Introduction to Microbiology

Anas Abu-Humaidan
M.D. Ph.D.

Lecture 19

Endopores

- When faced with harsh environmental conditions, like depletion of any of several nutrients (carbon, nitrogen, or phosphorous). **Some gram positive bacteria** undergo a cycle of differentiation called **sporulation.**

- Sporulation involves the production of many new structures, enzymes, and metabolites along with the disappearance of many vegetative cell components.

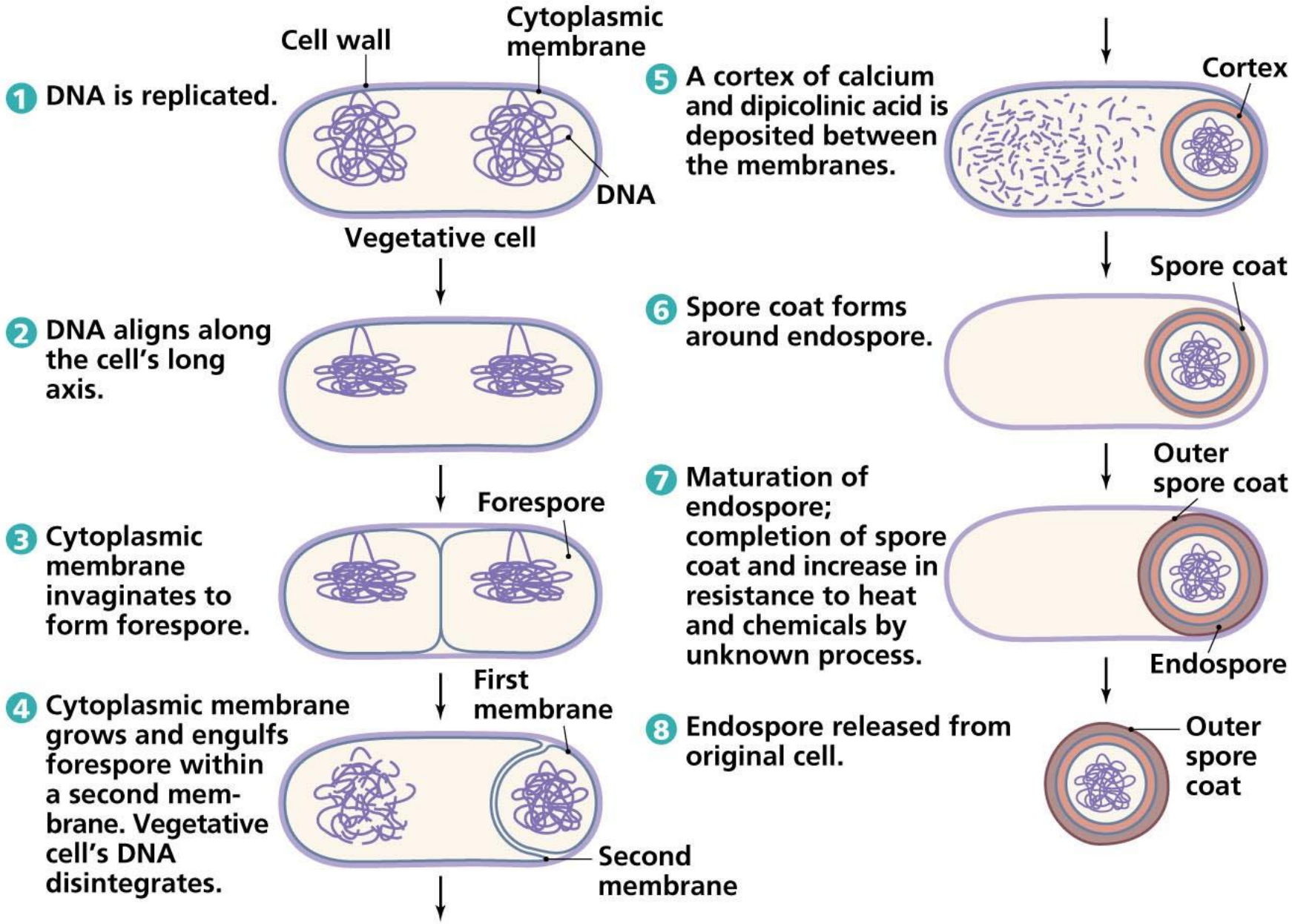
- The spore contains a complete copy of the chromosome, the bare minimum concentrations of essential proteins and ribosomes, and a high concentration of **calcium bound to dipicolinic acid**

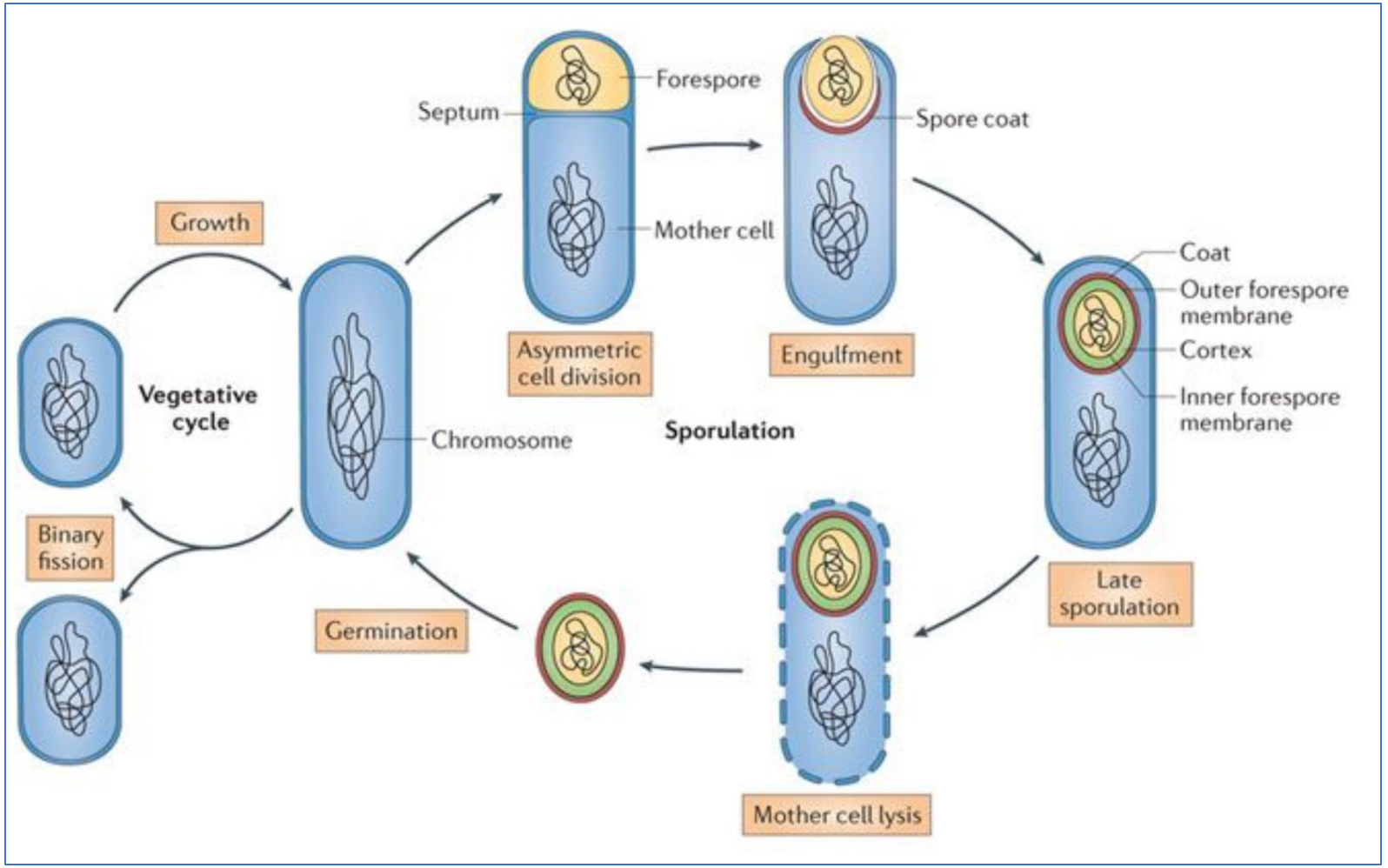
- The **spore** is a resting cell, highly resistant to desiccation, heat, and chemical agents. can exist for centuries as viable spores.

- When returned to favorable nutritional conditions, the spore **germinates** to produce a single vegetative cell.

- The location of the spore within a cell can assist in identification of the bacterium.

- The ultra structure and formation process of spores can vary from one species to another. (<u>exact detailed structure is not exam material</u>).





Sporulation begins when a sporangium divides asymmetrically to produce two compartments: the mother cell and the forespore, which are separated by a septum. Next, the mother cell engulfs the forespore, and following membrane fission at the opposite pole of the sporangium, a double-membrane bound forespore is formed. Coat assembly begins just after the initiation of engulfment and continues throughout sporulation. The peptidoglycan cortex between the inner and outer forespore membranes is assembled during late sporulation. In the final step, the mother cell lyses to release a mature spore into the environment. Spores are capable of quickly germinating and resuming vegetative growth in response to nutrients.

