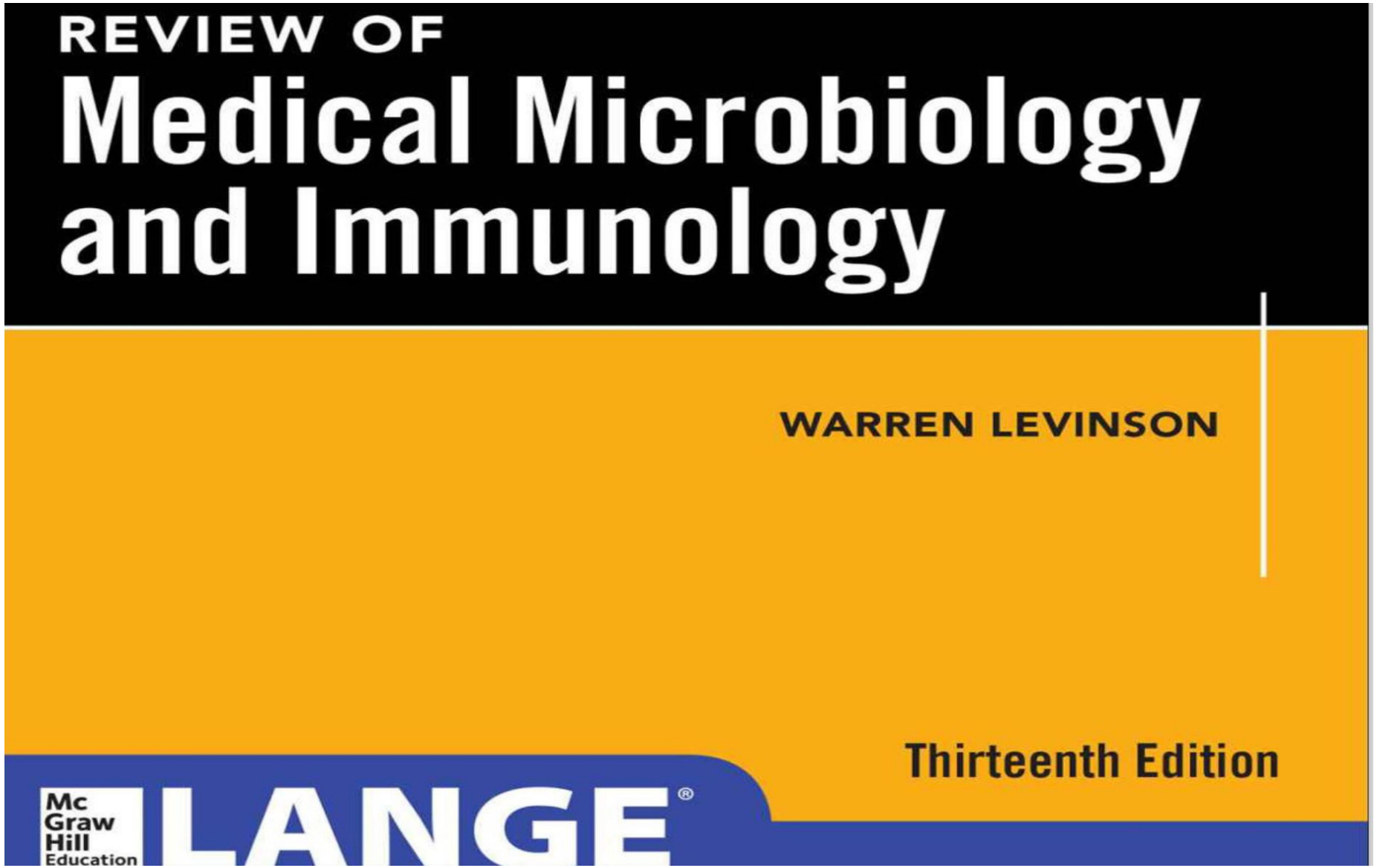
Levinson, W., Review of medical microbiology and immunology. Fourteenth edition. ed. 2016, New York: McGraw-Hill Education. ix, 821 pages.



Chapter 17, all tables and figures taken from this chapter

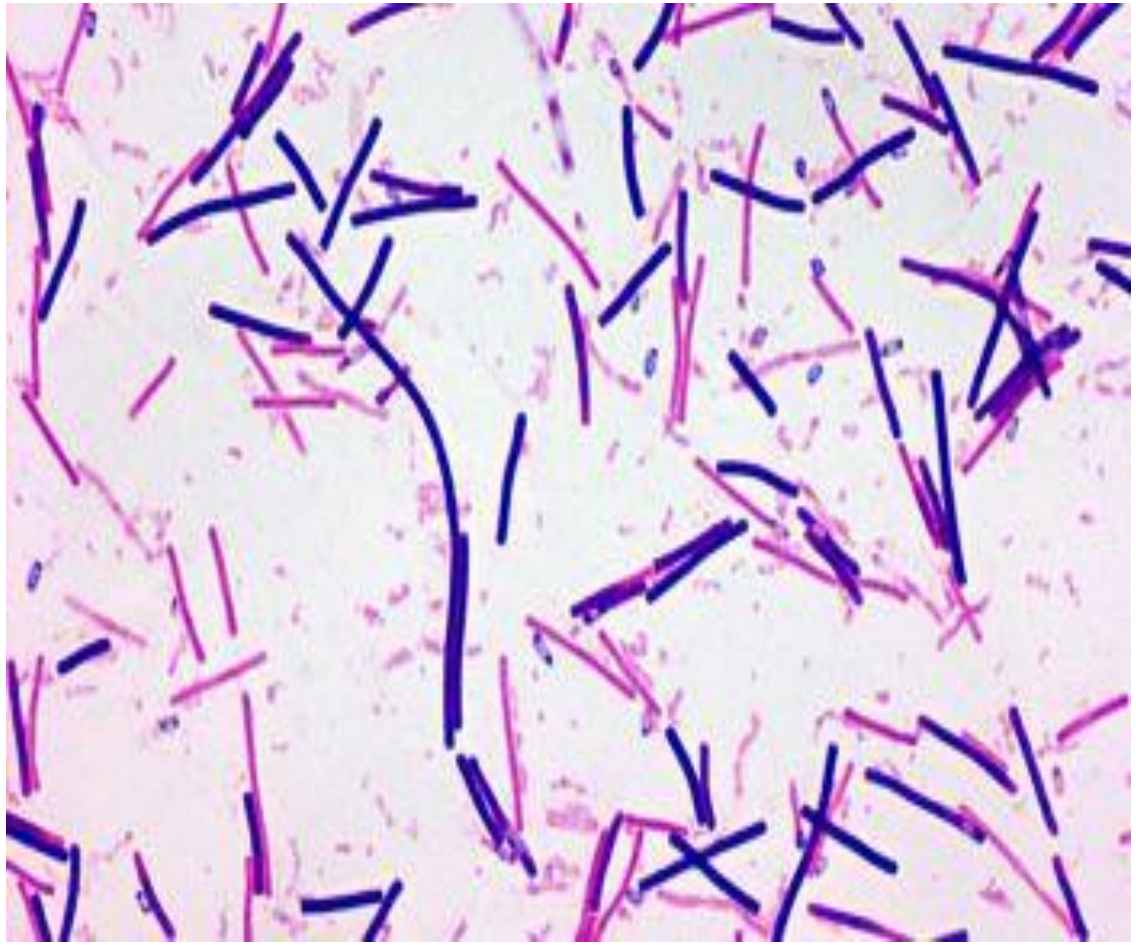
GRAM POSITIVE RODS

- Divided by their ability to form spores or not
- SPORE forming : Bacillus (AEROBIC) and Clostridium (NON AEROBIC)
- NON SPORE forming :
- *Corynebacterium diphtheriae* and *Listeria monocytogenes*

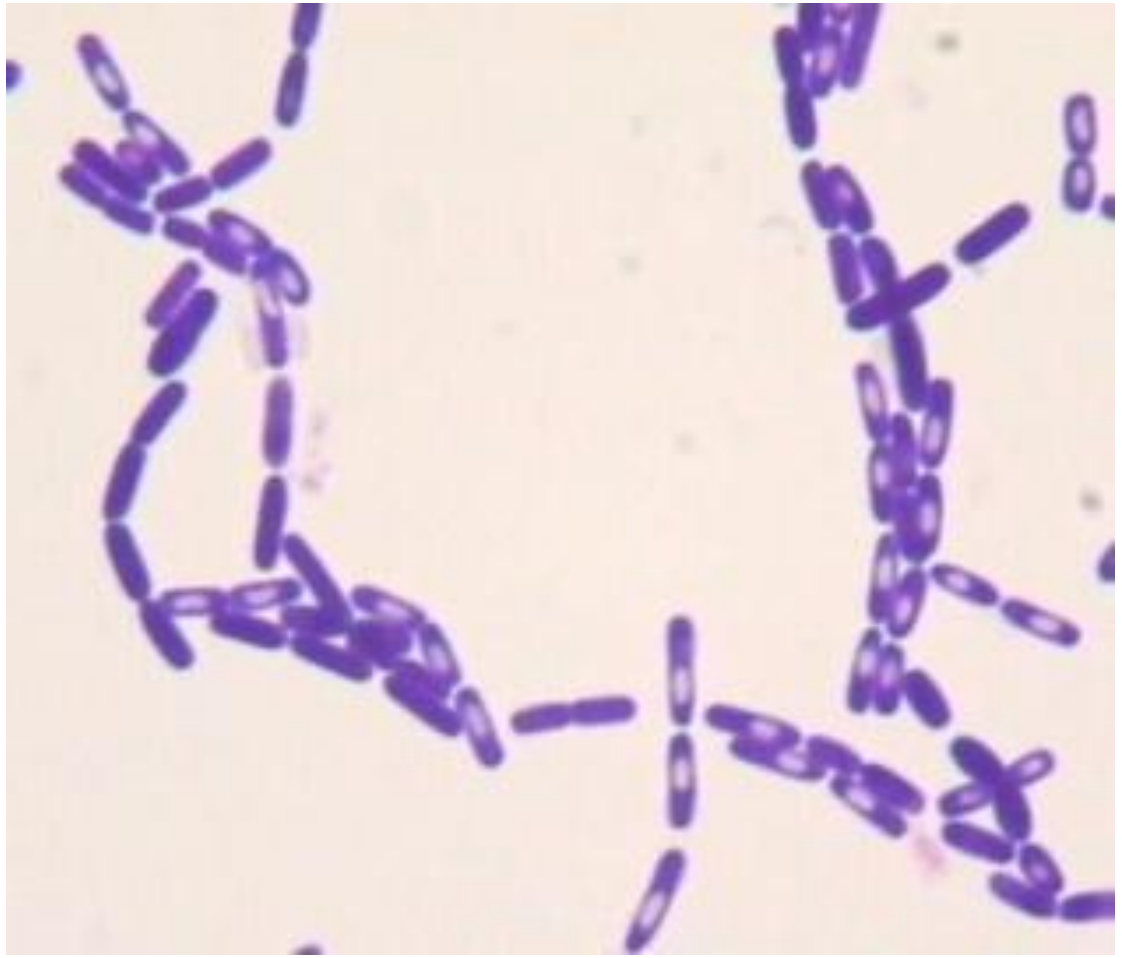
Growth	Anaerobic Growth	Spore Formation	Exotoxins Important in Pathogenesis
Bacillus	–	+	+
Clostridium	+	+	+
Corynebacterium	–	–	+
Listeria	–	–	–

- These two gram-positive rods can also be distinguished based on the appearance (size, stain intensity and spore size/position) on Gram stain

- 1)Bacillus and Clostridium species are longer and more deeply staining than Corynebacterium and Listeria species.
- Corynebacterium species are club shaped (i.e., they are thinner on one end than the other, the other end contains large granules which makes it look like a club).
- Corynebacterium and Listeria species characteristically appear as V- or L-shaped rods.



