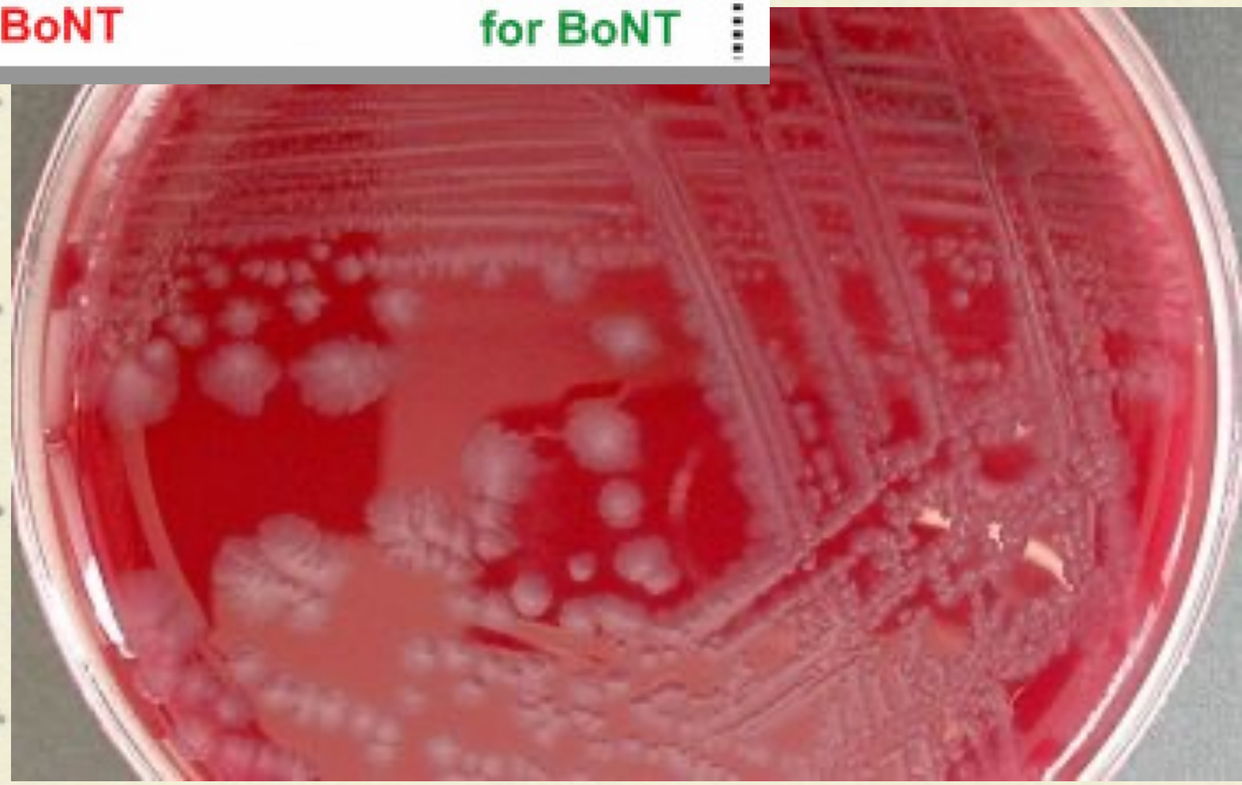
C. botulinum

Laboratory Diagnosis

- **Foodborne**
  - Diagnosed by confirming toxin activity in implicated food, patient's serum, feces, or gastric fluid
    - Serum test sensitivity is $\leq 60\%$
- **Infant**
  - Serum or feces test for toxin
    - 90% sensitivity
- **Wound**
  - Serum test for toxin, can culture bacteria from wound
  - Culturing is improved by heating sample (10 mins at 80°C) killing non-spore formers in sample
    - Nutritionally enriched anaerobic media
    - Toxin activity confirmed by mice bioassay