Overview

Pathogens that will be discussed this lecture are Spore-Forming Gram-Positive rods

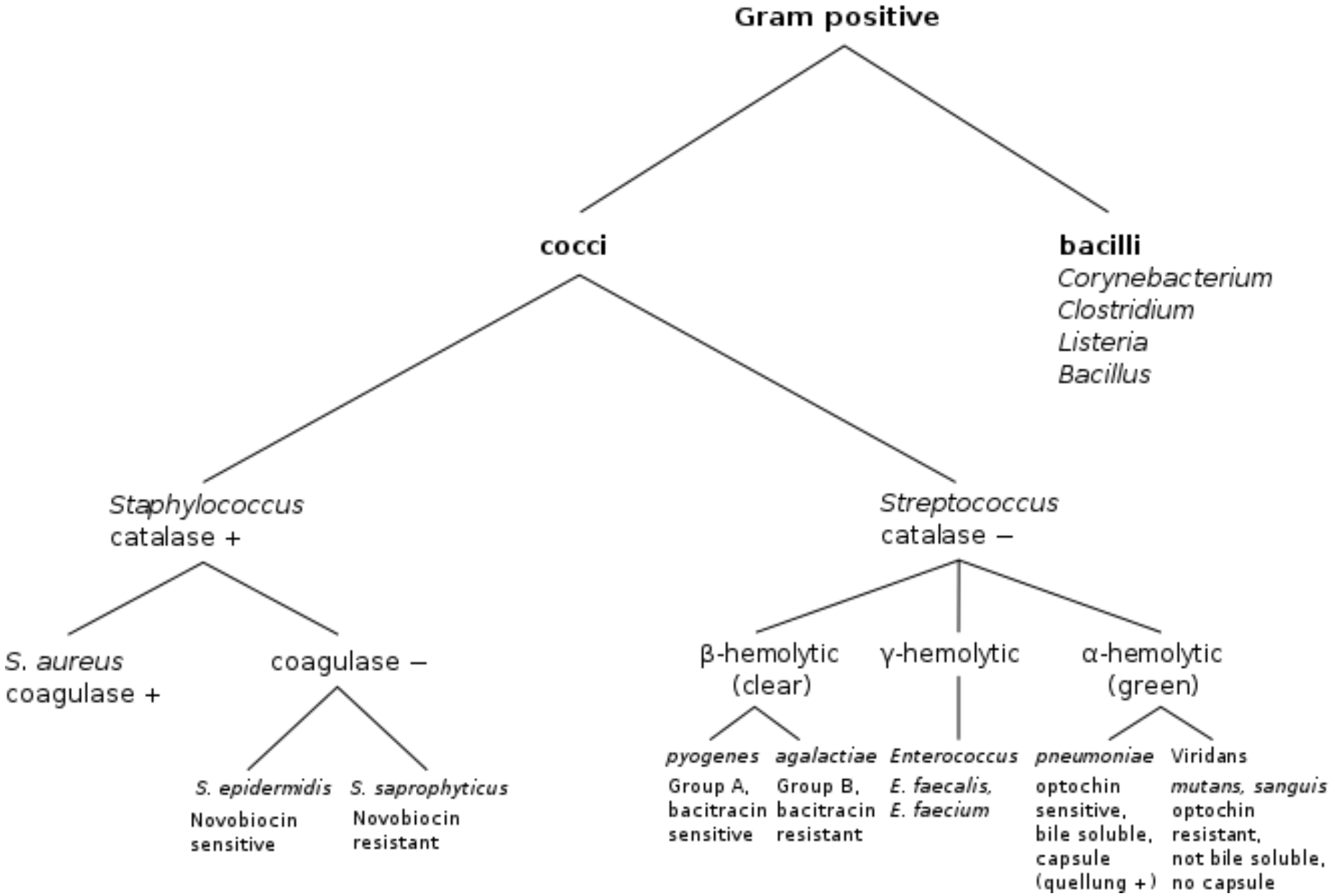
- ***Bacillus sepcies:*** The genus *Bacillus* includes large **aerobic**, gram-positive rods occurring in chains.

Major pathogens include ***Bacillus anthracis*** and ***Bacillus cereus.***

- ***Clostridium Species: a*** genus of Gram-positive rods***, are obligate anaerobes.***

Major pathogens include ***Clostridium difficile. Clostridium perfringens, Clostridium tetani, Clostridium botulinum***

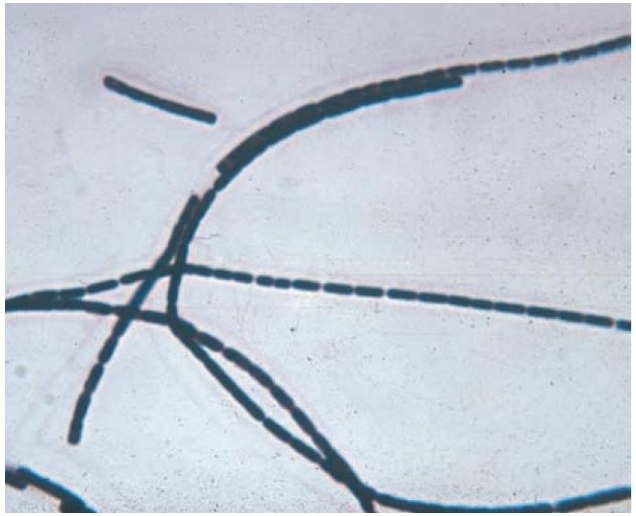
Overview


Gram positive
cocci
bacilli
Corynebacterium
Clostridium
Listeria
Bacillus
Staphylococcus
catalase +
Streptococcus
catalase −
S. aureus
coagulase +
coagulase −
β-hemolytic
(clear)
γ-hemolytic
α-hemolytic
(green)
S. epidermidis
Novobiocin
sensitive
S. saprophyticus
Novobiocin
resistant
pyogenes
Group A,
bacitracin
sensitive
agalactiae
Group B,
bacitracin
resistant
Enterococcus
E. faecalis,
E. faecium
pneumoniae
optochin
sensitive,
bile soluble,
capsule
(quellung +)
Viridans
mutans, sanguis
optochin
resistant,
not bile soluble,
no capsule

Bacillus anthracis

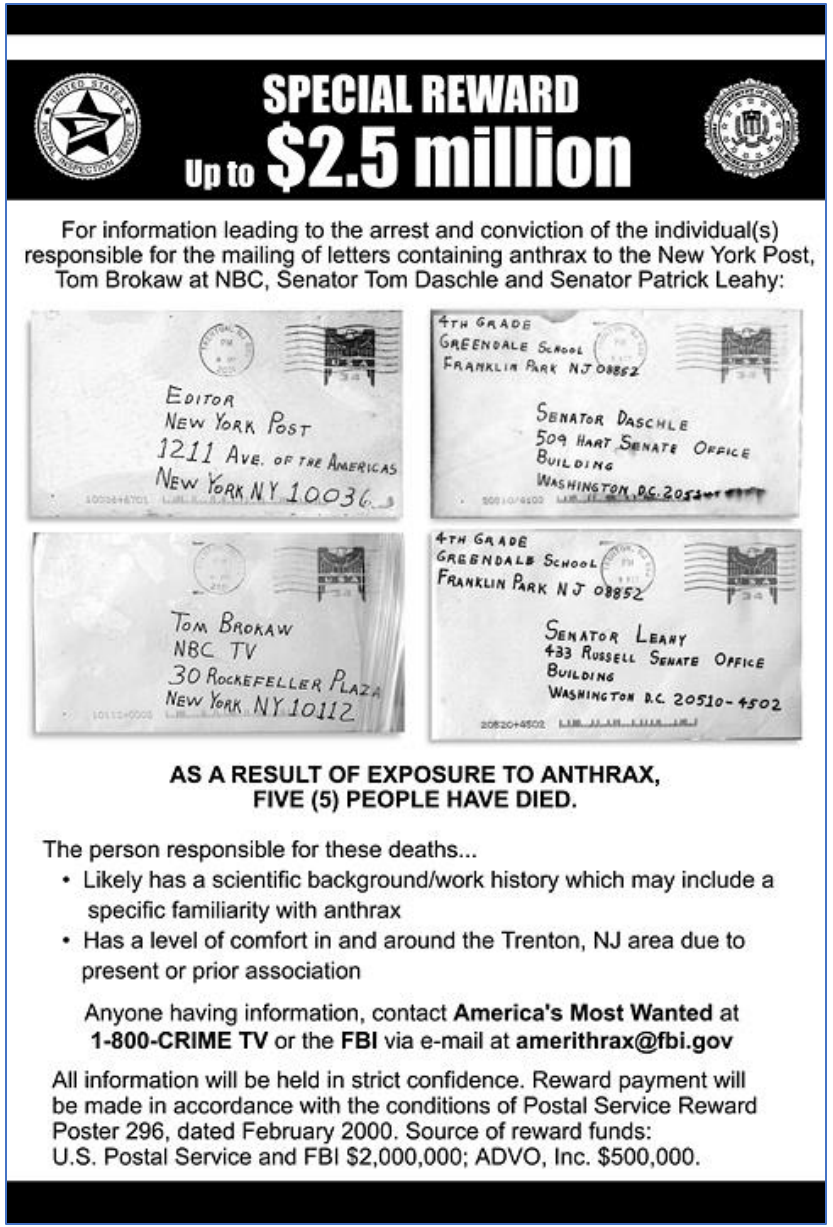
B. anthracis is a large (1 × 3 to 8 µm) organism arranged as single or paired **Gram positive rods** or as long, serpentine chains. Spores are are not seen in clinical specimens.

Anthrax is primarily a disease of herbivores; humans are infected through exposure to contaminated animals or animal products. Exposure can also be part of **biological warfare**.



Human *B. anthracis* disease is acquired by one of three routes: **inoculation (**Skin infections represent more than95% of cases)**, ingestion,** and **inhalation.**

Bacillus anthracis



SPECIAL REWARD
Up to $2.5 million

For information leading to the arrest and conviction of the individual(s) responsible for the mailing of letters containing anthrax to the New York Post, Tom Brokaw at NBC, Senator Tom Daschle and Senator Patrick Leahy:

AS A RESULT OF EXPOSURE TO ANTHRAX, FIVE (5) PEOPLE HAVE DIED.