Clostridium difficile

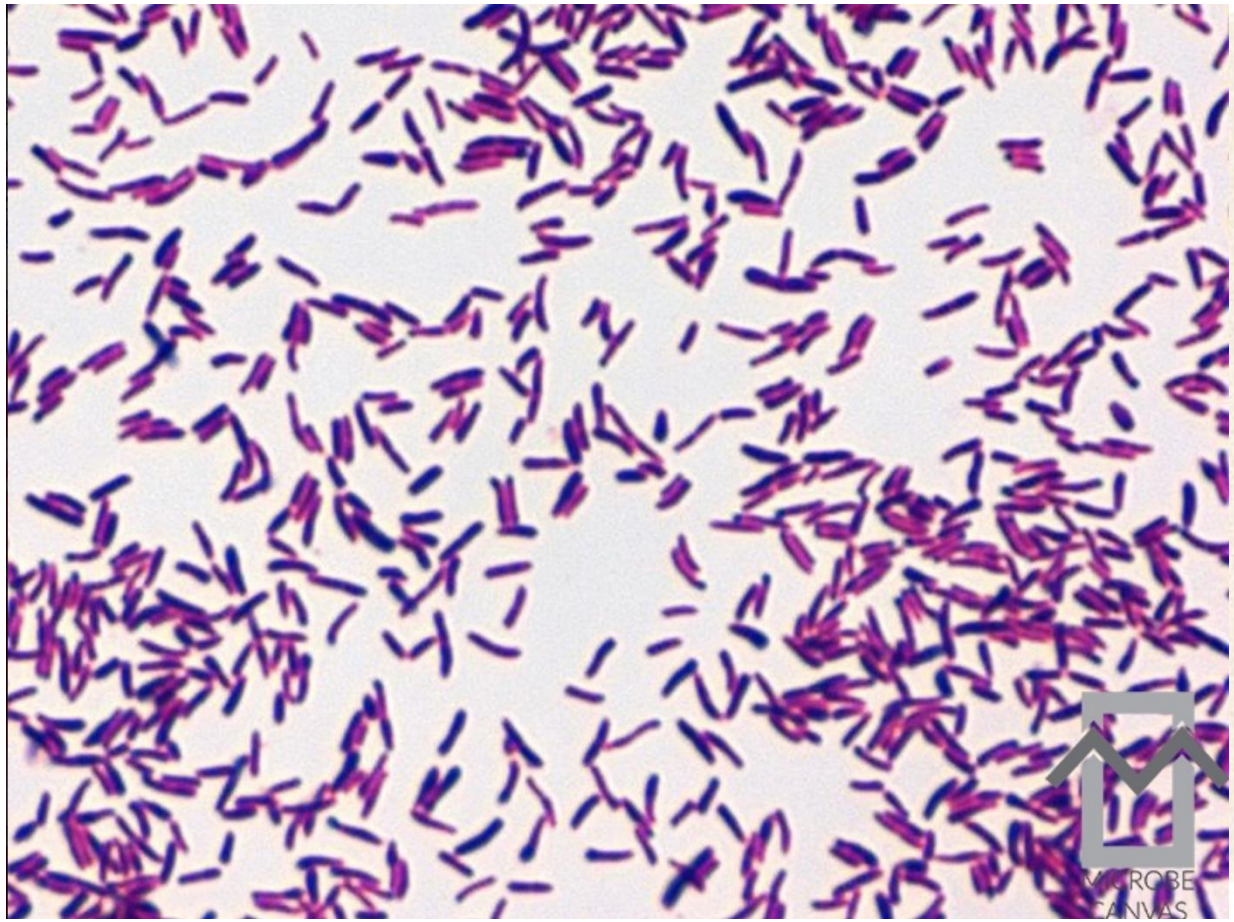


Bacillus Cereus

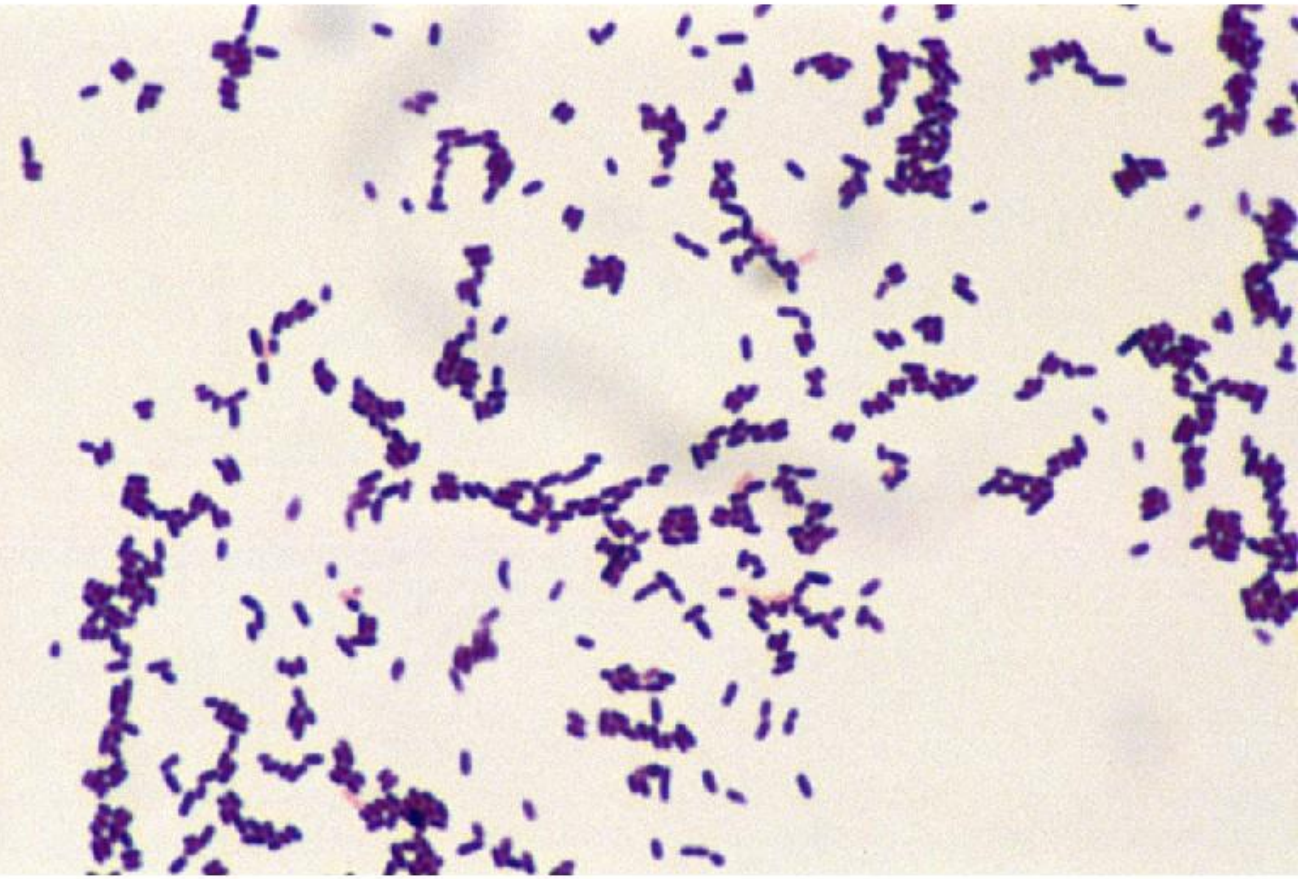
Notice central clearing of the spore

http://microbe-canvas.com/Bacteria/anaerobic-gram-positive-rods/spores-positive/lecithinase-negative-2/indole-negative-4/clostridium-difficile.html

https://s3.amazonaws.com/classconnection/236/flashcards/677236/jpg/jpg_bacillus_cereus_sporule_gram_1_-144c04c6194539ede81-154B85C5F2B73E4B9E7-thumb400.jpg



Corynbacterium



Listeria

http://microbe-canvas.com/Bacteria/gram-positive-rods/cells-difteroid/facultative-anaerobic-2/catalase-positive-4/corynebacterium-diphtheriae.html

SPORE-FORMING GRAM-POSITIVE RODS

1- BACILLUS species

TABLE 17–2 Important Features of Pathogenesis by *Bacillus* Species

Organism	Disease	Transmission/Predisposing Factor	Action of Toxin	Prevention
B. anthracis	Anthrax	1. Cutaneous anthrax: spores in soil enter wound 2. Pulmonary anthrax: spores are inhaled into lung	Exotoxin has three components: protective antigen binds to cells; edema factor is an adenylate cyclase; lethal factor is a protease that inhibits cell growth	Vaccine contains protective antigen as the immunogen
B. cereus	Food poisoning	Spores germinate in reheated rice, then bacteria produce exotoxins, which are ingested	Two exotoxins (enterotoxins): 1. Similar to cholera toxin, it increases cyclic AMP 2. Similar to staphylococcal enterotoxin, it is a superantigen	No vaccine

1. Bacillus anthracis

- Disease:
- *B. anthracis* causes anthrax (Figure 17–1), which is common in animals but rare in humans.
- Human disease occurs in three main forms: cutaneous, pulmonary (inhalation), and gastrointestinal.
- In 2001, an outbreak of both inhalation and cutaneous anthrax occurred in the United States.
- The outbreak was caused by sending spores of the organism through the mail. There were 18 cases, causing 5 deaths in this outbreak.

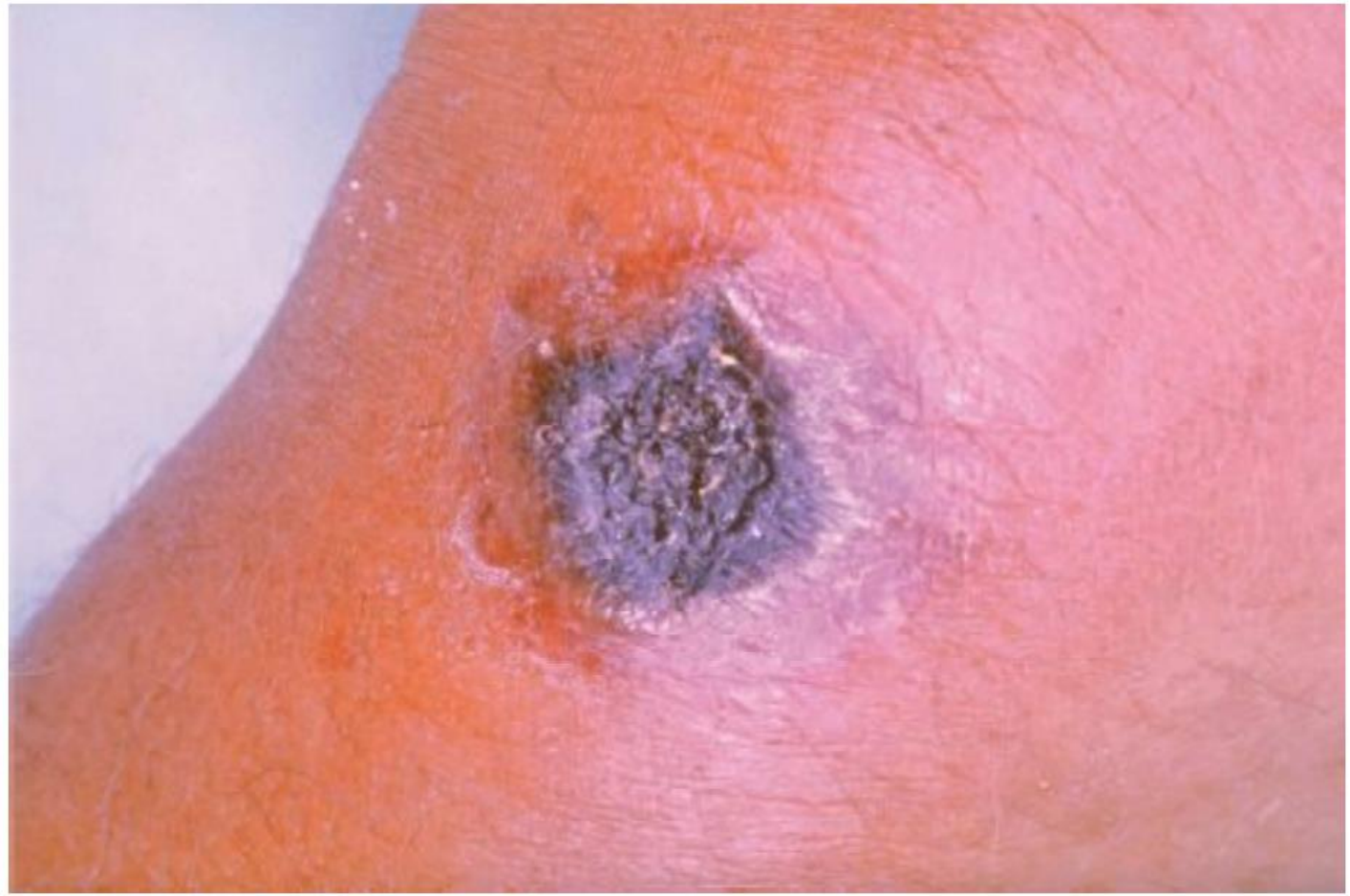
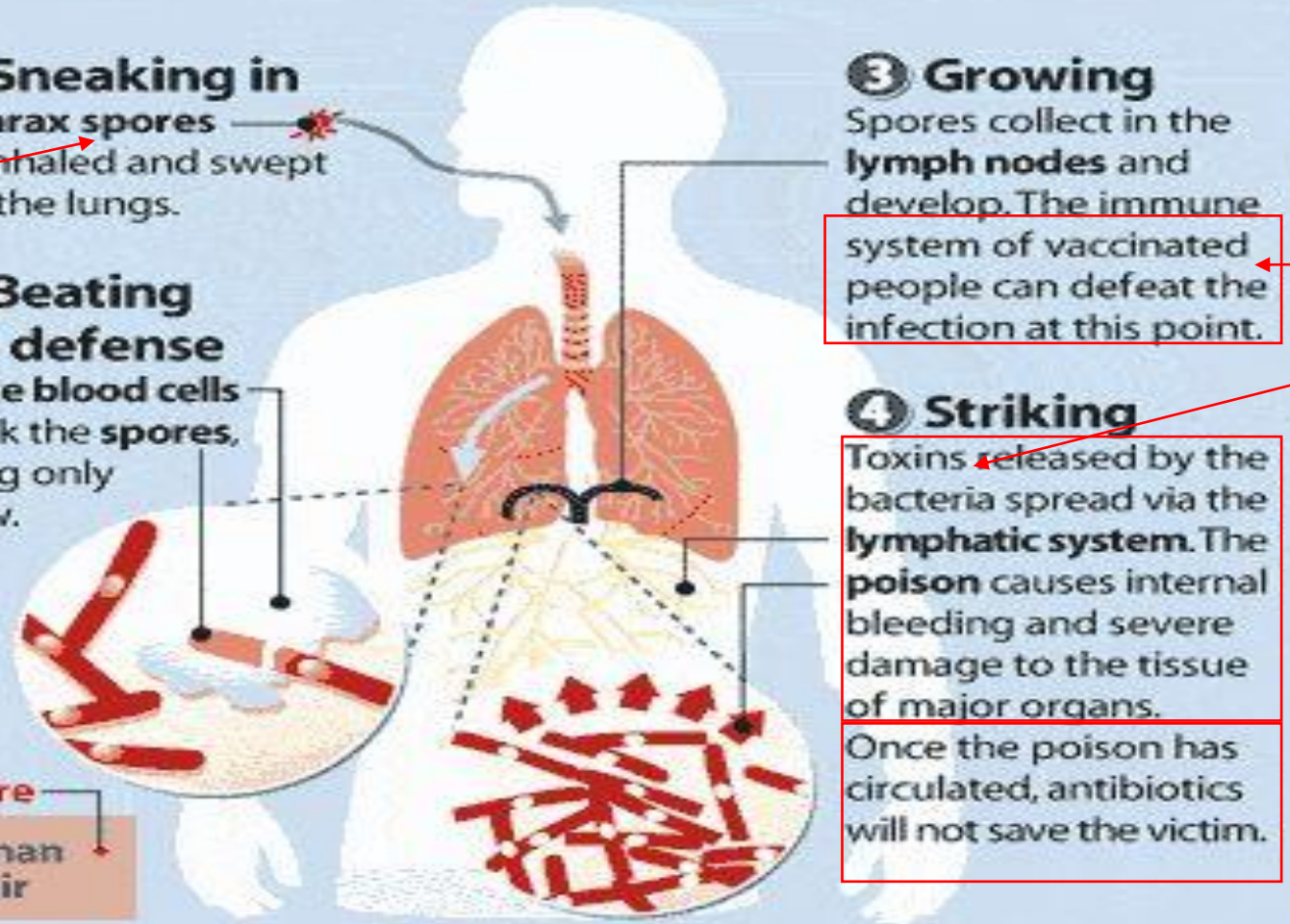


FIGURE 17–1 Skin lesion of anthrax. Note the *black eschar*, a necrotic lesion covered by a crust, caused by lethal factor, an exotoxin produced by *Bacillus anthracis*. Note the area of edema surrounding the eschar, which is caused by another exotoxin called *edema factor*. (Source: Centers for Disease Control and Prevention. CDC # 2033. CDC Provider: Dr. James H. Steele.)

HOW ANTHRAX ATTACKS

Anthrax is a naturally occuring bacterium that plagues farm animals and, occasionally, agricultural workers. An airborne form of the disease, however, can be harnessed as a potent biological weapon.



Source: "The World's Best Anatomical Charts"; "Zoology"; Anthrax Vaccine Immunization Program; Journal of the American Medical Association

ADRIAN HOLOVATY/Missourian

https://i.pinimg.com/474x/79/24/2d/79242d9fe4b88f5fc02eaa3090d74973--simple-sugar-medical-conditions.jpg

- Important *Properties B. anthracis* is a large gram-positive rod with square ends, frequently found in chains .
- Its antiphagocytic capsule is composed of D-glutamate/(*This is unique—capsules of other bacteria are polysaccharides.)*
- It is nonmotile, whereas other members of the genus are motile.
- Anthrax toxin is encoded on one plasmid, and the polyglutamate capsule is encoded on a different plasmid.

Transmission

- Spores of the organism persist in soil for years. The route of entry determines type of disease: Skin/GI/RT
- Skin→ Humans are most often infected cutaneously, at the time of trauma to the skin , humans will get spores to enter and cause disease cutaneously, this source is usually animal hides.
- Lung→ Spores inhaled into the respiratory tract cause Pulmonary (inhalation) anthrax.
- GI → Gastrointestinal anthrax occurs when contaminated meat is ingested
- Inhalation anthrax is not communicable from person to person, despite the severity of the infection after being inhaled into the lung, the organism moves rapidly to the mediastinal lymph nodes, where it causes hemorrhagic mediastinitis.
- Because it leaves the lung so rapidly, it is not transmitted by the respiratory route to others.

Pathogenesis

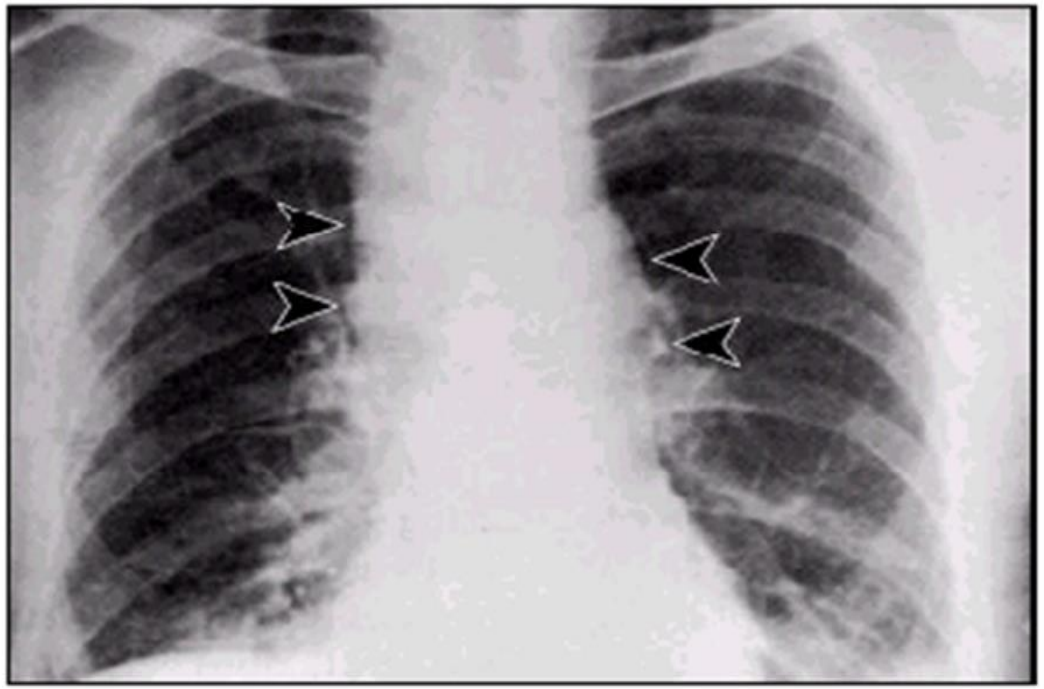
- Pathogensis is based on exotoxin production (primarily two exotoxins, which are both collectively known as anthrax toxin).
- edema factor and lethal factor, both these exotoxin are made up of two subunits (A–B subunits).
- The B (binding) subunit in is a protective antigen.
- The A (active) subunit has the enzymatic activity.