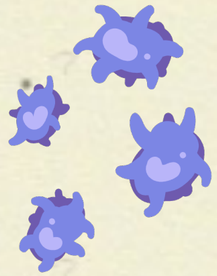


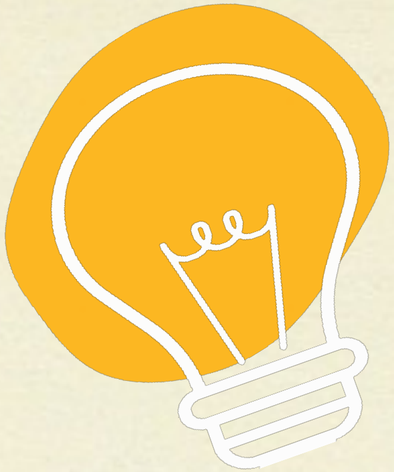




C. botulinum

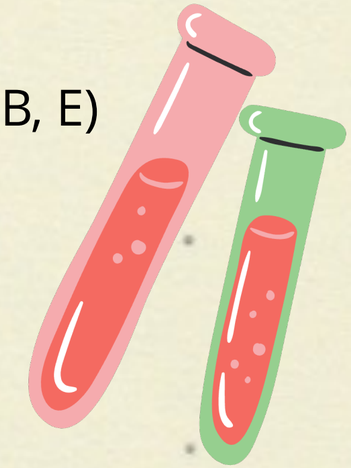
Treatment & Prevention





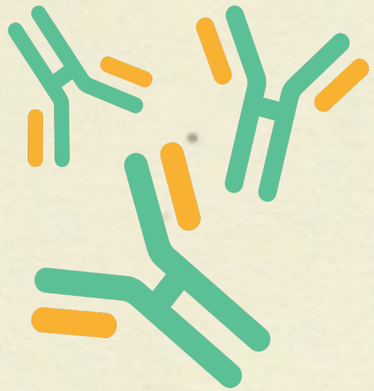
Treatment:

- **Ventilatory support**
  - Significantly reduces mortality
- **Gastric lavage** and antibiotics like **metronidazole or penicillin**
  - Flush organisms from GI tract
- **Trivalent botulism antitoxin** (A, B, E)
  - Inactivate unbound toxin



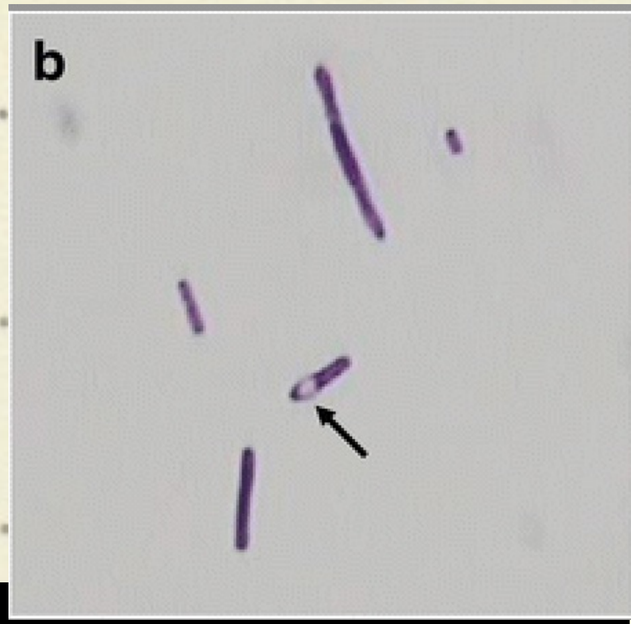
Prevention:

- Infection does not confer immunity!
- Preventing spore germination on/in foods (acidic pH, storage 4°C or below, heating to 60°C - 100°C for 10 mins)
- Keep honey away from infants under 1!
- No FDA approved vaccine