The person responsible for these deaths...

- Likely has a scientific background/work history which may include a specific familiarity with anthrax
- Has a level of comfort in and around the Trenton, NJ area due to present or prior association

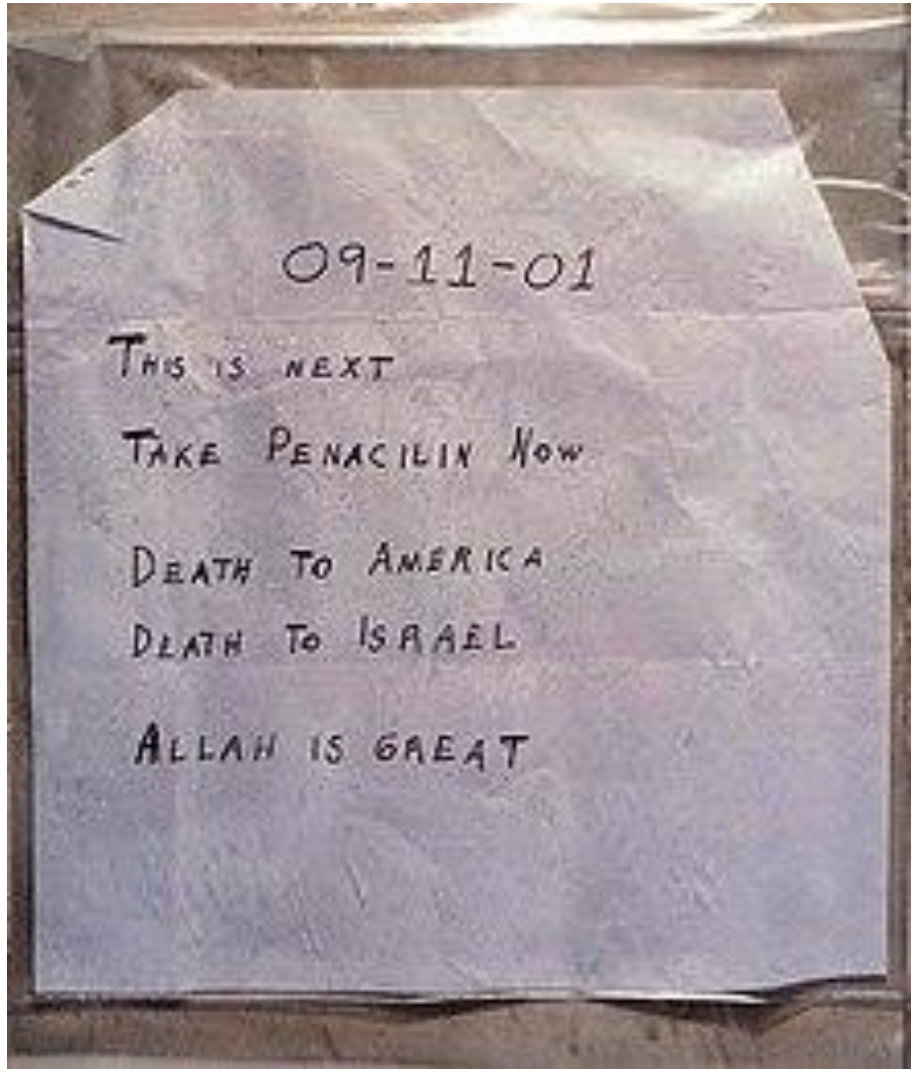
Anyone having information, contact **America's Most Wanted** at **1-800-CRIME TV** or the **FBI** via e-mail at **amerithrax@fbi.gov**

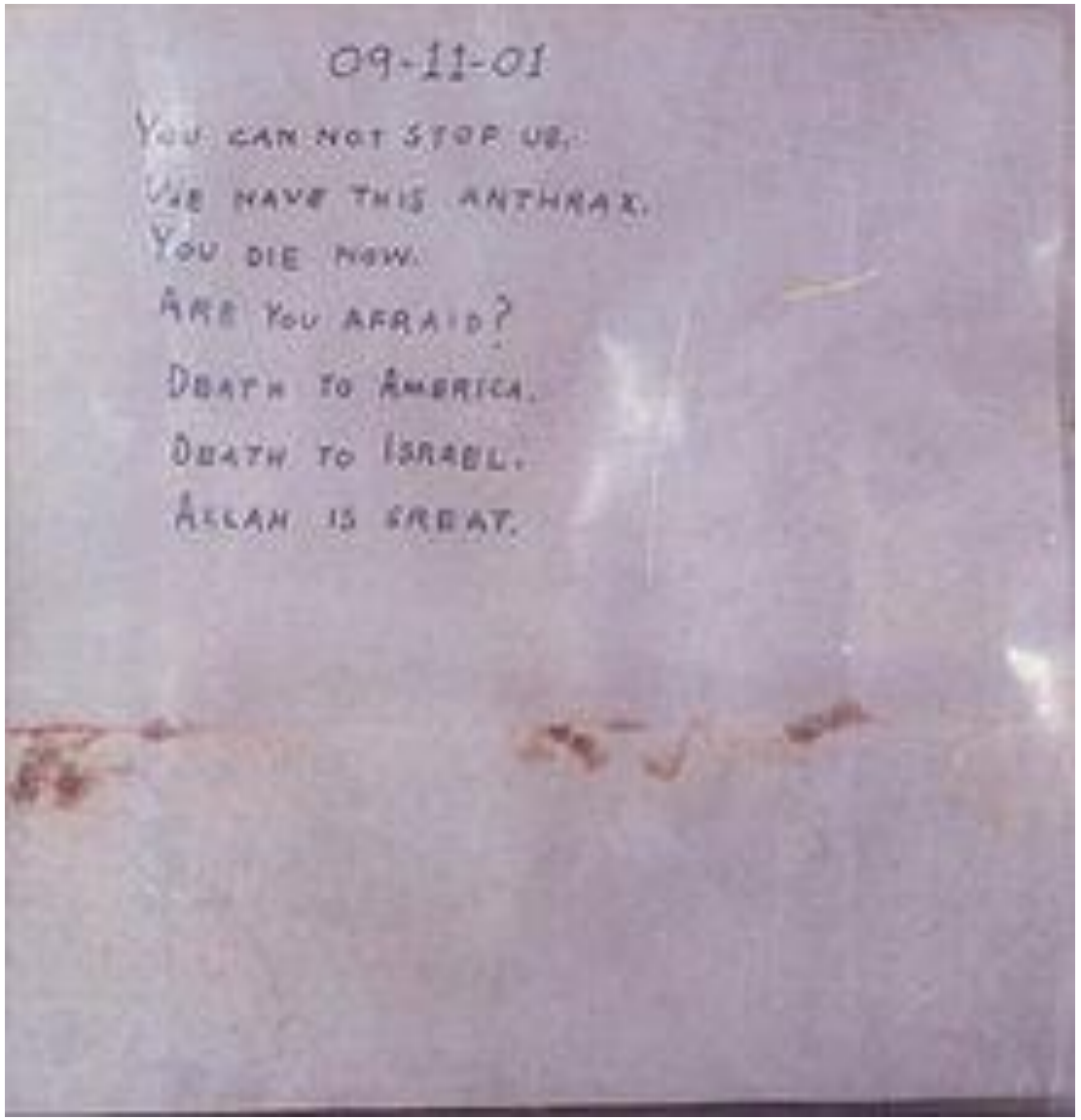
All information will be held in strict confidence. Reward payment will be made in accordance with the conditions of Postal Service Reward Poster 296, dated February 2000. Source of reward funds: U.S. Postal Service and FBI $2,000,000; ADVO, Inc. $500,000.

Clinical Case 20-1 Inhalation Anthrax

Bush and associates (*N Engl J Med* 345:1607–1610, 2001) reported the first case of inhalation anthrax in the 2001 bioterrorism attack in the United States. The patient was a 63-year-old man living in Florida who had a 4-day history of fever, myalgias, and malaise without localizing symptoms. His wife brought him to the regional hospital because he awoke from sleep with fever, emesis, and confusion. On physical examination, he had a temperature of 39° C, blood pressure of 150/80 mm Hg, pulse of 110 beats/min, and respiration of 18 breaths/min. No respiratory distress was noted. Treatment was initiated for presumed bacterial meningitis. Basilar infiltrates and a widened mediastinum were noted on the initial chest radiograph. Gram stain of cerebrospinal fluid (CSF) revealed many neutrophils and large gram-positive rods. Anthrax was suspected, and penicillin treatment was initiated. Within 24 hours of admission, CSF and blood cultures were positive for *Bacillus anthracis*. During the first day of hospitalization, the patient had a grand mal seizure and was intubated. On the second hospital day, hypotension and azotemia developed, with subsequent renal failure. On the third hospital day, refractory hypotension developed and the patient had a fatal cardiac arrest. This patient illustrates the rapidity with which patients with inhalation anthrax can deteriorate despite a rapid diagnosis and appropriate antimicrobial therapy. Although the route of exposure is via the respiratory tract, patients do not develop pneumonia; rather, the abnormal chest radiograph is caused by hemorrhagic mediastinitis.