- 1- Edema factor exotoxin:
- increases the intracellular cyclic adenosine monophosphate (cAMP) as it works as an adenylate cyclase.
- This causes an outpouring of fluid (recall *B. pertussis*?) from the cell into the extracellular space, which manifests as edema (this is similar to the mechanism of diarrhea causes by vibrio cholera)
- 2- Lethal factor exotoxin:
- This has a protease activity causes the inhibition of the MAPK signal transduction pathway (mitogen-activated protein kinase). MAPK is the signal pathway that promotes human cell growth, the cleavage of MAPK and inhibition of this pathway thus inhibits cell growth.
- The *Binding antigen causes the formation of small pores on cell membrane of target cells which then allows edema factor and lethal factor exotoxins to enter the cell.*
- The binding antigen is called the protective antigen due to the fact that ABs against this antigen are protective against the disease.

Clinical Findings

- The typical lesion of cutaneous anthrax is a painless ulcer with a black eschar (crust, scab), with a striking Local edema (usually exaggerated).
- The lesion is called a malignant pustule.
- Skin anthrax → Untreated cases progress to bacteremia and death – the lesions are full of bacteria and we have a small window of treatment before they reach the blood...
- Pulmonary (inhalation) anthrax → also known as "wool-sorter's disease (inhaled from breathing spores present on infected sheep), begins with nonspecific respiratory tract symptoms resembling influenza, especially a dry cough and **substernal pressure**, which then progresses to hemorrhagic mediastinitis, bloody pleural effusions, septic shock, and death.
- Although the lungs are infected, the classic signs and symptoms and Xray features of pneumonia are not present, however mediastinal widening seen on chest X-ray is an important diagnostic criterion. Hemorrhagic mediastinitis and hemorrhagic meningitis are severe life-threatening complications.
- → The symptoms of gastrointestinal anthrax include vomiting, abdominal pain, and bloody diarrhea.

Anthrax: Inhalational



✍Mediastinal widening

JAMA 1999;281:1735–1745

http://www.nejm.org/doi/full/10.1056/NEJMicm0802093

https://www.slideshare.net/doctorrao/anthrax-teaching

Laboratory Diagnosis

- Smears (microscopy of samples from lesions) show the typical large, gram-positive rods in long chains.
- Spores are usually not seen in smears of exudate because spores form when nutrients are insufficient, and **nutrients are plentiful in infected** tissue.
- Nonhemolytic (gamma) colonies form on blood agar aerobically.
- In case of a bioterror attack, rapid diagnosis can be performed in special laboratories using polymerase chain reaction (PCR)–based assays.
- Another rapid diagnostic procedure is the direct fluorescent antibody test that detects antigens of the organism in the lesion.
- Serologic tests, such as an enzyme-linked immunosorbent assay (ELISA) test for antibodies, require acute and convalescent serum samples and can only be used to make a diagnosis retrospectively.

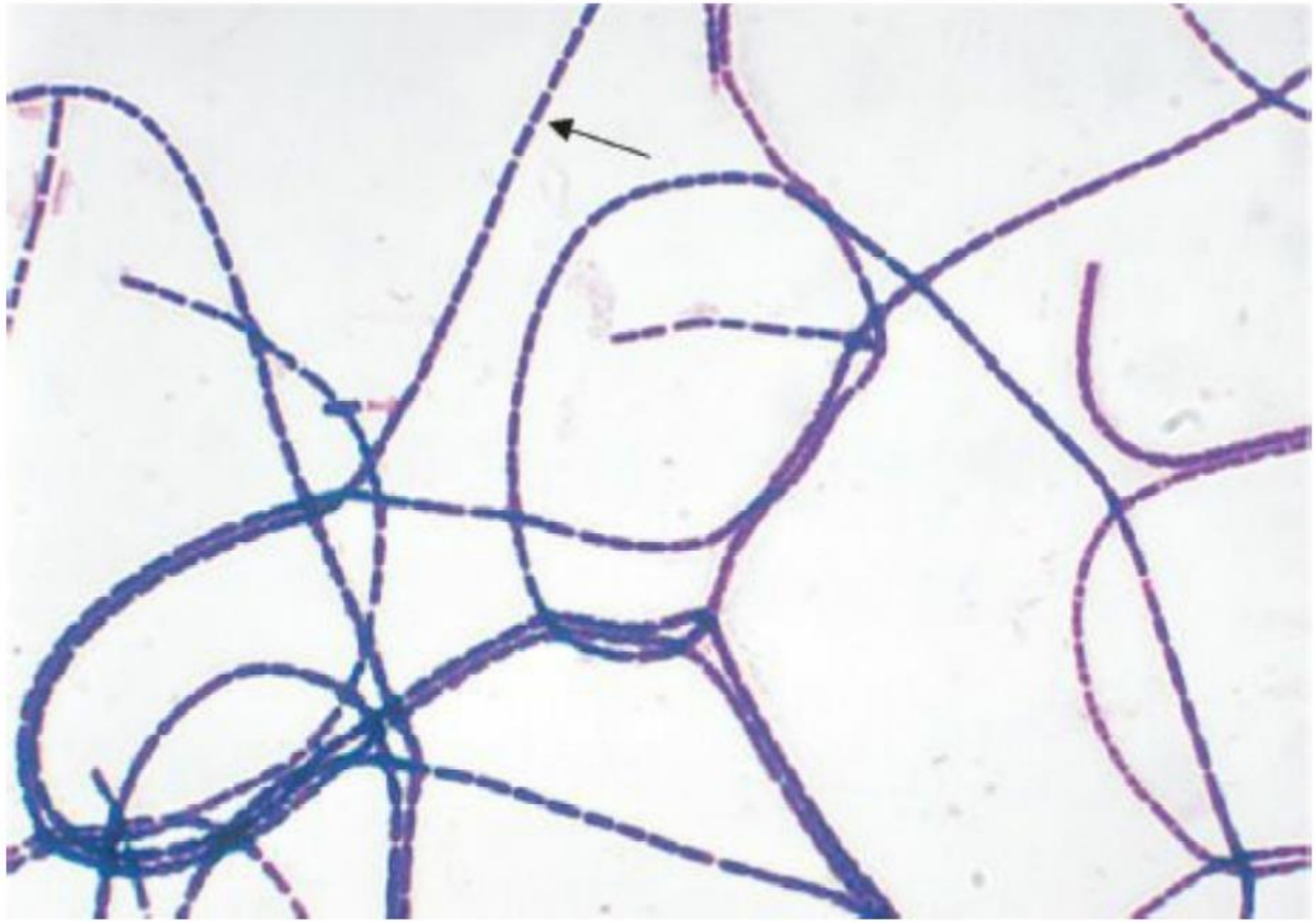


FIGURE 17–2 *Bacillus anthracis*—Gram stain. Arrow points to one large "box car–like" gram-positive rod within a long chain. (Figure courtesy of Public Health Image Library, Centers for Disease Control and Prevention.)

Treatment and Prevention

- Ciprofloxacin or doxycycline was used as prophylaxis in those exposed during the outbreak in the United States in 2001.
- People at high risk can be immunized with cell-free vaccine containing purified protective antigen as immunogen, the vaccine is weakly immunogenic, and six doses of vaccine over an 18-month period are given. Annual boosters are also given to maintain protection.
- Incinerating animals that die of anthrax, rather than burying them, will prevent the soil from becoming contaminated with spores.
- Antibiotics (Ciprofloxacin + another antibiotic) given IV as treatment, also antitoxins are also given

2. Bacillus cereus

- Disease *B. cereus* causes food poisoning (also toxin mediated!).

- Transmission:
- This is caused by spores that survive heating, the classic food item is **rice** (especially rice that is kept warm for a long time –reheated fried rice- buffets) , **spores survive steaming and quick frying**.

- When the rice is kept warm, the spores germinate , they grow and start producing the exotoxin,

Pathogenesis

- *B. cereus* produces two **entero**toxins (enteric targeting toxin).
- one of the enterotoxins is the same as that of cholera toxin (*it adds adenosine diphosphate ribose, a process called ADP-ribosylation, to a G protein, which stimulates adenylate cyclase and leads to an increased concentration of cyclic AMP within the enterocyte*).
- The other enterotoxin resembles that of staphylococcal enterotoxin (acts as a superantigen).

Clinical Findings-

- There are two syndromes.
- (1) *One syndrome has a short incubation period (4 hours) and consists primarily of nausea and vomiting, similar to staphylococcal food poisoning*. (recall staph food poisoning has prominent vomiting)
- (2) The other has a long incubation period (18 hours) and features watery, non bloody diarrhea, resembling clostridial gastroenteritis.

- No laboratory diagnosis is usually done
- Treatment is only symptomatic/ self limited, once the toxin does its damage, the symptoms are gone, this is due to the fact that **you ingest the toxin and not the bacteria (in sum)**
- Prevention : rice!

CLOSTRIDIUM

- There are four medically important species:
- *Clostridium tetani, Clostridium botulinum, Clostridium perfringens* (which causes either gas gangrene or food poisoning), and *Clostridium difficile (pseudomembranous colitis).*

- All clostridia are anaerobic (unlike Bacillus), spore-forming, Gram-positive rods

Organism	Disease	Transmission/ Predisposing Factor	Action of Toxin	Prevention
C. tetani	Tetanus	Spores in soil enter wound	Blocks release of inhibitory transmitters (e.g., glycine)	Toxoid vaccine
C. botulinum	Botulism	Exotoxin in food is ingested	Blocks release of acetylcholine	Proper canning; cook food
C. perfringens	1. Gas gangrene	Spores in soil enter wound	Lecithinase	Debride wounds
	2. Food poisoning	Exotoxin in food is ingested	Superantigen	Cook food
C. difficile	Pseudomembranous colitis	Antibiotics suppress normal flora	Cytotoxin damages colon mucosa	Appropriate use of antibiotics

TABLE 7–9 Main Features of Exotoxins and Endotoxins

	Comparison of Properties	
Property	**Exotoxin**	**Endotoxin**
Source	Certain species of gram-positive and gram-negative bacteria	Cell wall of gram-negative bacteria
Secreted from cell	Yes	No
Chemistry	Polypeptide	Lipopolysaccharide
Location of genes	Plasmid or bacteriophage	Bacterial chromosome
Toxicity	High (fatal dose on the order of 1 μg)	Low (fatal dose on the order of hundreds of micrograms)
Clinical effects	Various effects (see text)	Fever, shock
Mode of action	Various modes (see text)	Includes TNF and interleukin-1
Antigenicity	Induces high-titer antibodies called antitoxins	Poorly antigenic
Vaccines	Toxoids used as vaccines	No toxoids formed and no vaccine available
Heat stability	Destroyed rapidly at 60°C (except staphylococcal enterotoxin)	Stable at 100°C for 1 hour
Typical diseases	Tetanus, botulism, diphtheria	Meningococcemia, sepsis by gram-negative rods

TNF = tumor necrosis factor.

Bacterium	Disease	Mode of Action	Toxoid Vaccine
Gram-positive rods			
Corynebacterium diphtheriae	Diphtheria	Inactivates EF-2 by ADP-ribosylation	Yes
Clostridium tetani	Tetanus	Blocks release of the inhibitory neurotransmitter glycine by proteolytic cleavage of releasing proteins	Yes
Clostridium botulinum	Botulism	Blocks release of acetylcholine by proteolytic cleavage of releasing proteins	Yes[1]
Clostridium difficile	Pseudomembranous colitis	Exotoxins A and B inactivate GTPases by glucosylation	No
Clostridium perfringens	Gas gangrene	Alpha toxin is a lecithinase; enterotoxin is a superantigen	No
Bacillus anthracis	Anthrax	Edema factor is an adenylate cyclase; lethal factor is a protease that cleaves MAP kinase, which is required for cell division	No
Gram-positive cocci			
Staphylococcus aureus	1. Toxic shock syndrome	Is a superantigen; binds to class II MHC protein and T-cell receptor; induces IL-1 and IL-2	No
	2. Food poisoning	Is a superantigen acting locally in the gastrointestinal tract	No
	3. Scalded skin syndrome	Is a protease that cleaves desmoglein in desmosomes	No
Streptococcus pyogenes	Scarlet fever	Is a superantigen; action similar to toxic shock syndrome toxin of *S aureus*	No
Gram-negative rods			
Escherichia coli	1. Watery diarrhea	Labile toxin stimulates adenylate cyclase by ADP-ribosylation; stable toxin stimulates guanylate cyclase	No
	2. Bloody diarrhea	Shiga toxin inhibits protein synthesis in enterocytes by removing adenine from 28S ribosomal RNA	No
Shigella dysenteriae	Bloody diarrhea	Shiga toxin inhibits protein synthesis in enterocytes by removing adenine from 28S ribosomal RNA	No
Vibrio cholerae	Cholera	Stimulates adenylate cyclase by ADP-ribosylation	No
Bordetella pertussis	Whooping cough	Stimulates adenylate cyclase by ADP-ribosylation; inhibits chemokine receptor	Yes[2]

[1]For high-risk individuals only.

[2]The acellular vaccine contains pertussis toxoid and four other proteins.

1. *Clostridium tetani*, cause of tetanus

Transmission

- **Spores are widespread in soil** (spores and soil are always intimate, not really rusty nails, but soil, rusty nails are usually discarded on soil, and thus have higher potential of containing the spores).
- The portal of entry is usually a wound site (e.g., where a nail penetrates the foot), but the spores can also be introduced during "skinpopping," a technique used by drug addicts to inject drugs into the skin.
- Germination of spores is favored by necrotic tissue and poor blood supply in the wound (why? Less blood = less oxygen).
- Neonatal tetanus, in which the organism enters through a contaminated umbilicus or circumcision wound, is a major problem in some developing countries.

Pathogenesis

- Tetanus toxin (tetanospasmin) is an exotoxin produced by **vegetative** cells at the **wound site**.
- This polypeptide toxin is carried (retrograde) through the axons of neurons to the central nervous system, where it binds to ganglioside receptors and blocks release of inhibitory mediators (e.g., glycine and γ-aminobutyric acid [GABA]) at spinal synapses- so it inhibits the inhibitors substance= over excitation.
- Tetanus toxin and botulinum toxin (see later, both are clostridial toxins) are among the most toxic substances known.
- Tetanus toxin has one antigenic type (so the vaccine has one antigenic toxoid), unlike botulinum toxin, which has eight.