Miscellaneous Clostridium

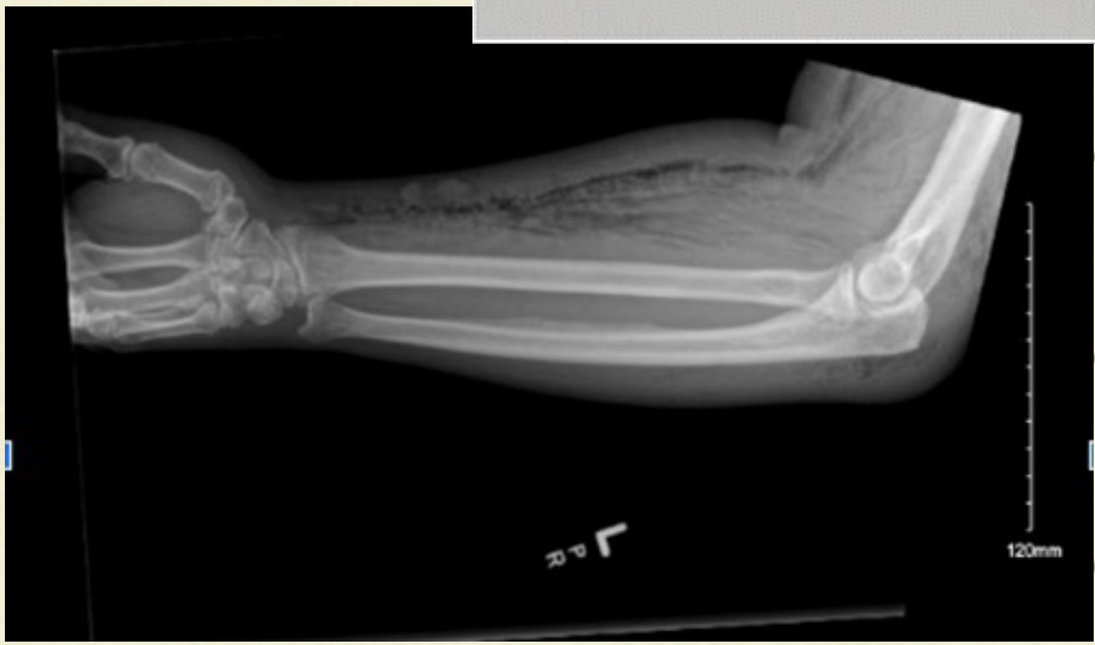
The End of the Chapter



- *C. septicum*: nontraumatic myonecrosis
  - Risk factors include colon cancer, acute leukemia, diabetes
  - Patients often die with 1-2 days of initial presentation

- *C. sordellii*: associated with fatal toxic shock syndrome (natural childbirth and medically-induced abortion)

- *C. tertium*: traumatic wound infections, aerotolerance complicates diagnosis
  - Usually diagnosed when spores are viewed

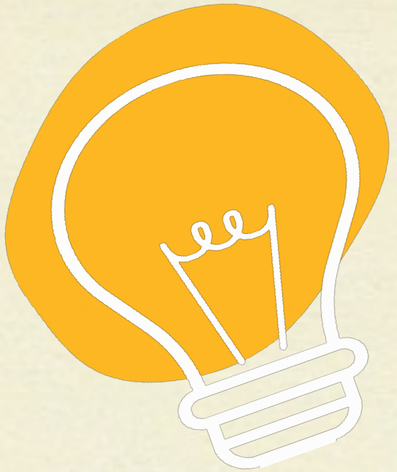


Helpful?

Organism	Gram Stain	Oxygen requirement	Shape	Spore Formation	Special Identifiers
C. difficile	Positive with some variability	Anaerobic	Rod/bacillus	Yes	Barnyard smell
C. perfringens	Positive with some variability	Anaerobic	Rectangular shaped rods	Weakly	Rectangular shape, weak spore former, beta-hemolytic
C. tetani	Positive	Anaerobic	Drumstick shaped rods	Yes	Non-fermenting, motile
C. botulinum	Positive	Anaerobic	Rod/bacillus	Yes	Multiple species make up this historic category

Thank you!

References



Thomas, A.K., Peritore-Galve, F.C., Ehni, A.G. *et al.* Mucosal vaccination clears *Clostridioides difficile* colonization. *Nature* (2026). https://doi.org/10.1038/s41586-026-10138-x