Bacillus anthracis





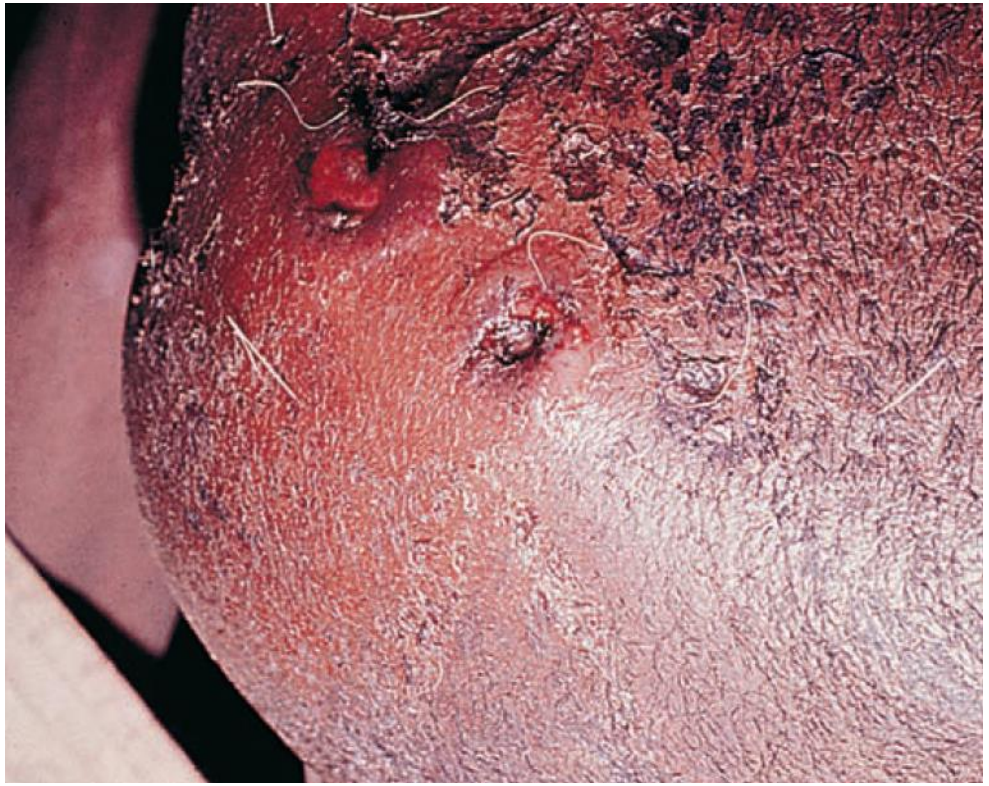
Bacillus anthracis

The major factors responsible for the virulence of *B. anthracis* are the **capsule, edema toxin, and lethal toxin**. The capsule made of **poly-d-glutamic acid** inhibits phagocytosis of replicating cells. **Edema toxin** is responsible for the fluid accumulation observed in anthrax. **Lethal toxin is** cytotoxic and stimulates macrophages to release proinflammatory cytokines.

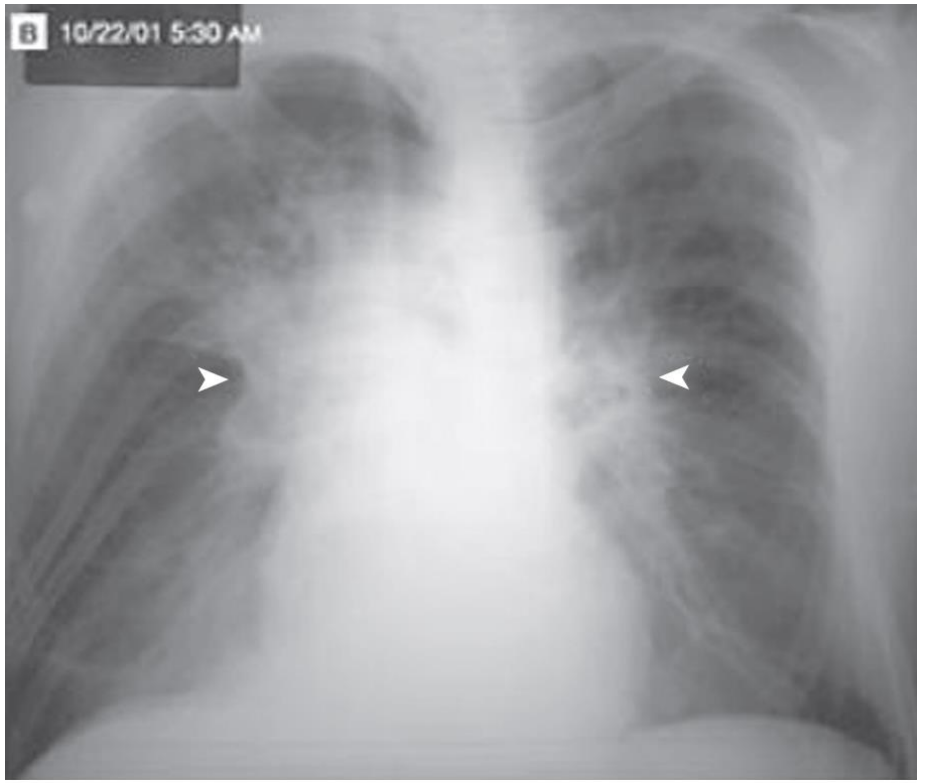
The spores germinate in the tissue at the site of entry, and growth of the vegetative organisms results in formation of a gelatinous edema and congestion.

Almost all cases progress to **shock and death within 3 days of initial symptoms** unless anthrax is suspected and treatment is initiated immediately

Bacillus anthracis



Typically, **cutaneous anthrax** starts with the development of a painless papule at the site of inoculation that rapidly progresses to an ulcer surrounded by vesicles and then to a necrotic eschar.



Inhalation anthrax can be associated with a **prolonged latent period** (2 months or more), during which the infected patient remains asymptomatic. Spores phagocytosed in the lungs; and transported by the lymphatic drainage to the **mediastinal lymph nodes, where germination occurs**. Hemorrhagic **necrosis** and **edema** of the mediastinum are early manifistations, Sepsis occurs and spread to other organs (GI ulcerations, meningitis) can take place.

Bacillus cereus

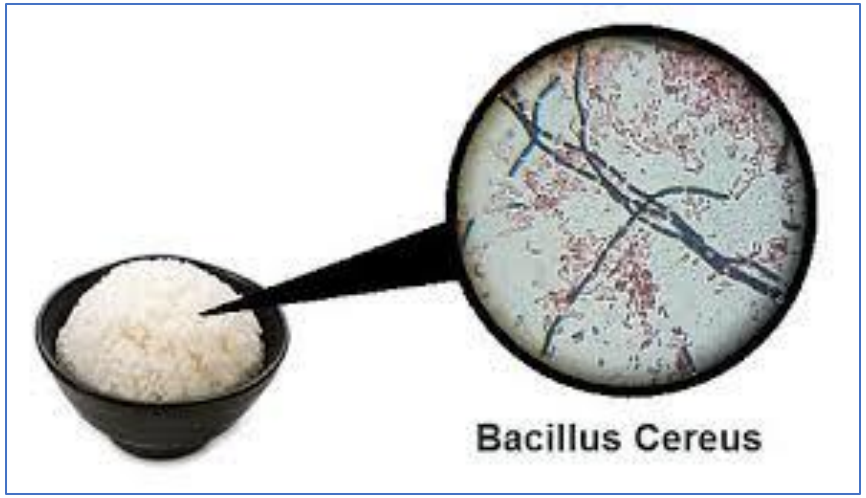
B. cereus and other *Bacillus* species are ubiquitous organisms, present in virtually **all environments.**

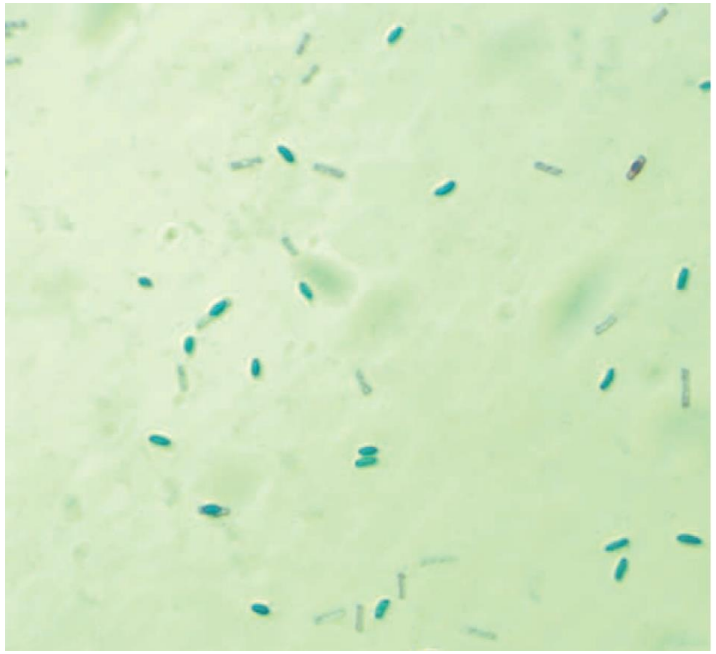
B. cereus is responsible for two forms of food poisoning: **vomiting disease (emetic form)** and **diarrheal disease (diarrheal form).**

The **emetic form** of disease results from consumption of **contaminated rice. An intoxication** caused by ingestion of the enterotoxin, not the bacteria. Thus the incubation period after eating the contaminated rice is **short (1 to 6 hours)**, and the duration of illness is also short (<24 hours).

The **diarrheal form** of *B. cereus* food poisoning is a true infection resulting from ingestion of the bacteria in contaminated meat, vegetables, or sauces. With longer incubation period.

B. cereus **ocular infections** usually occur after traumatic, penetrating injuries of the eye with a soil-contaminated object.