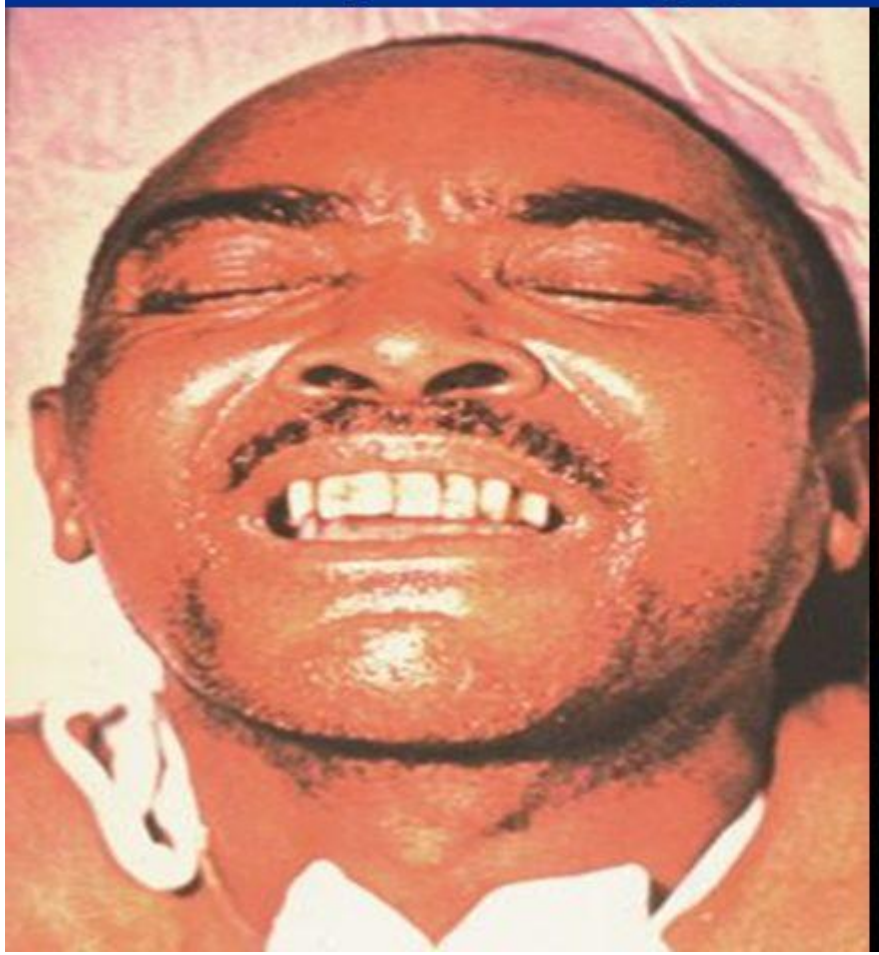
Clinical Findings

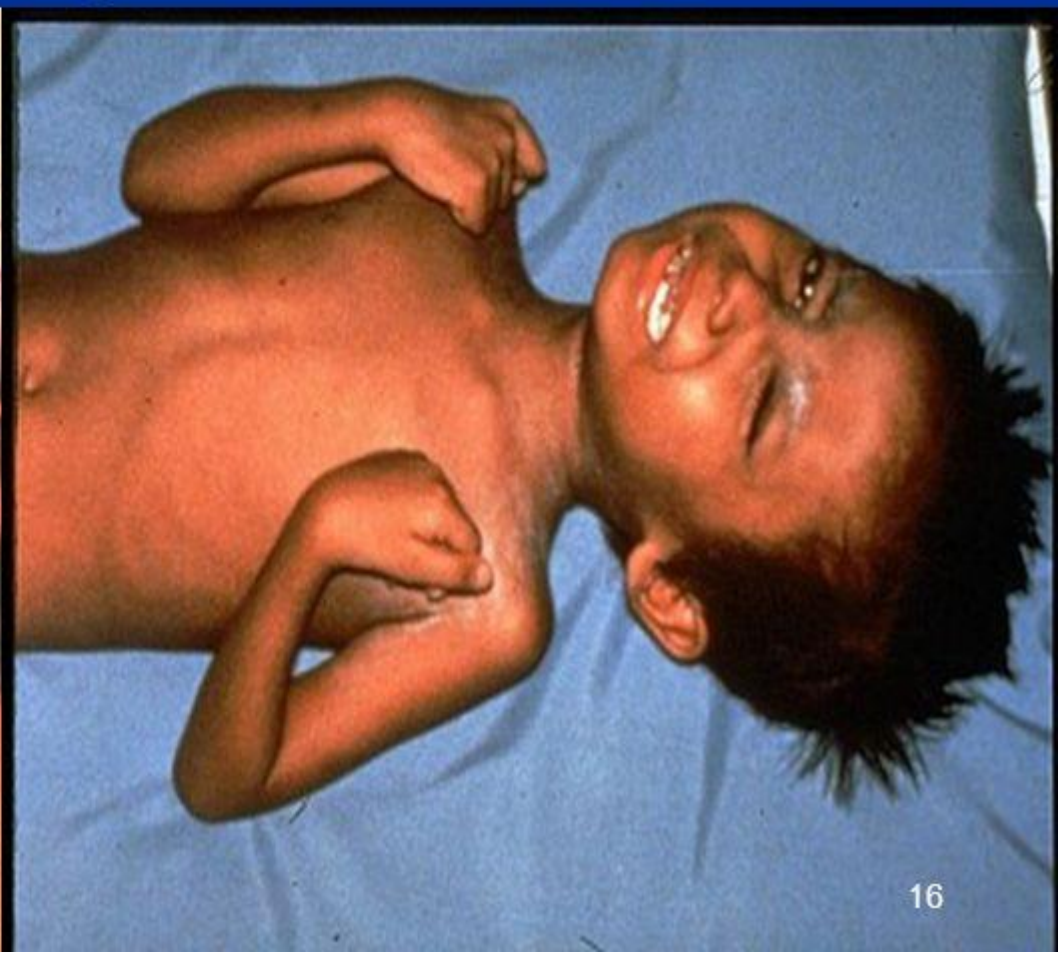
- Tetanus is characterized by strong muscle spasms (spastic paralysis, tetany).
- Specific clinical features include lockjaw (trismus) due to rigid contraction of the jaw muscles, which prevents the mouth from opening; a characteristic grimace known as risus sardonicus; and exaggerated reflexes.
- Opisthotonos, a pronounced arching of the back due to spasm of the strong extensor muscles of the back, is often seen (Figure 17–4).
- Respiratory failure ensues
- A high mortality rate is associated with this disease.
- **in tetanus, spastic paralysis (strong muscle contractions) occurs, whereas in botulism, flaccid paralysis (weak or absent muscle contractions) occurs**.

Risus sardonicus

Risus Sardonicus :

Spasm of facial muscles (frontalis & angle of mouth muscles) producing grinning facies





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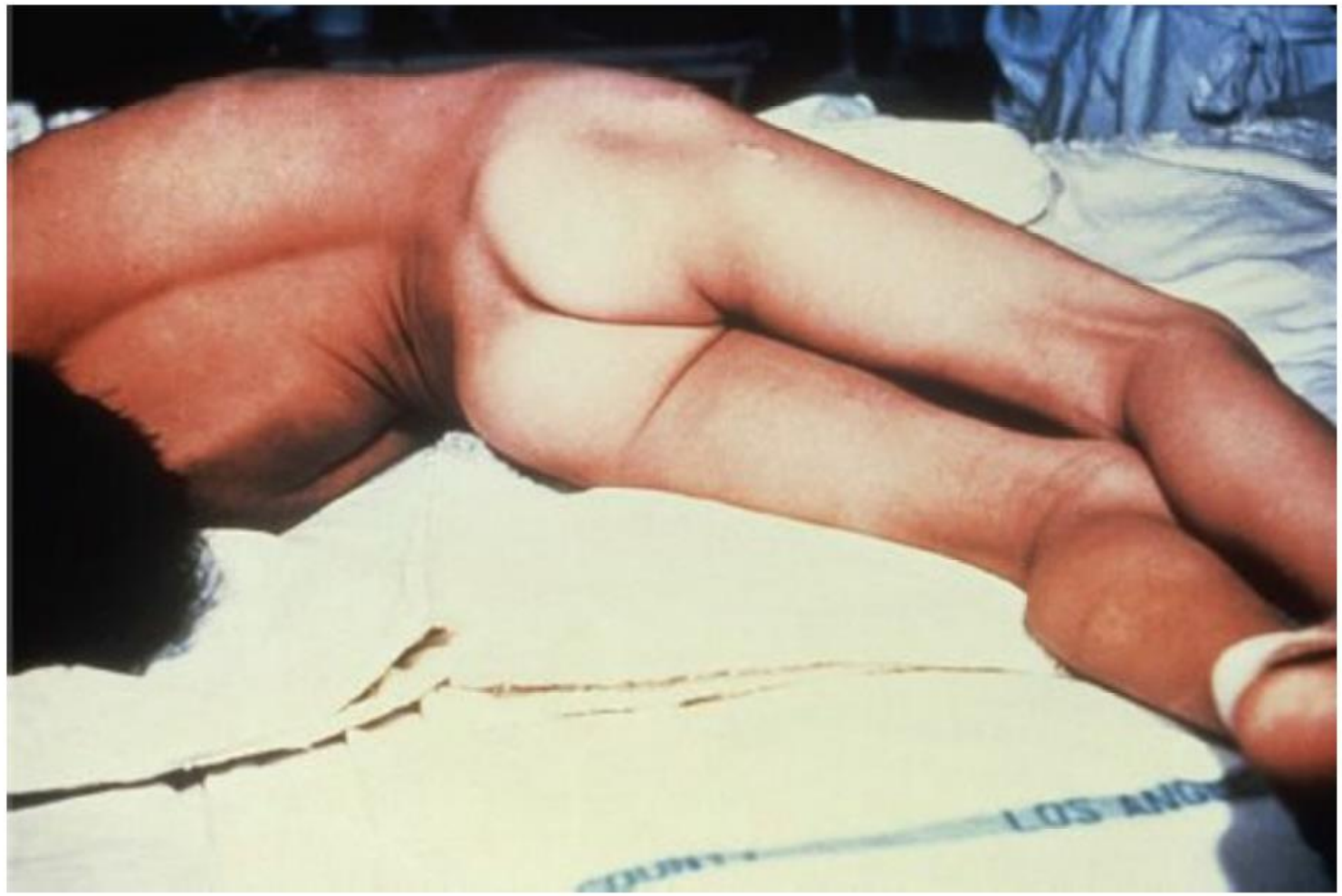
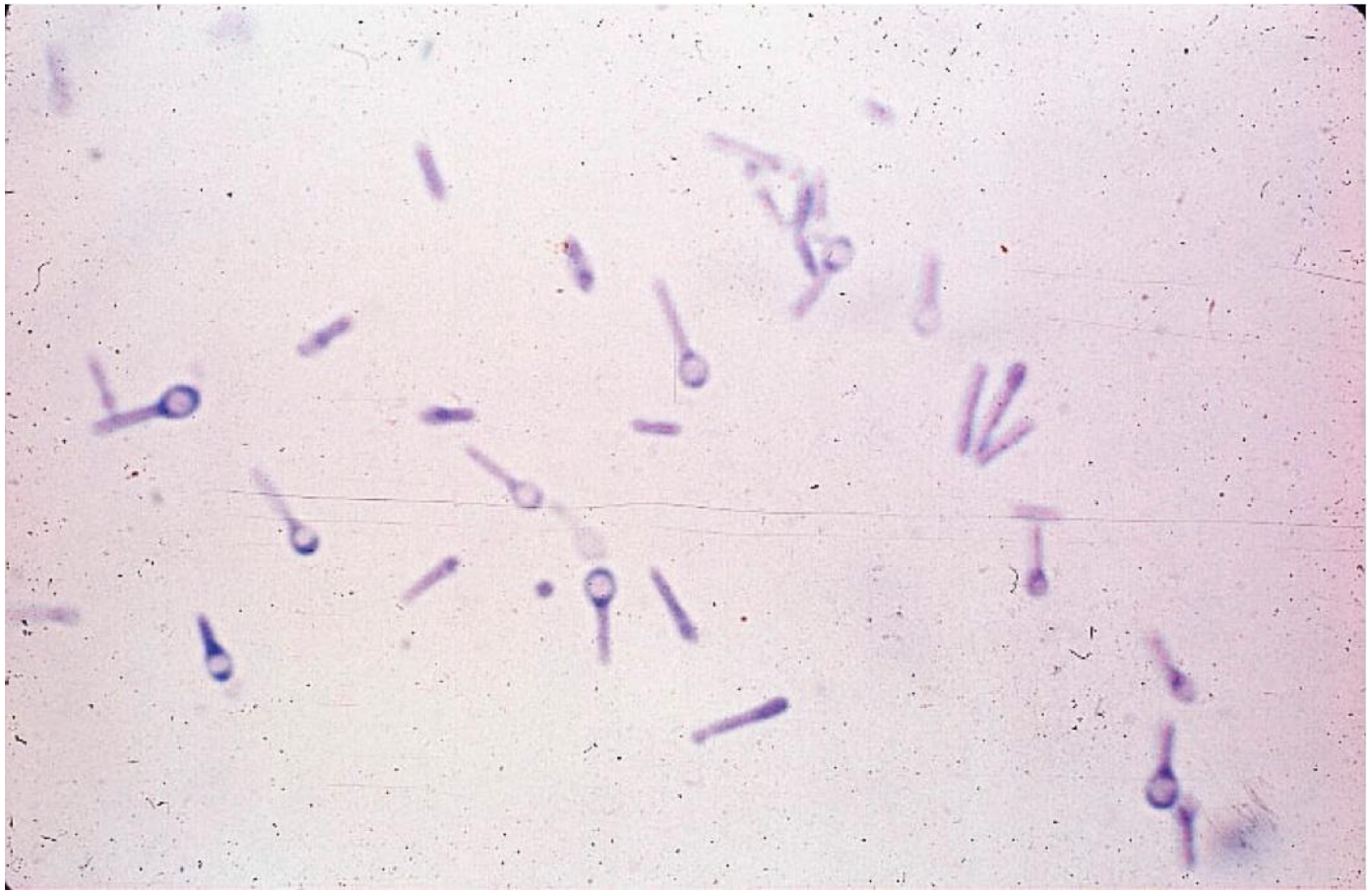


FIGURE 17–4 Tetanus. Note the marked hyperextension of the back, a position called *opisthotonos*, caused by tetanus toxin, an exotoxin that inhibits the release of mediators of the inhibitory neurons in the spinal cord. (Source: Centers for Disease Control and Prevention. CDC # 6373.)

Laboratory Diagnosis and Treatment

- There is no microbiologic or serologic diagnosis (diagnosed clinically, LOCKJAW is the first symptom usually).
- Organisms are rarely isolated from the wound site (again disease is due to toxin production not infection).
- *C. tetani* produces a large terminal spore (spore at the end of the rod), this gives the organism the characteristic appearance of a "tennis racket." see next slide
- Treatment:
- Tetanus immune globulin (**tetanus antitoxin**) is used to neutralize the toxin, the role of antibiotics is uncertain(metronidazole or penicillin G can be given, however not proven to be helpful).
- An adequate airway must be maintained and respiratory support given, with Benzodiazepines (e.g., diazepam [Valium]) to prevent spasms.



Large and terminal spore, *C. tetani*

http://classconnection.s3.amazonaws.com/344/flashcards/1212344/jpg/clostridiumtetani1349841271090.jpg

Prevention

- Tetanus is prevented by immunization with tetanus toxoid (formaldehyde-treated toxin) in childhood and every 10 years thereafter.
- Tetanus toxoid is part of the scheduled vaccines that are usually given to children (in combination with diphtheria toxoid and the acellular pertussis vaccine, all three are called –DTaP).
- Clean wound → When trauma occurs, the wound should be cleaned and debrided, and tetanus toxoid (TT) booster should be given (active immunity boost).
- All other wounds (contaminated especially) → tetanus immune globulin the toxoid booster and penicillin administered. (active + passive immunity + chemotherapy)

Wound management and tetanus prophylaxis

Previous doses of tetanus toxoid*	Clean and minor wound		All other wounds¶	
	Tetanus toxoid-containing vaccine[Δ]	Human tetanus immune globulin	Tetanus toxoid-containing vaccine[Δ]	Human tetanus immune globulin[◊]
<3 doses or unknown	Yes[§]	No	Yes[§]	Yes
≥3 doses	Only if last dose given ≥10 years ago	No	Only if last dose given ≥5 years ago[¥]	No

Appropriate tetanus prophylaxis should be administered as soon as possible following a wound but should be given even to patients who present late for medical attention. This is because the incubation period is quite variable; most cases occur within 8 days, but the incubation period can be as short as 3 days or as long as 21 days. For patients who have been vaccinated against tetanus previously but who are not up to date, there is likely to be little benefit in administering human tetanus immune globulin more than one week or so after the injury. However, for patients thought to be completely unvaccinated, human tetanus immune globulin should be given up to 21 days following the injury; Td or Tdap should be given concurrently to such patients.

* Tetanus toxoid may have been administered as diphtheria-tetanus toxoids adsorbed (DT), diphtheria-tetanus-whole cell pertussis (DTP, DTwP; no longer available in the United States), diphtheria-tetanus-acellular pertussis (DTaP), tetanus-diphtheria toxoids adsorbed (Td), booster tetanus toxoid-reduced diphtheria toxoid-acellular pertussis (Tdap), or tetanus toxoid (TT).

¶ Such as, but not limited to, wounds contaminated with dirt, feces, soil, or saliva; puncture wounds; avulsions; wounds resulting from missiles, crushing, burns, or frostbite.

Δ The preferred vaccine preparation depends upon the age and vaccination history of the patient:

- <7 years: DTaP
- Underimmunized children ≥7 and <11 years who have not received Tdap previously: Tdap. Children who receive Tdap between age 7 and 11 years do not require revaccination at age 11 years.
- ≥11 years: A single dose of Tdap is preferred to Td for all individuals in this age group who have not previously received Tdap. Pregnant women should receive Tdap during each pregnancy.
- Td is preferred to TT for those who received Tdap previously and when Tdap is not available.

◊ 250 units intramuscularly at a different site than tetanus toxoid; intravenous immune globulin should be administered if human tetanus immune globulin is not available.

§ The vaccine series should be continued through completion as necessary.

¥ Booster doses given more frequently than every five years are not needed and can increase adverse effects.

http://www.uptodate.com/contents/image?imageKey=PEDS/61087

2. *Clostridium botulinum*, cause of botulism

- Transmission Spores:
- Similarly, spores are found in soil but also contaminate vegetables and meats.
- This is especially true with canned food (or vacuum sealed foods),beware of misshapen cans – remember that's an anaerobic condition.
- When foods are canned or vacuum-packed without adequate **sterilization**, spores survive and germinate in the anaerobic environment.
- Toxin is produced within the canned food and ingested preformed.
- The highest-risk foods
- (1) alkaline vegetables such as green beans, peppers, and mushrooms
- (2) smoked fish.
- The toxin is relatively heat-labile; it is inactivated by boiling for several minutes. Thus, disease can be prevented by sufficient cooking (in essence, don't eat out of the can, don't eat without proper boiling of canned goods, avoid swollen or misshapen cans).

http://www.sasionline.org/prepping/storing-your-canned-goods-safely-how-to-avoid-botulism/

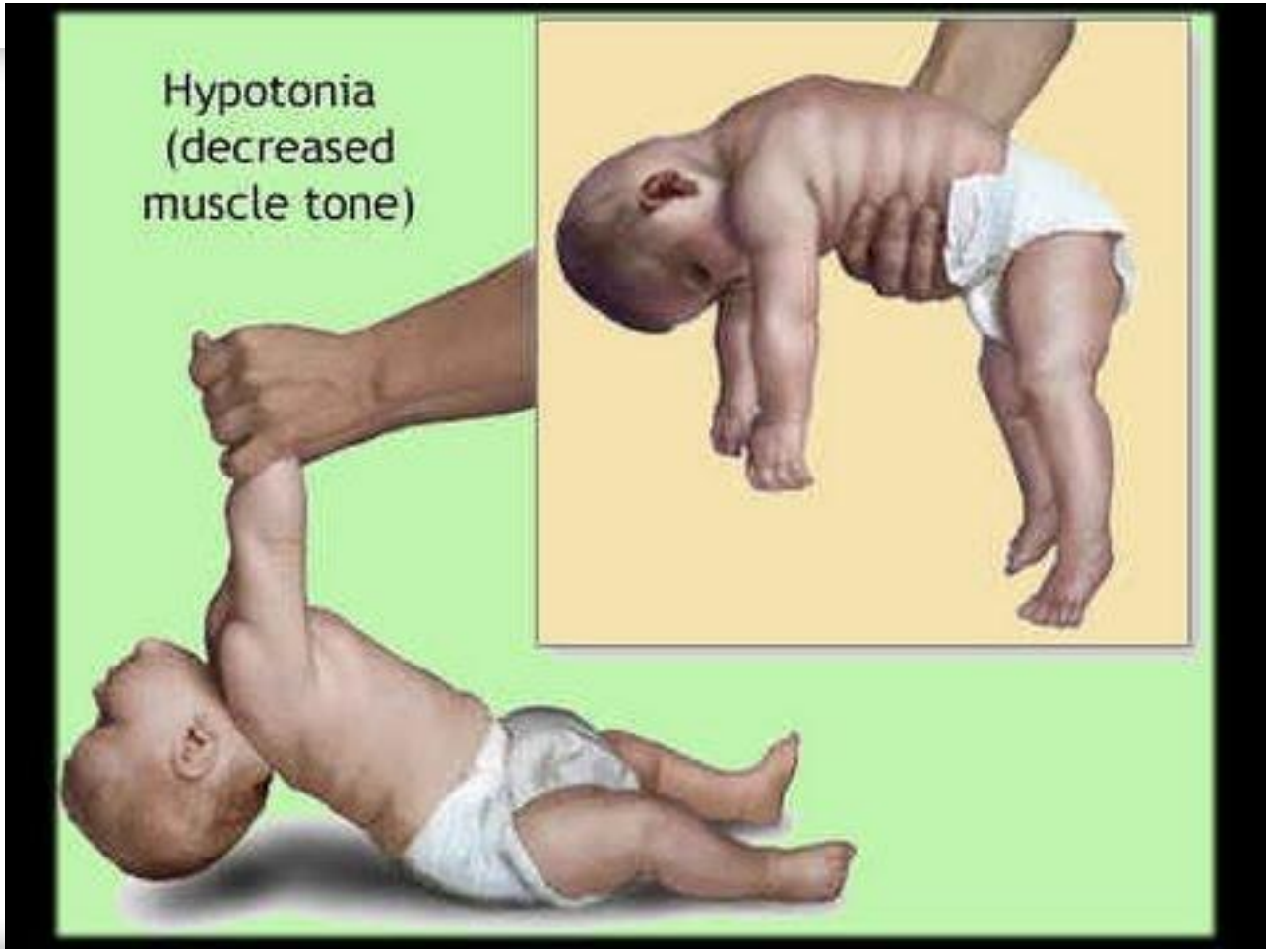
http://www.sciencephoto.com/image/11863/530wm/B2201300-Botulism_bacteria-SPL.jpg

Pathogenesis

- The botulinum toxin is absorbed from the gut (once ingested, preformed) and carried **via the blood (unlike Tetanus toxin)** to **peripheral** nerve synapses, where it **blocks release of acetylcholine (blocks the activating neurotransmitter= loss of tone)**.
- the toxin acts as protease that cleaves the proteins involved in acetylcholine release.
- The toxin is a polypeptide encoded by a lysogenic phage.
- Along with tetanus toxin, it is among the most toxic substances known.
- *There are eight immunologic types of toxin; types A, B, and E are the most common in human illness*.
- Medical uses:
- In plastic surgery (Botox is a commercial preparation of exotoxin A used to remove wrinkles on the face (induced hypotonia)).
- Minute amounts of the toxin are effective in the treatment of certain spasmodic muscle disorders such as torticollis, “writer’s cramp,” and blepharospasm.

Medical use Vs. disease

https://www.epainassist.com/images/treatment-of-benign-essential-blepharospasm.jpg



https://i.ytimg.com/vi/OX97penRbWc/hqdefault.jpg

Clinical Findings

- Descending muscle weakness and paralysis (descends from head and goes lower)
- (diplopia, dysphagia, and respiratory muscle failure)
- No fever is present.
- In contrast, Guillain-Barré syndrome (post viral syndrome) is an ascending paralysis(starts at legs and goes higher)
- Clinical forms
- (1) Wound botulism, in which spores contaminate a wound, germinate, and produce toxin at the site, associated with drug abuse (skin popping)
- (2) Infant botulism, in which the organisms grow in the gut and produce the toxin there.
- Ingestion of honey containing the organism is implicated in transmission of infant botulism, affected infants develop weakness or paralysis and may need respiratory support but usually recover spontaneously.

Laboratory Diagnosis

- The organism is usually not cultured.
- Botulinum toxin is demonstrable in uneaten food and the patient's serum by mouse protection tests *(we give mice a sample from the patient and we see the symptoms develop, we give them antitoxin and they are saved = Dx made).*

Treatment

- Trivalent antitoxin (types A, B, and E) is given
- respiratory support.
- The antitoxin is made in horses (whereas tetanus antitoxin is made in humans) and serum sickness occurs in about 15% of recipients

Prevention

- **Sterilization of food prior to introducing it in anaerobic conditions**.
- **Adequate cooking to inactivate the toxin.**
- Swollen cans must be discarded (clostridial proteolytic enzymes form gas, which swells cans).

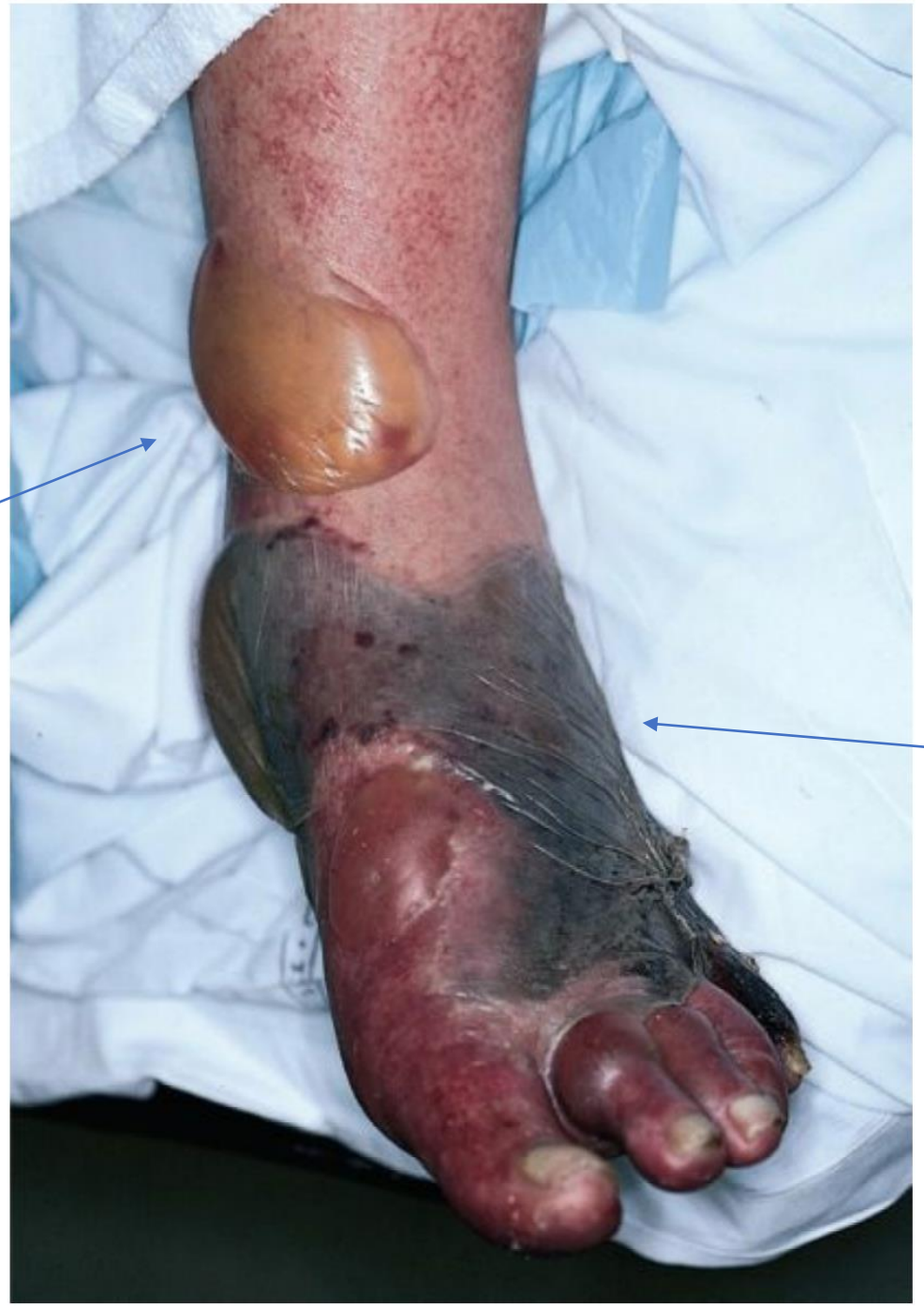
3. *Clostridium perfringens*

- *C. perfringens* causes clinical forms,
- In wounds = gas gangrene
- If ingested = food poisoning

1- Gas Gangrene

- **Gas gangrene** (myonecrosis, necrotizing fasciitis) is one of the two diseases caused by *C. perfringens*
- Gas gangrene is also caused by other histotoxic clostridia such as *Clostridium histolyticum, Clostridium septicum, Clostridium novyi*, and *Clostridium sordellii*.
- *C. sordellii* also causes toxic shock syndrome in postpartum and postabortion women.

A large gas- and fluid-filled bulla is seen near the ankle



large area of necrosis on lateral aspect of foot. Necrosis is mainly caused by lecithinase produced by Clostridium perfringens.

Gas in tissue is a feature of gangrene produced by these anaerobic bacteria

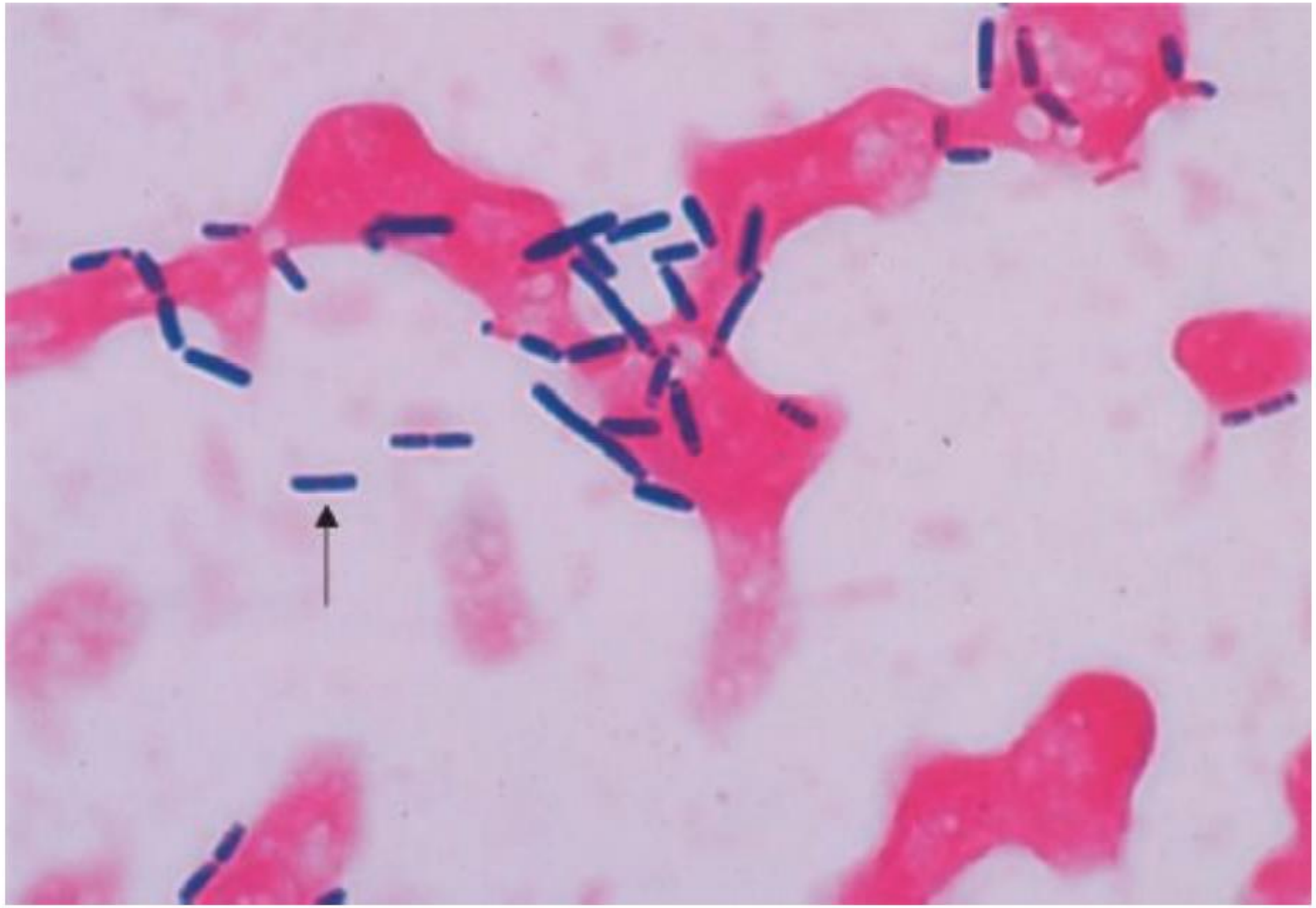
- Transmission :
- Spores are located in the soil (surprise); HOWEVER = normal vegetative cells part normal flora of the colon and vagina (**also 3% of people carry *C. diff*, but no one should carry tetani or botulism**).
- Gas gangrene is associated with **war wounds, road traffic accidents, diabetic foot and septic abortions** (endometritis).
- Pathogenesis :
- Organisms grow in traumatized tissue (especially muscle where oxygen is less!) and produce a variety of toxins (notice so far, all these organisms cause illness by exotoxin production).
- The most important is alpha toxin (lecithinase), which damages cell membranes, including those of erythrocytes, resulting in hemolysis. Degradative enzymes produce gas in tissues

Clinical Findings

- Pain, edema, cellulitis, and gangrene (necrosis) occur in the wound area
- Crepitation indicates the presence of gas in tissues.
- Hemolysis and jaundice are common, as are blood-tinged exudates.
- Shock and death can ensue.
- Mortality rates are high

Laboratory Diagnosis

- Microscopy of exudate from wounds will show large Gram-positive rods.
- Spores are not usually seen because they are formed primarily under nutritionally deficient conditions.
- The organisms are cultured anaerobically and then identified by sugar fermentation reactions and organic acid production. *C. perfringens* colonies exhibit a double zone of hemolysis on blood agar.
- Egg yolk agar is used to demonstrate the presence of the lecithinase. Serologic tests are not useful.