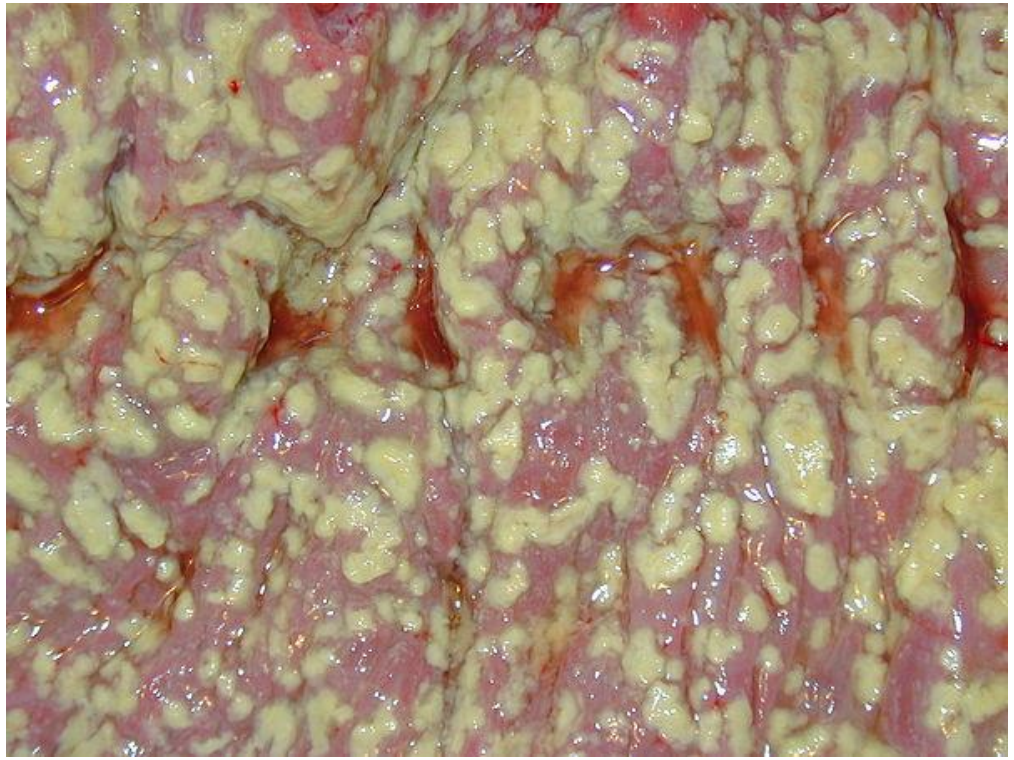


Spores retain the malachite green dye in this special spore stain, and the vegetative cells are gray or colorless.

Clostridium difficile

The disease develops in people **taking antibiotics**, because the drugs **alter the normal enteric flora**, either **permitting overgrowth** of these relatively resistant organisms or making the patient more **susceptible to exogenous acquisition** of *C. difficile.*

Remarkable success with “fecal transplants” has been demonstrated, illustrating the fact that *C. difficile* does not become established when a healthy enteric population of bacteria is present.



Pseudomembranous colitis is an inflammatory condition of the colon characterized by elevated yellow-white plaques that coalesce to form pseudomembranes on the mucosa.

Clostridium difficile

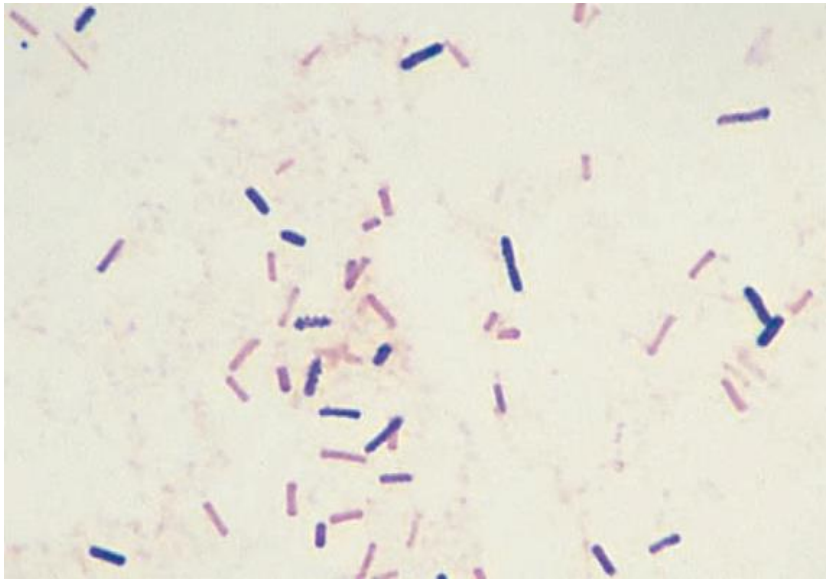
Clinical Case 30-1 *Clostridium difficile* Colitis

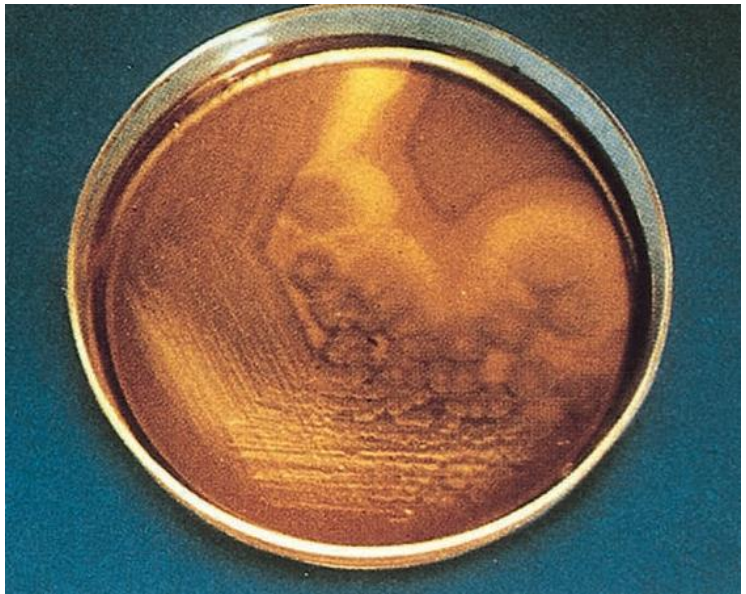
Limaye and colleagues (*J Clin Microbiol* 38:1696, 2000) presented a classic presentation of *C. difficile* disease in a 60-year-old man who received a transplanted liver 5 years previous to his hospital admission for evaluation of crampy abdominal pain and severe diarrhea. Three weeks prior to admission he received a 10-day course of oral trimethoprim-sulfamethoxazole for sinusitis. On physical examination, the patient was febrile and had moderate abdominal tenderness. Abdominal computed tomography scan revealed right colon thickening but no abscess. Colonoscopy showed numerous whitish plaques and friable erythematous mucosa consistent with pseudomembranous colitis. Empirical therapy with oral metronidazole and intravenous levofloxacin was initiated. A stool immunoassay for *C. difficile* toxin A was negative, but *C. difficile* toxin was detected by both culture and cytotoxicity assay (demonstration stool filtrate causes cytotoxicity to cell cultures that is neutralized by specific antisera against *C. difficile* toxins). Therapy was changed to oral vancomycin, and the patient responded with resolution of diarrhea and abdominal pain. This is an example of severe *C. difficile* disease following antibiotic exposure in an immunocompromised patient, with a characteristic presentation of pseudomembranous colitis. The diagnostic problems with immunoassays are well known and have now been replaced by polymerase chain reaction assays that target the toxin genes. Treatment with metronidazole is currently preferred, although vancomycin is an acceptable alternative.

Clostridium perfringens

C. perfringens is a large (0.6 to 2.4 × 1.3 to 19.0 µm), rectangular, gram-positive rod, with **spores rarely observed** either in vivo or after in vitro cultivation, an important characteristic that differentiates this species from most other clostridia. **Colonies** of *C. perfringens* are also distinctive, with their **rapid, spreading growth.**

Type A *C. perfringens* commonly inhabits the intestinal tract of humans and animals and is **widely distributed in nature**, particularly in soil and water contaminated with feces. Spores are formed under adverse environmental conditions and can survive for prolonged periods. Strains of types B through E do not survive in soil but colonize the intestinal tracts of animals and occasionally humans





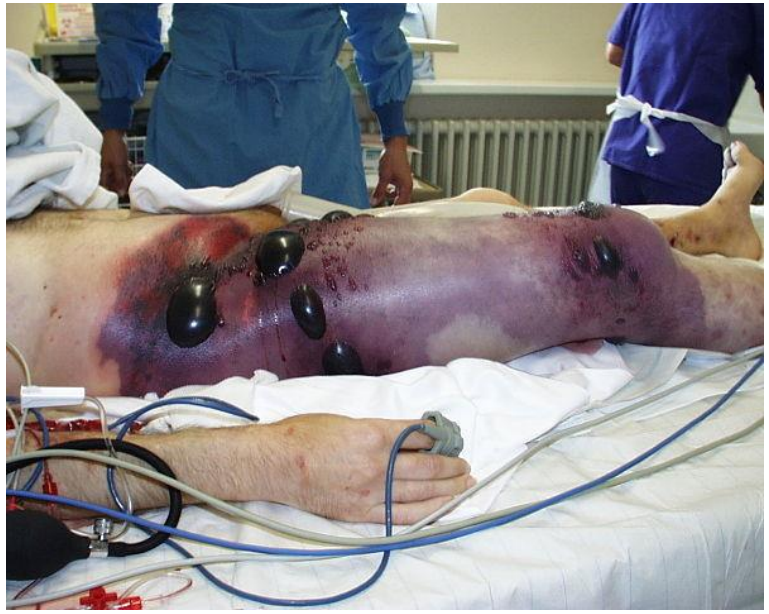
A presumptive identification of *C. perfringens* can be made by detection of a zone of **complete hemolysis** (caused by the theta toxin) and a wider zone of **partial hemolysis** (caused by the alpha toxin), combined with the characteristic microscopic morphology.