E 17–3 *Clostridium perfringens*—Gram stain. Arrow points

Treatment and Prevention:

- Penicillin G is the antibiotic of choice for Treatment and prevention.
- Wounds should be debrided as a treatment and prophylactic option.

Disease: Food Poisoning, caused by *C. perfringens*.

- Transmission:
- The heat-resistant spores survive cooking and germinate, the contaminate food from the soil.
- The organisms grow to large numbers in **reheated** foods, especially meat dishes.

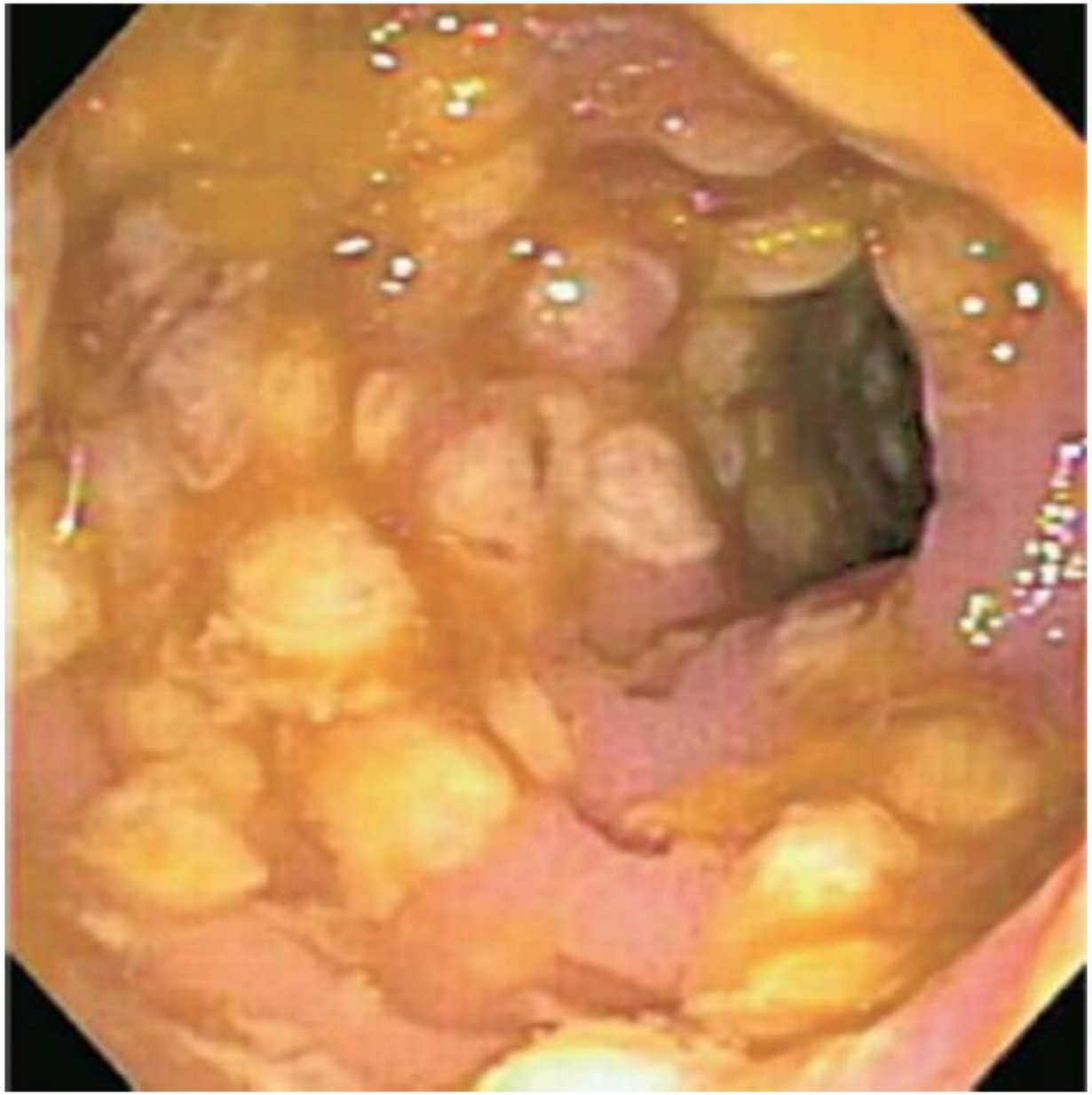
- Pathogenesis:
- *C. perfringens* is a member of the normal flora in the colon (large bowel) **but not in the small bowel**, where the enterotoxin acts to cause diarrhea.
- *The mode of action of the enterotoxin is the same as that of the enterotoxin of S. aureus, and exotoxin 2 of B. cereus (acts as a superantigen).*

- **Clinical Findings:**
- The disease has an 8- to 16-hour incubation period and is characterized by watery diarrhea with cramps and little vomiting.
- Incubation period is essentially the time needed for the toxin to reach the site of action (small bowel), no vomiting since it doesn't act on the stomach,
- It spontaneously resolves in 24 hours.
- **Laboratory Diagnosis:**
- Not usually done, we can find the organism in number from the uneaten contaminated food.
- **Treatment:**
- Symptomatic (supportive) treatment only
- **Prevention:**
- adequate cooking of food, especially reheating

4. *Clostridium difficile*

- Disease:
- *C. difficile* causes **antibiotic-associated pseudomembranous colitis**
- *C. difficile* is the most common nosocomial (hospital-acquired) cause of diarrhea.
- Remember this is caused primarily due to the overuse of broad spectrum antibiotics

Pseudomembranous colitis



yellowish plaquelike lesions in colon. Caused by an exotoxin produced by *Clostridium difficile* that inhibits a signal transduction protein, leading to death of enterocytes

Levinsons, figure 17-6 p 319

Transmission

- 3% of in the community carry this organism
- 30% in hospitalized patients.
- Since 97% of people are not colonized, they will not develop pseudomembranous colitis, in the hospital however, 30% of people are at risk after use of broad specturm antibiotics.
- Actual transmission is by fecal–oral route.
- The hands of hospital personnel are important intermediaries (prevention is aimed here).

Pathogenesis

- Antibiotics suppress drug-sensitive members of the normal flora, allowing *C. difficile* to multiply and produce exotoxins A and B.
- Both exotoxin A and exotoxin B are glucosyltransferases, they glucosylate Rho GTPases proteins that control the actin filaments.
- The main effect of exotoxin B in particular is to cause depolymerization of actin, resulting in a loss of cytoskeletal integrity, apoptosis, and death of the enterocytes.

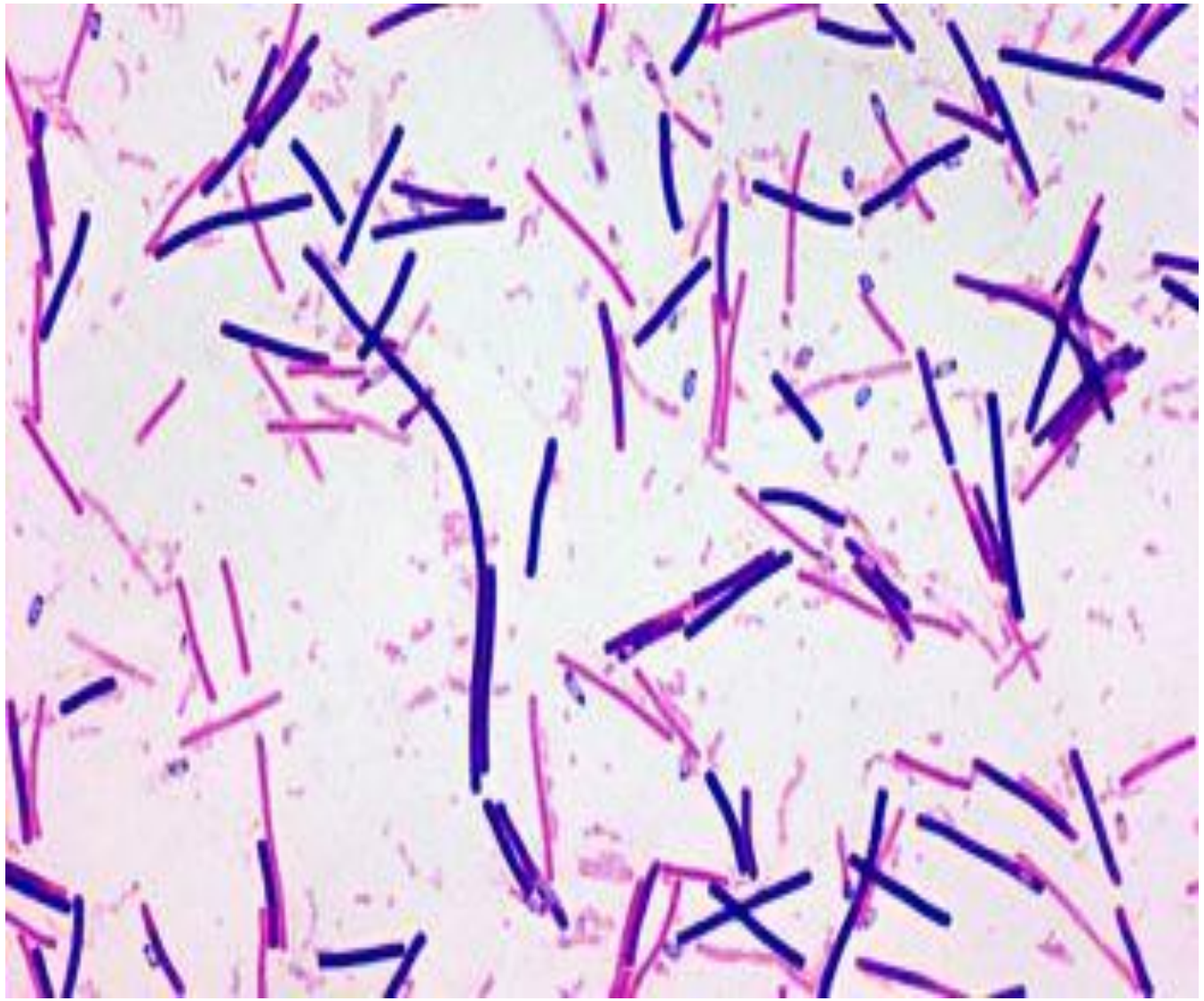
- **Clindamycin** was the first antibiotic linked as a cause of pseudomembranous colitis.
- **Third-generation cephalosporins** (target G-ve mostly and anaerobes) the most common cause (commonly used drugs).
- Ampicillin and fluoroquinolones are also commonly implicated.
- In addition to antibiotics, cancer chemotherapy also predisposes to pseudomembranous colitis.
- *C. difficile* rarely invades the intestinal mucosa

Clinical Findings

- Diarrhea (non bloody with neutrophils seen in 50% of cases) + pseudomembranes (yellow-white plaques seen in colonoscopy) on the colonic mucosa.
- Fever and abdominal pain often occur.
- Toxic megacolon (enlarged swollen colon) can occur, and surgical resection of the colon may be necessary.
- To differentiate pseudomembranous colitis from transient post antibiotic therapy diarrhea, the *C. diff* toxin must be isolated from the stool

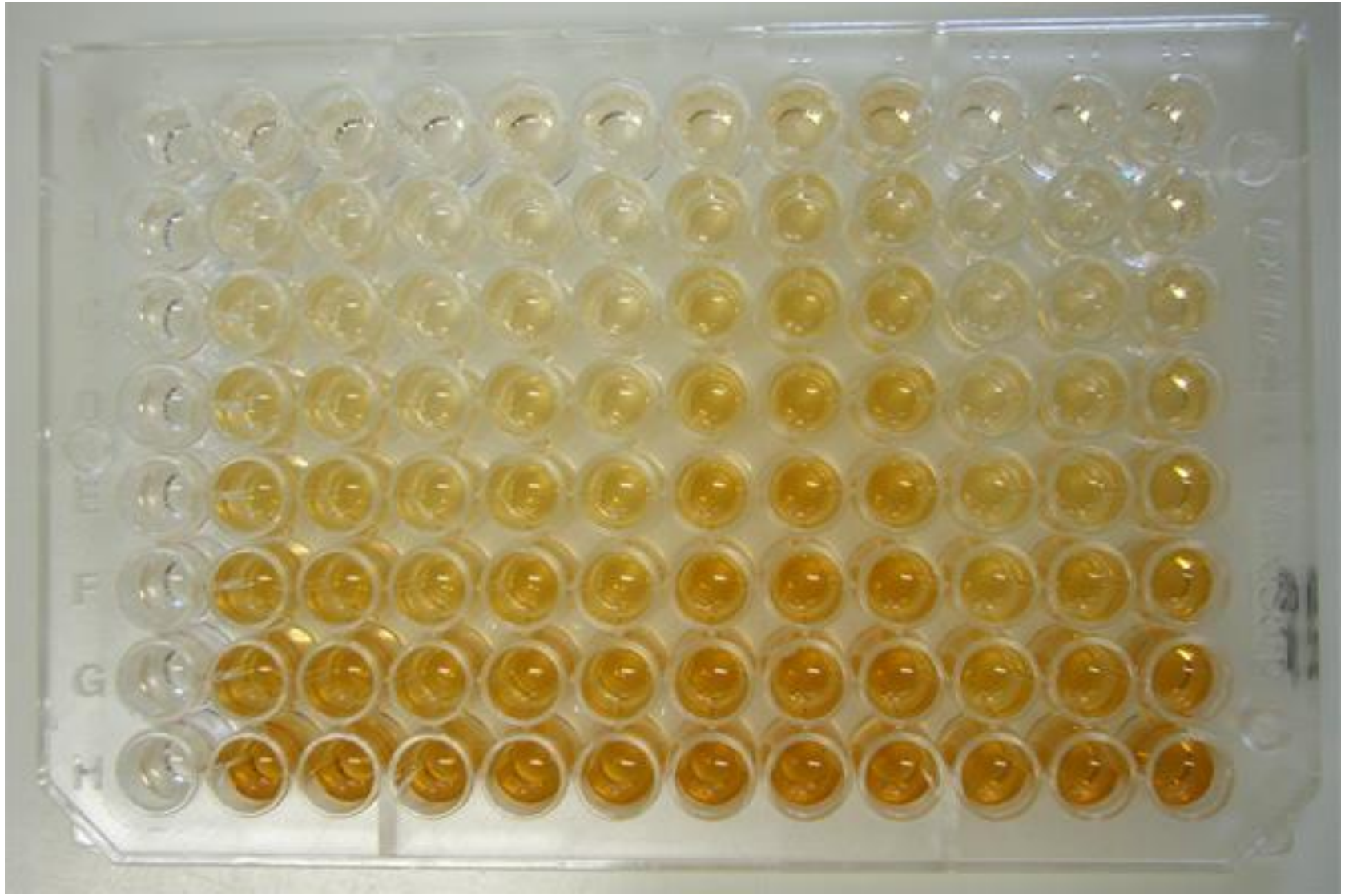
Laboratory Diagnosis

- As mentioned the basis of Dx is to find (filtrate) the exotoxin in stool.
- We cannot only depend on culture of *C. difficile* (due to the fact that some people are asymptomatic carriers and any diarrhea or GI symptom may not necessarily be caused by it)
- It would take time to link the culture to exotoxin, and thus we just go for the exotoxin



Exotoxin detection

- There are two types of tests usually used to detect the exotoxins.
- ELISA (antibody against exotoxin) The ELISA tests are rapid but are less sensitive than the cytotoxicity test.
- Cytotoxicity test (we use cultured human cells and expose them to the patients stool FILTRATE and observe the actin filament destabilization. It is more sensitive and specific but takes more time (up to 2 days!), we have to also use an antitoxin on the another culture and observe protection effect.
- We can use PCR to detect the gene for the exotoxin



https://upload.wikimedia.org/wikipedia/commons/a/a9/ELISA.jpg

Treatment

- The antibiotic causing the diarrhea should be stopped and replaced with metronidazole > vancomycin (we use it less to reduce the chance of VRE)

- Fluid loss should be replaced

- However, in life threatening cases, vancomycin should be used because it is more effective than metronidazole (we may even have to resect the colon in these extreme cases Life>colon)

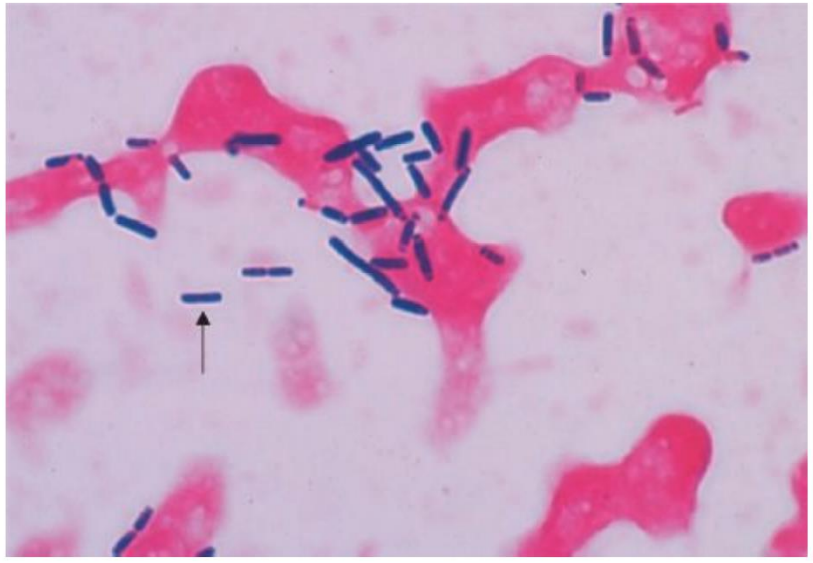
- Carrier state is not removed in many patients (recurrence of disease ensues).

- Fidaxomicin (Dificid) is both treatment and preventative measure, also can be used in extreme cases

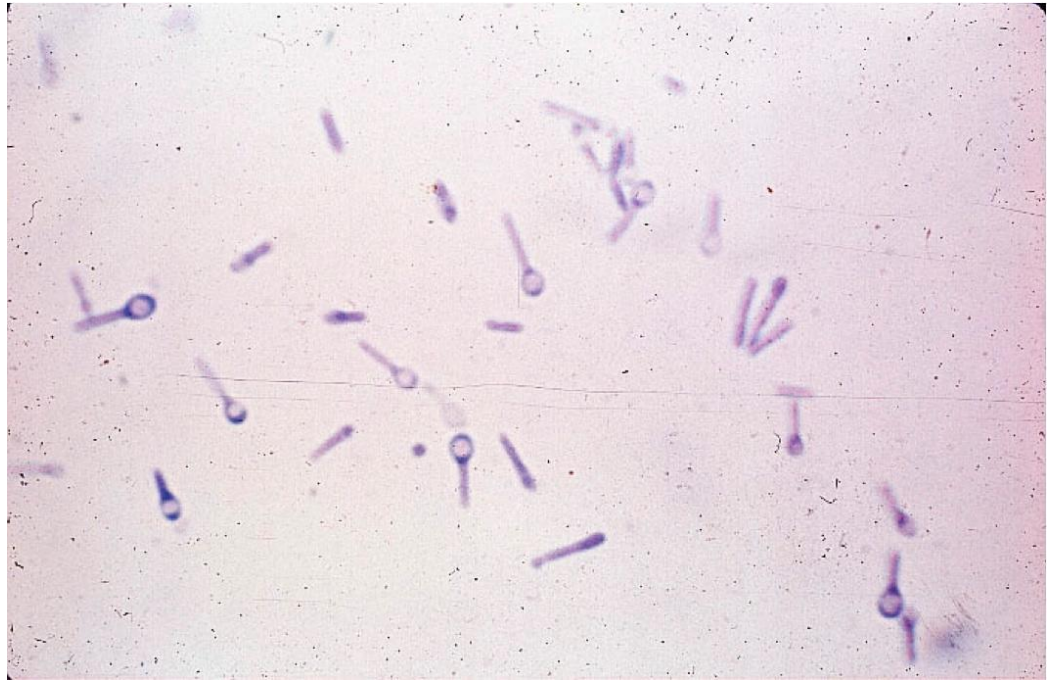
- Fecal bacteriotherapy approach can be used to replace the normal flora (which we take from a normal patient like a transplant). Very high cure rates are claimed for this approach but safety is a major concern.

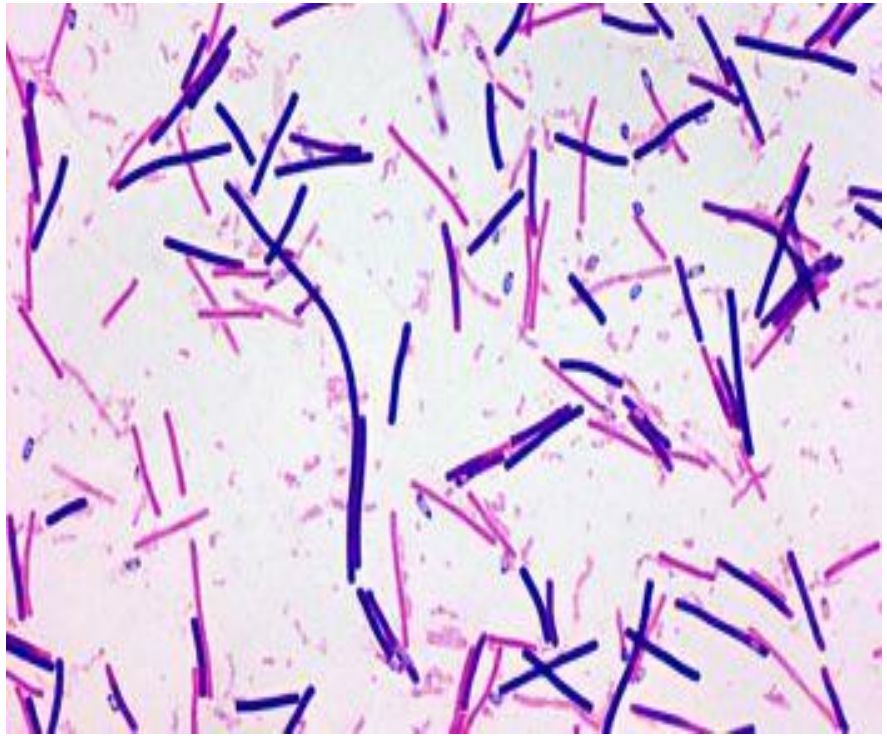
Prevention

- We don’t have vaccines or antimicrobial to prevent
- Antibiotic control: especially in the hospital setting is becoming the standard approach to control C diff.
- Infection control procedures (we should not have 3% to 30%) →rigorous handwashing.
- Probiotics such as Lactobacillus, Bifidobacterium, or the yeast Saccharomyces may be useful to prevent pseudomembranous colitis.



E 17–3 *Clostridium perfringens*—Gram stain. Arrow points





NON–SPORE-FORMING GRAM-POSITIVE RODS

- There are two important pathogens in this group:

- 1 *Corynebacterium diphtheriae*
- *2 Listeria monocytogenes*

Organism	Type of Pathogenesis	Typical Disease	Predisposing Factor	Mode of Prevention
C. diphtheriae	Toxigenic	Diphtheria	Failure to immunize	Toxoid vaccine
L. monocytogenes	Pyogenic	Meningitis; sepsis	Neonate; immunosuppression	No vaccine; pasteurize milk products

CORYNEBACTERIUM DIPHTHERIAE

- *C. diphtheriae* causes diphtheria

- Other Corynebacterium species (diphtheroids) are implicated in opportunistic infections.

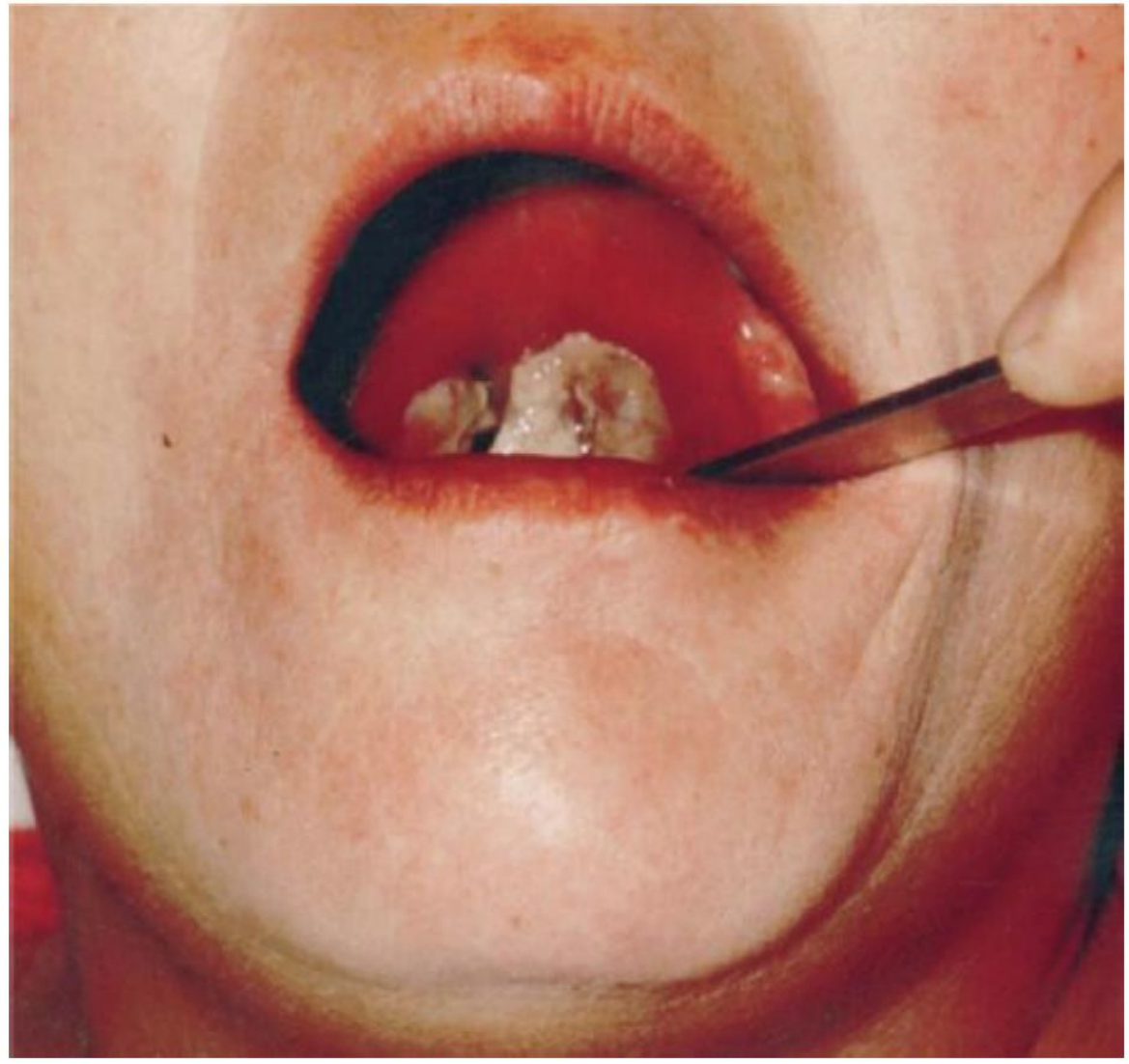
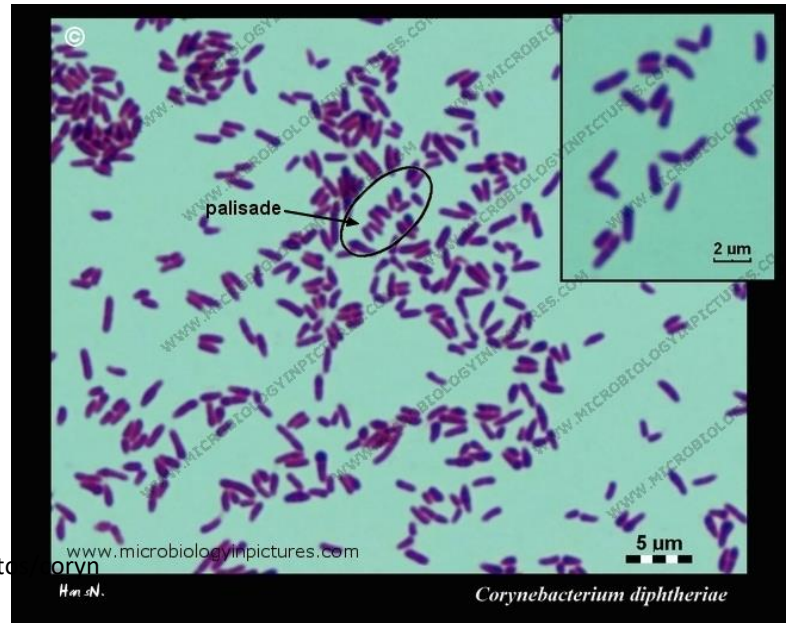


FIGURE 17–7 Diphtheria. Note whitish-gray pseudomembrane covering posterior pharynx and marked inflammation of palate and pharynx. Caused by diphtheria toxin, an exotoxin that inhibits protein synthesis by inhibiting elongation factor-2. (Courtesy of Dr. Peter Strebel.)

Important Properties

- Corynebacteria , club shaped Gram positive rods (wider at one end) and are arranged in palisades or in V- or L-shaped formations (or chinse letters)
- The rods have a beaded appearance. The beads consist of granules of highly polymerized polyphosphate—a storage mechanism for high-energy phosphate bonds.
- The granules stain metachromatically (i.e., a dye that stains the rest of the cell blue will stain the granules red).



https://www.microbiologyinpictures.com/bacteria%20photos/corynebacterium%20diphtheriae%20photo...oryn
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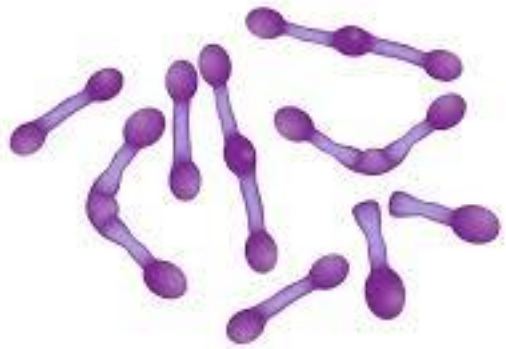
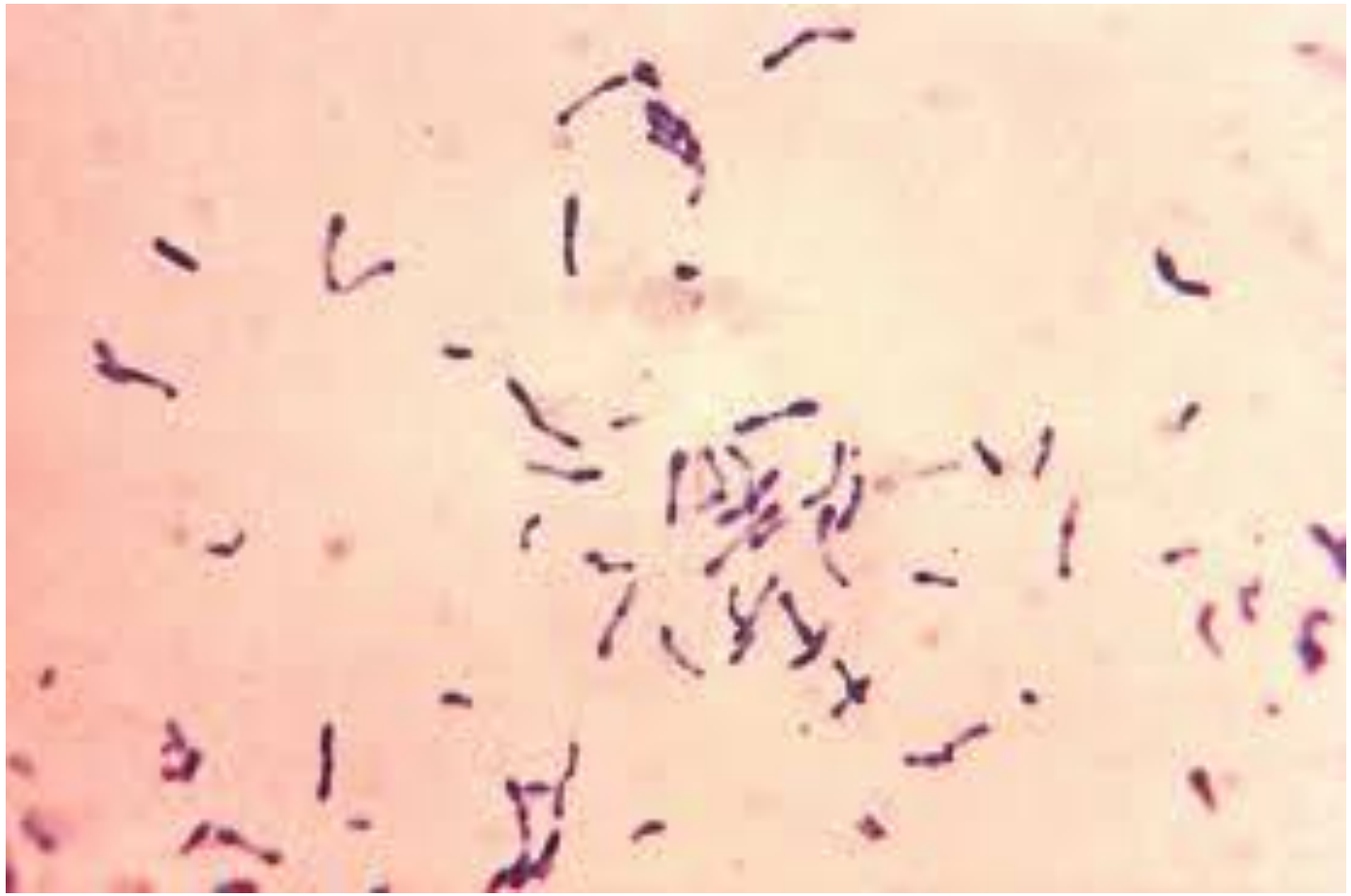
Club Shaped G+Ve rods