Clostridium perfringens

- *C. perfringens* is responsible for a range of soft-tissue infections including **cellulitis,** fasciitis or suppurative **myositis,** and **myonecrosis** with gas formation (caused by the metabolic activity of the rapidly dividing bacteria) in the soft tissue (**gas gangrene**). The toxin involved in gas gangrene is known as **α-toxin**, which inserts into the plasma membrane of cells, producing gaps in the membrane that disrupt normal cellular function

- **Clostridial food poisoning,** an **intoxication** characterized by (1) a short incubation period (8 to 12 hours), (2) a clinical presentation that includes abdominal cramps. (3) a clinical course lasting less than 24 hours.

- *C. perfringens* produces **enterotoxin**, The enterotoxin is produced during the phase transition from vegetative cells to spores and is released in the alkaline environment of the small intestine when the cells undergo the terminal stages of spore formation **(sporulation).**



Treatment is usually **debridement and excision**, with amputation necessary in many cases. Water-soluble antibiotics (such as penicillin) alone are not effective because they **do not penetrate ischaemic muscles** sufficiently to be effective.

Clostridium perfringens

- Spore forming bacteria are ubiquitous in nature and can easily contaminate sharp objects, subsequently causing infections in wounds.

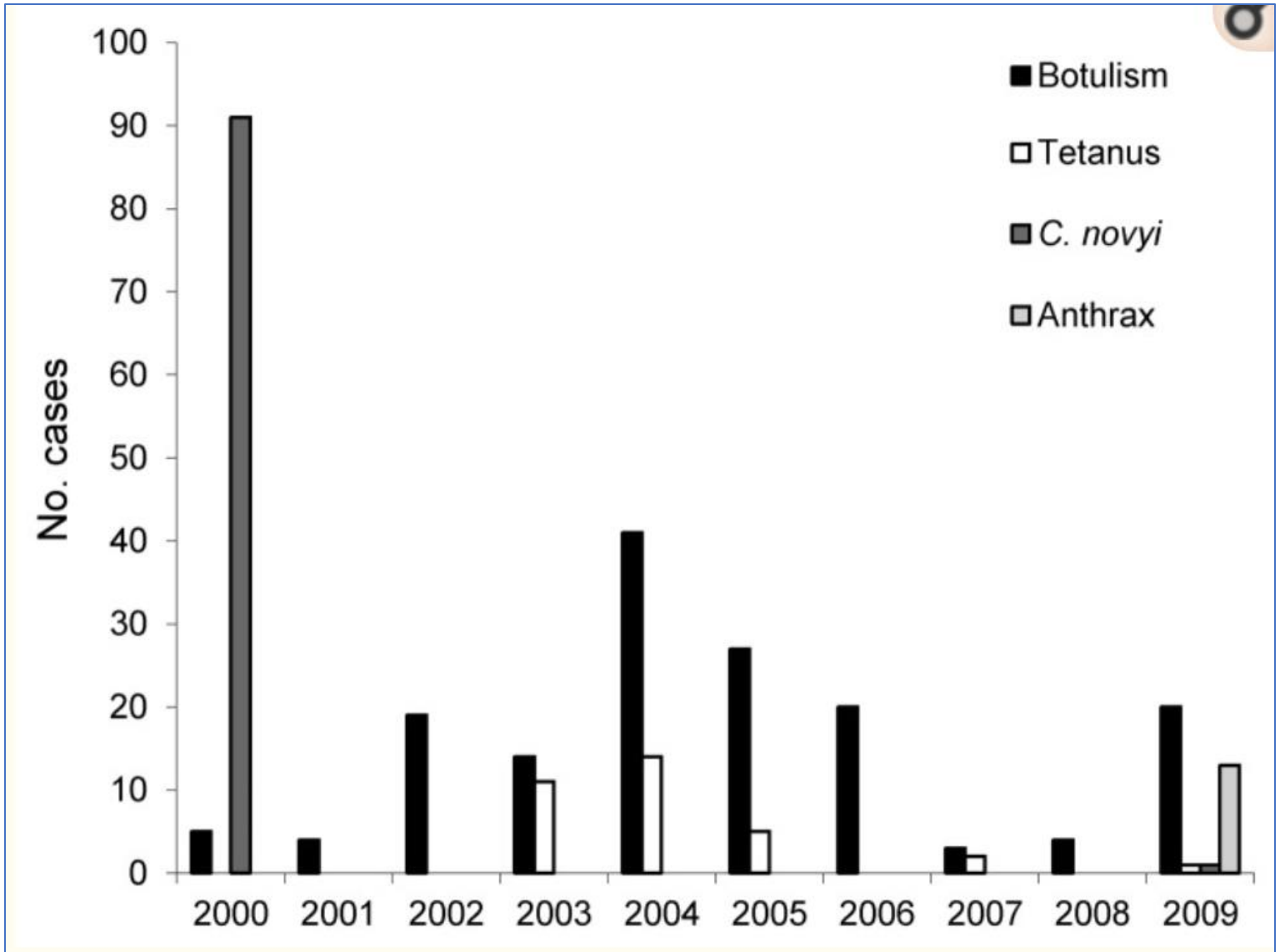


Figure 1

Annual numbers of cases of botulism, tetanus, *Clostridium novyi* infection, and anthrax among persons who inject drugs, England and Scotland, 2000–2009.

Overview

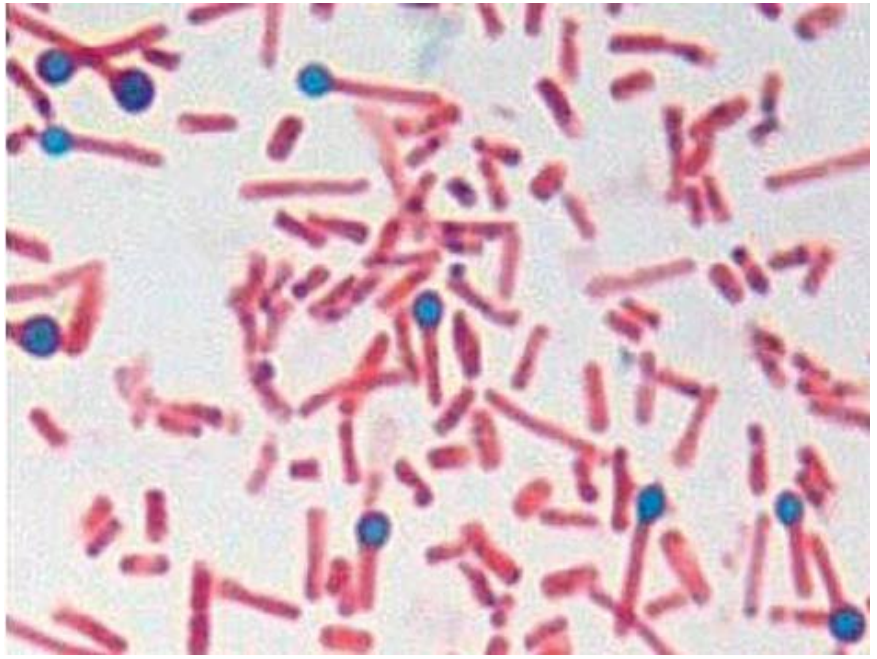
Clinical Case 30-3 Tetanus

The following is a typical history of a patient with tetanus (CDC, *MMWR Morb Mortal Wkly Rep* 51:613–615, 2002). An 86-year-old man saw a physician for care of a splinter wound in his right hand, acquired 3 days earlier while gardening. He was not treated with either a tetanus toxoid vaccine or tetanus immune globulin. Seven days later he developed pharyngitis, and after an additional 3 days, he presented to the local hospital with difficulty talking, swallowing, and breathing, and with chest pain and disorientation. He was admitted to the hospital with the diagnosis of stroke. On his fourth hospital day, he had developed neck rigidity and respiratory failure, requiring tracheostomy and mechanical ventilation. He was transferred to the medical intensive care unit, where the clinical diagnosis of tetanus was made. Despite treatment with tetanus toxoid and immune globulin, the patient died 1 month after admission to the hospital. This case illustrates that *Clostridium tetani* is ubiquitous in soil and can contaminate relatively minor wounds; it also illustrates the unrelenting progression of neurologic disease in untreated patients.

Clostridium tetani

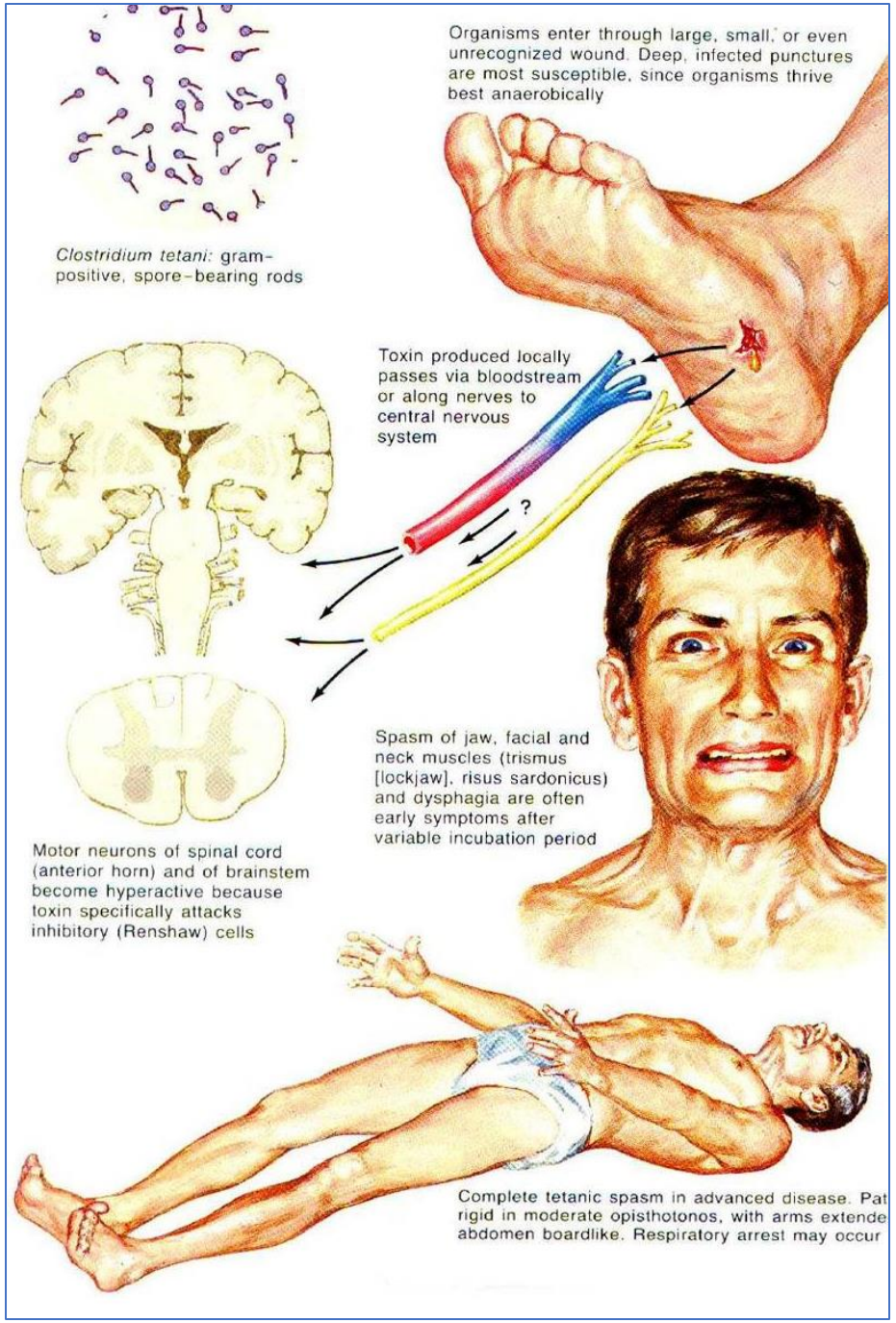
- *C. tetani* is a large (0.5 to 2 × 2 to 18 µm), motile, spore-forming rod. The organism produces round, terminal spores that give it the appearance of a drumstick. *C. tetani* is **ubiquitous.** It is found in fertile soil and transiently colonizes the GI tracts of many animals, including humans.

- *C. tetani* produces two toxins, an oxygen-labile hemolysin **(tetanolysin)** and a plasmid-encoded, heat-labile neurotoxin **(tetanospasmin).**

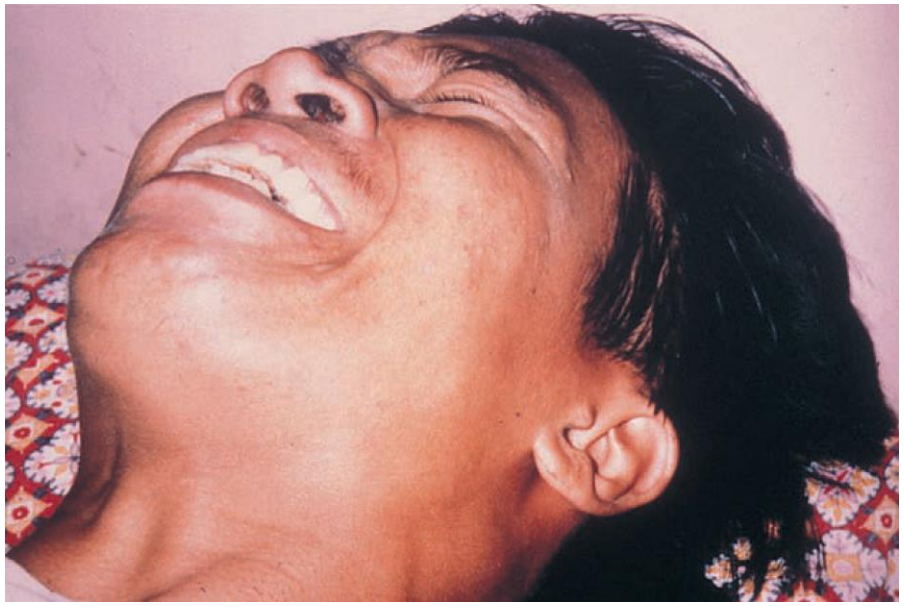


- Tetanospasmin **inactivates proteins that regulate release of the inhibitory neurotransmitters** glycine and gamma-aminobutyric acid (GABA). This leads to unregulated excitatory synaptic activity in the motor neurons, resulting in **spastic paralysis.**

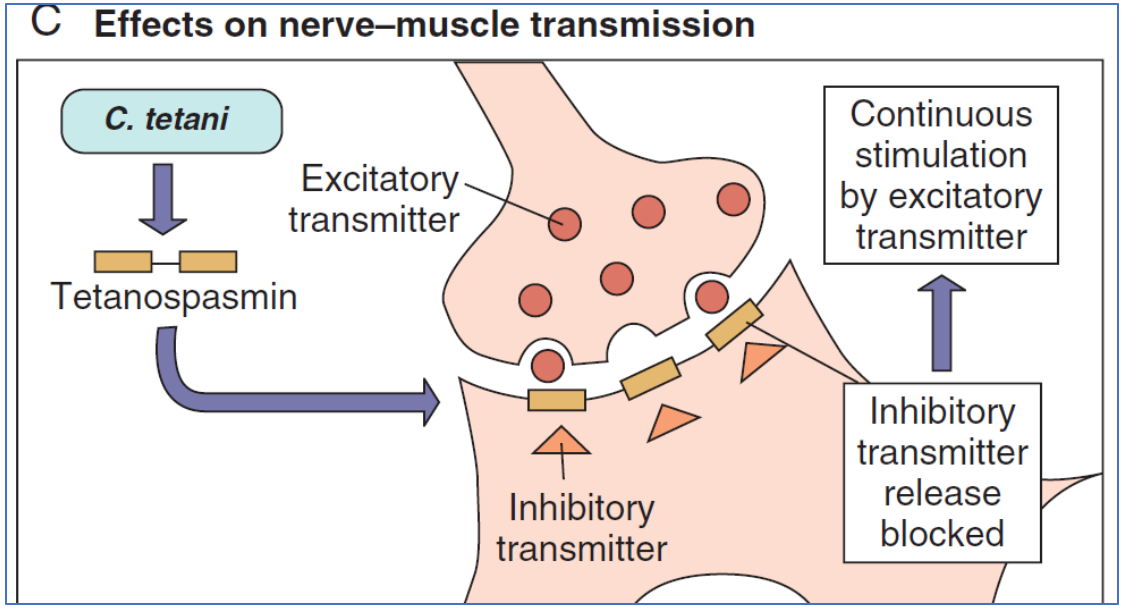
- Disease is relatively rare because of the high incidence of **vaccine-induced immunity.**

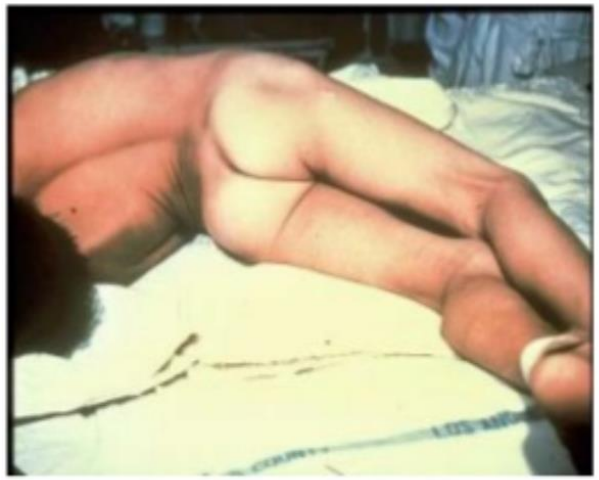


Clostridium tetani



Involvement of the masseter muscles (trismus or **lockjaw**) is the presenting sign in most patients. The characteristic **sardonic smile** that results from the sustained contraction of the facial muscles.





unregulated excitatory synaptic activity in the motor neurons, resulting in **spastic paralysis. Generalized tetanus** is the most common form.

Clostridium botulinum

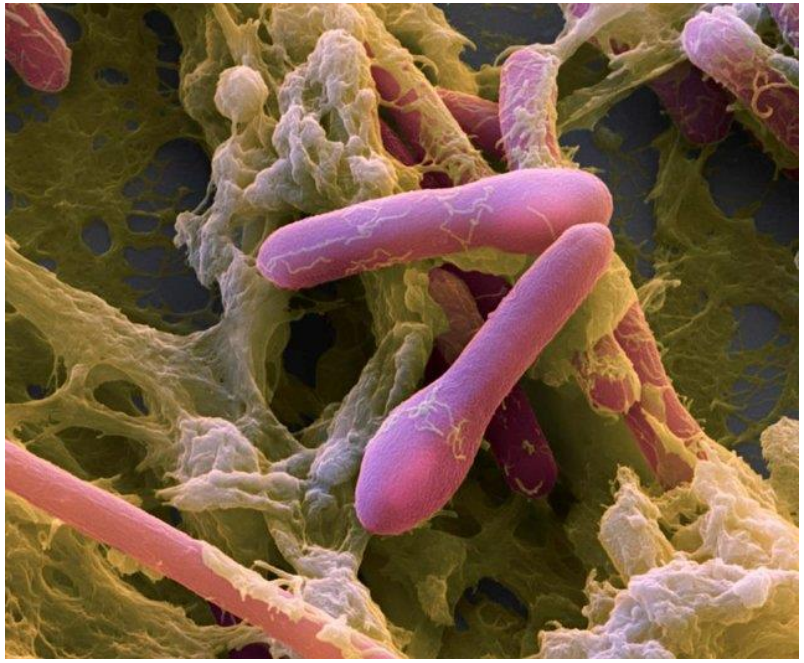
Clinical Case 30-4 Foodborne Botulism with Commercial Carrot Juice

The Centers for Disease Control and Prevention reported an outbreak of foodborne botulism associated with contaminated carrot juice (*MMWR Morb Mortal Wkly Rep* 55:1098, 2006). On September 8, 2006, three patients went to a hospital in Washington County, Georgia, with cranial nerve palsies and progressive descending flaccid paralysis resulting in respiratory failure. The patients had shared meals on the previous day. Because botulism was suspected, the patients were treated with botulinum antitoxin. The patients had no progression of their neurologic symptoms, but they remained hospitalized and on ventilators. An investigation determined that the patients had consumed contaminated carrot juice produced by a commercial vendor. Botulinum toxin type A was detected in the serum and stool of all three patients and in leftover carrot juice. An additional patient in Florida was also hospitalized with respiratory failure and descending paralysis after drinking carrot juice sold in Florida. Because carrot juice has a low acid content (pH 6.0), *Clostridium botulinum* spores can germinate and produce toxin if contaminated juice is left at room temperature.

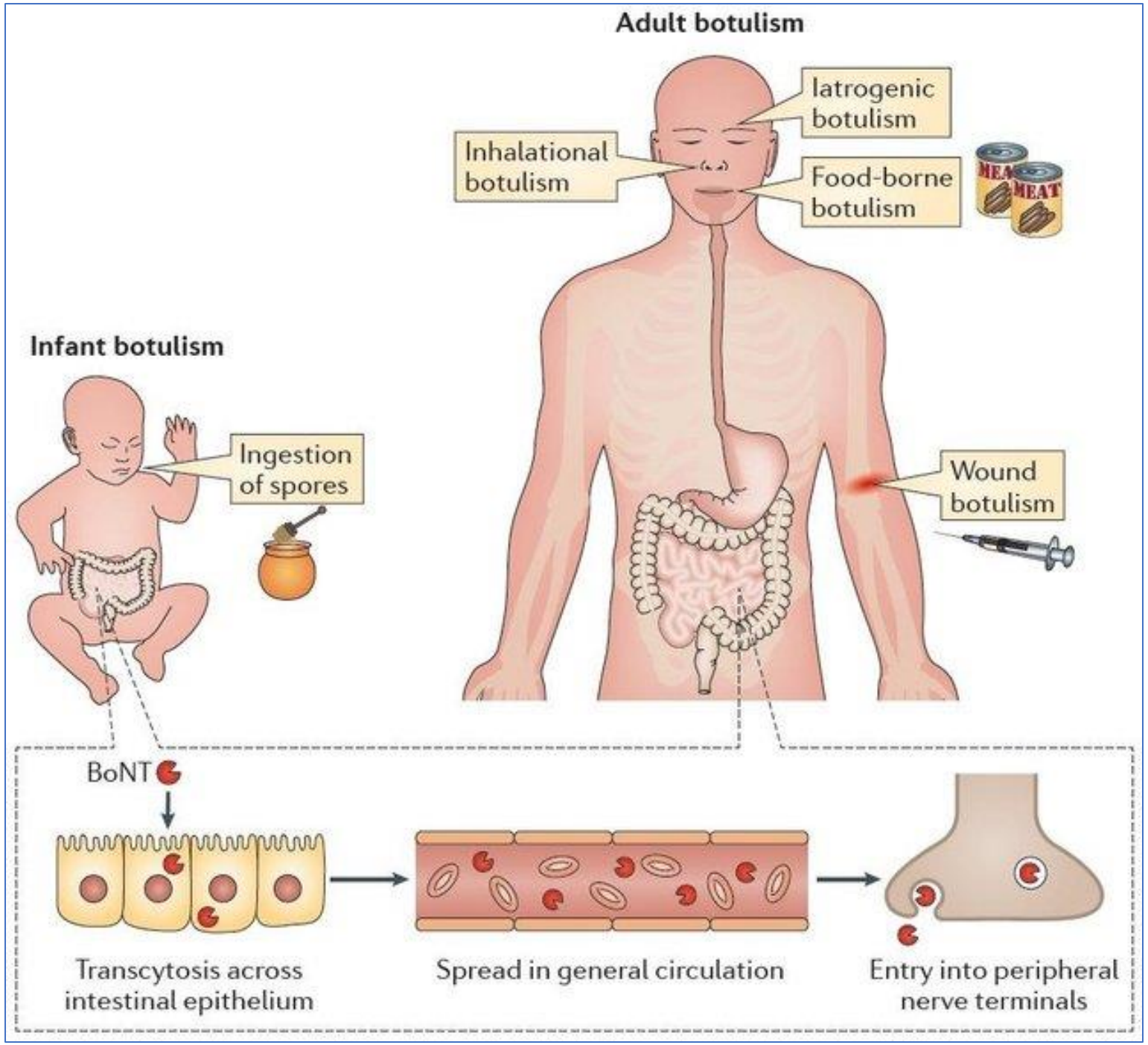
Clostridium botulinum

- The etiologic agents of botulism are a heterogeneous collection of large (0.6 to 1.4 × 3.0 to 20.2 µm), fastidious, spore-forming, anaerobic rods. *C. botulinum* is commonly isolated in soil and water samples throughout the world.

- Patients with **foodborne botulism (**most are associated with consumption of home-canned foods) typically become weak and dizzy 1 to 3 days after consuming the contaminated food. Bilateral descending **weakness of the peripheral muscles** develops in patients with progressive disease (flaccid paralysis), and death is most commonly attributed to **respiratory paralysis**.



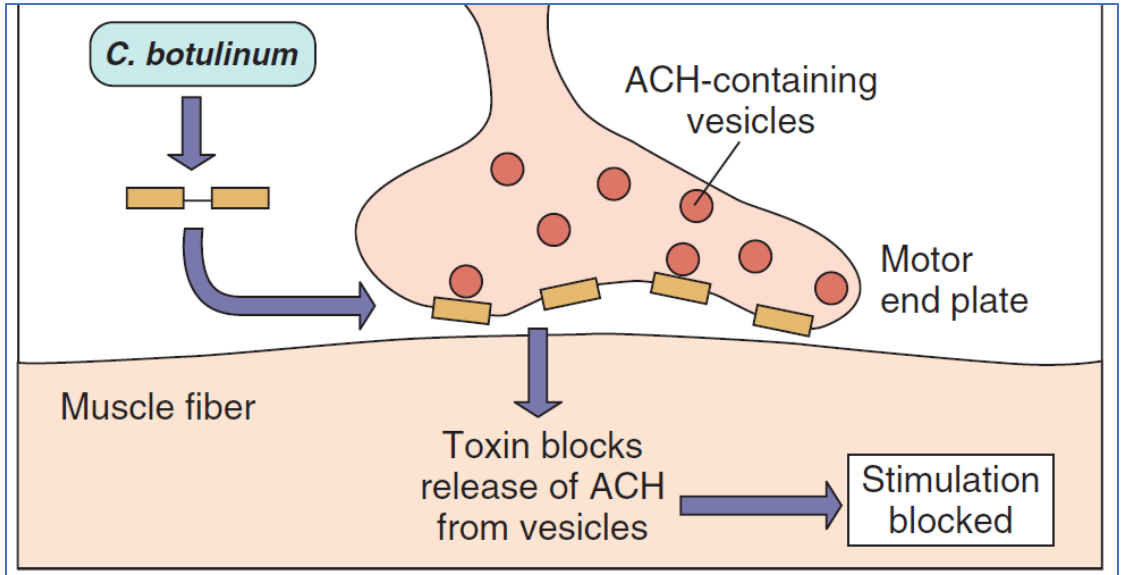
- **Infant botulism:** Associated with consumption of foods (e.g., honey, infant milk powder) contaminated with botulinum spores and ingestion of spore-contaminated soil and dust. In contrast with foodborne botulism, this disease is caused by neurotoxin produced in vivo by *C. botulinum* colonizing the GI tracts of infants.



Clostridium botulinum

- Seven antigenically distinct botulinum toxins (A to G), human disease is associated with types A, B, E, and F.

- The botulinum neurotoxin remains at the neuromuscular junction, The botulinum endopeptidase then **inactivates the proteins that regulate release of acetylcholine,** blocking neurotransmission at peripheral cholinergic synapses. The resulting clinical presentation of botulism is a **flaccid paralysis.**



Further reading:

- Murray - Medical Microbiology 8th Edition
  Section 4: Bacteriology
    Chapter 20: Bacillus
    Chapter 30: Clostridium