Corynebacterium diphtheriae



http://www.dovemed.com/diseases-conditions/diphtheria/

https://www.google.jo/imgres?imgurl=https%3A%2F%2Fupload.wikimedia.org%2Fwikipedia%2Fcommons%2Fthumb%2F0%2F06%2FCorynebacterium_diphtheriae_Gram_stain.jpg%2F1200px-Corynebacterium_diphtheriae_Gram_stain.jpg&imgrefurl=https%3A%2F%2Fen.wikipedia.org%2Fwiki%2FCorynebacterium_diphtheriae&docid=khth4_c1qMetCM&tbnid=QZibeFEPsW87gM%3A&vet=10ahUKEwjh5qP23KnXAhVJlxoKHaY9Cm8QMwiSASgAMAA..i&w=1200&h=796&bih=711&biw=870&q=corynebacterium%20diphtheriae&ved=0ahUKEwjh5qP23KnXAhVJlxoKHaY9Cm8QMwiSASgAMAA&iact=mrc&uact=8

Transmission

- Humans are the only natural host of *C. diphtheriae*
- Both toxigenic and nontoxigenic organisms reside in the upper respiratory tract and are transmitted by airborne droplets (similar to other respiratory pathogens).
- The organism can also infect the skin at the site of a preexisting skin lesion.
- This occurs primarily in the tropics but can occur worldwide in indigent persons with poor skin hygiene.

Pathogenesis

- Mainly exotoxin mediated (similar to other G+ve rods), however, the bug must establish itself in the throat first (invasiveness) prior to exotoxin production.
- Diphtheria toxin inhibits protein synthesis by ADP-ribosylation of elongation factor-2 (EF-2) used to maintain elongation of the peptide chain = no protein synthesis in eukaryotic cell.
- Similar to other toxins it is formed in an A- B fashion (active/binding)
- As mentioned, the toxin is encoded on a gene transmitted by transduction on a temperate phage

- The host response to *C. diphtheriae* consists of the following:
- (1) A local inflammation in the throat which forms fibrinous exudate that gives the characteristic tough, adherent, gray pseudomembrane
- (2) Antibody production against the exotoxin , which hinders the exotoxin activity by blocking the interaction of the binding domain (the B in the A-B config) with the receptors (no binding = no cell entry)

Clinical Findings/complications

- Diphteria is rare now thanks to vaccines, however we should be aware of the thick throat psuedomembrane
- The other aspects are: *nonspecific fever, sore throat, and cervical adenopathy*. There are three prominent complications:
- (1) Extension of the membrane into the larynx and trachea, causing airway obstruction.
- (2) Myocarditis accompanied by arrhythmias and circulatory collapse.
- (3) Nerve weakness or paralysis, especially of the cranial nerves.

- Paralysis of the muscles of the soft palate and pharynx can lead to regurgitation of fluids through the nose.
- Peripheral neuritis affecting the muscles of the extremities also occurs.
- Cutaneous diphtheria causes ulcerating skin lesions covered by a gray membrane.
- These lesions are often indolent and often do not invade surrounding tissue. Systemic symptoms rarely occur.

Laboratory Diagnosis

- For diphtheria we need to show the presence of the **organism and production of the toxin** (due to presence of atoxigenic strains).
- Due to the quick nature of toxin mediated disease, the decision to treat with an antitoxin should be clinical and not wait for lab confirmation.
- A throat swab should be cultured on Loeffler's medium (cream colored colonies are shown in the slant) , a tellurite plate (black colonies seen a tellurium salt that is reduced to elemental tellurium within the organism thus black colored colonies), and a blood agar plate.
- The typical gray-black color of tellurium in the colony is a telltale diagnostic criterion.
- If *C. diphtheriae* is recovered from the cultures then we can confirm toxin (either animal inoculation, antibody-based gel diffusion precipitin test or PCR test for the presence of the gene).

http://www.medical-labs.net/wp-content/uploads/2014/05/b350-3-Corynebacterium-diphtheriae-on-tellurite.jpg

Loefflers medium enchances staining of metachromatic granules and helps diagnosis esp with methylene blue stain

- Smears of the throat swab should be stained with both Gram stain and methylene blue.
- Although the diagnosis of diphtheria cannot be made by examination of the smear, the finding of many tapered, pleomorphic Gram-positive rods can be suggestive.
- The methylene blue stain is excellent for revealing the typical metachromatic granules (the club shape is due to these granules).

Treatment

- 1)ANTITOXIN) The treatment of choice is **antitoxin**, which should be given **immediately** on the basis of **clinical impression** (not on lab confirmation, this takes while to get both isolation of organism and detection of toxin)
- The need for immediate treatment with antitoxin is due to the toxin's RAPID and IRREVERSIBLE action on cells, thus antitoxin will work on unbound toxin in the blood only
- 2) ANTIBIOTICS) Treatment with penicillin G or erythromycin is **also recommended** with antitoxin but not as a substitute.
- Antibiotics will reduce bacterial count and thus toxin production, they will also reduce the chance of a carrier state – since it requires being established before producing toxin.

Prevention

- Diphtheria is now rare in the world due to its inclusion in the scheduled vaccine regiment (DTaP) with diphtheria toxoid.
- **In warzones or areas with lapse in immunization, reemergence (and atypical symptoms) are on the rise**
- formaldehyde treatment of the toxin, destroys the toxin but leaves intact the antigenicity
- Immunization consists of three doses given at 2, 4, and 6 months of age, with boosters at 1 and 6 years of age.
- Because immunity wanes, a booster every 10 years is recommended.
- Immunization does not prevent nasopharyngeal carriage of the organism.

LISTERIA MONOCYTOGENES

- Diseases :
- *L. monocytogenes* has similar spectrum of illness to other respiratory pathogens, namely sepsis and meningitis.
- However, it is known to be dangerous and targeting newborn, pregnant women and immunocompromised patients
- It is mainly transmitted on contaminated **food** (vegetables and poorly cooked meats), thus it can cause febrile gastroenteritis
- It is a major cause of concern for the food industry (along with *Staph, botulism, Shigella, Salmonella, C perfringens* and *E. coli,* and *norvovirus*).

Important Properties:

- *L. monocytogenes* is a small Gram-positive rod arranged in V- or L-shaped formations similar to corynebacteria.
- The organism exhibits an unusual tumbling movement that distinguishes it from the corynebacteria, which are nonmotile.
- Colonies on a blood agar plate produce a narrow zone of β-hemolysis that resembles the hemolysis of some streptococci.
- Listeria grows well at cold temperatures, so storage of contaminated food in the refrigerator can increase the risk of gastroenteritis.
- This paradoxical growth in the cold is called "cold enhancement."

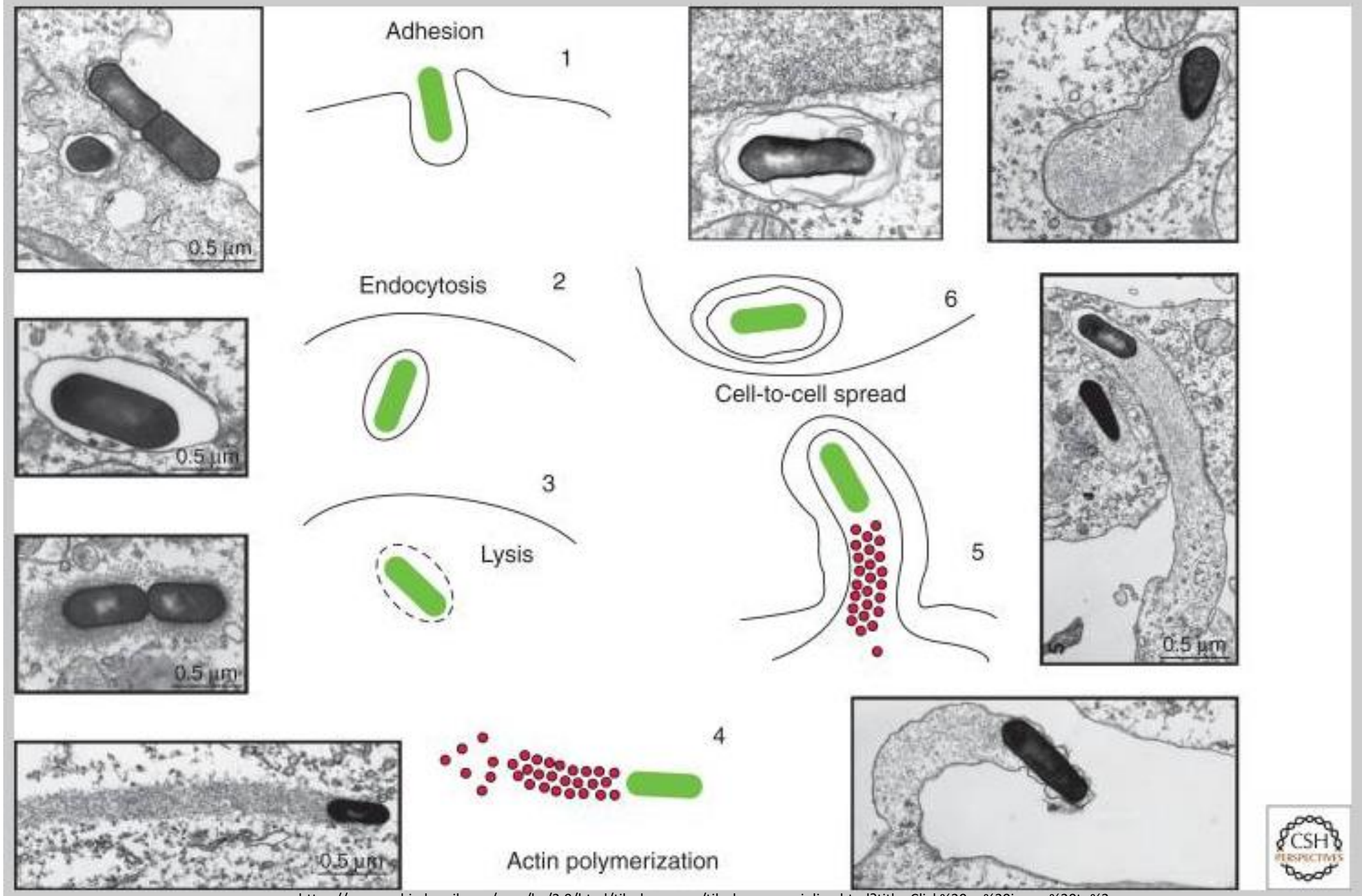

http://10minus6cosm.tumblr.com

Pathogenesis

- Listeria infections occur primarily in two clinical settings:
- (1) in **the fetus** or in a newborn as a result of transmission across the placenta or during delivery
- (2) in **pregnant** women and immunosuppressed adults, especially renal transplant patients.
- (Note that pregnant women have reduced cell-mediated immunity during the third trimester.)

- The organism is distributed worldwide in animals, plants, and soil. From these reservoirs, it is transmitted to humans primarily by ingestion of **unpasteurized milk products, undercooked meat, and raw vegetables** (its both a zoonotic concern as well as food health issue).
- Contact with domestic farm animals and their feces is also an important source.
- In the United States, listeriosis is primarily a food-borne disease associated with eating unpasteurized cheese and delicatessen meats.
- Following ingestion, the bacteria appear in the colon and then can colonize the female genital tract.
- From this location, they can infect the fetus if membranes rupture or infect the neonate during passage through the birth canal

- The pathogenesis of Listeria:
- As an **intracellular pathogen**, listeria virulence depends on its ability to escape the defenses of the cell, thrive intracellularly and then invade other cells.
- Invasion of cells is mediated by bacterial factor internalin (Listeria side) + E-cadherin (Human cell surface).
- The ability of Listeria to pass the placenta, enter the meninges, and invade the gastrointestinal tract depends on the interaction of internalin and E-cadherin on those tissues.
- Upon entering the cell, the organism produces listeriolysin,(this enzyme allows it to break away from the phagosome into the cytoplasm)
- This to clear Listeria cell-mediated immunity > humoral immunity.
- *L. monocytogenes* can move from cell to cell by means of actin rockets — filaments of actin polymerize and propel the bacteria through the membrane of one human cell and into another.



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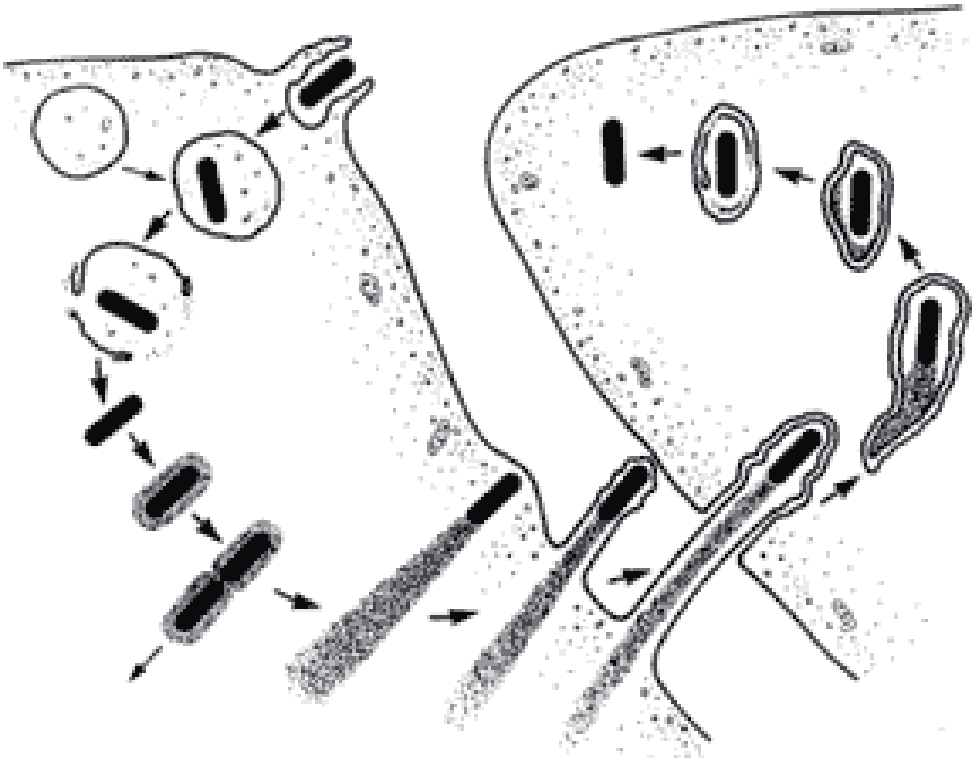
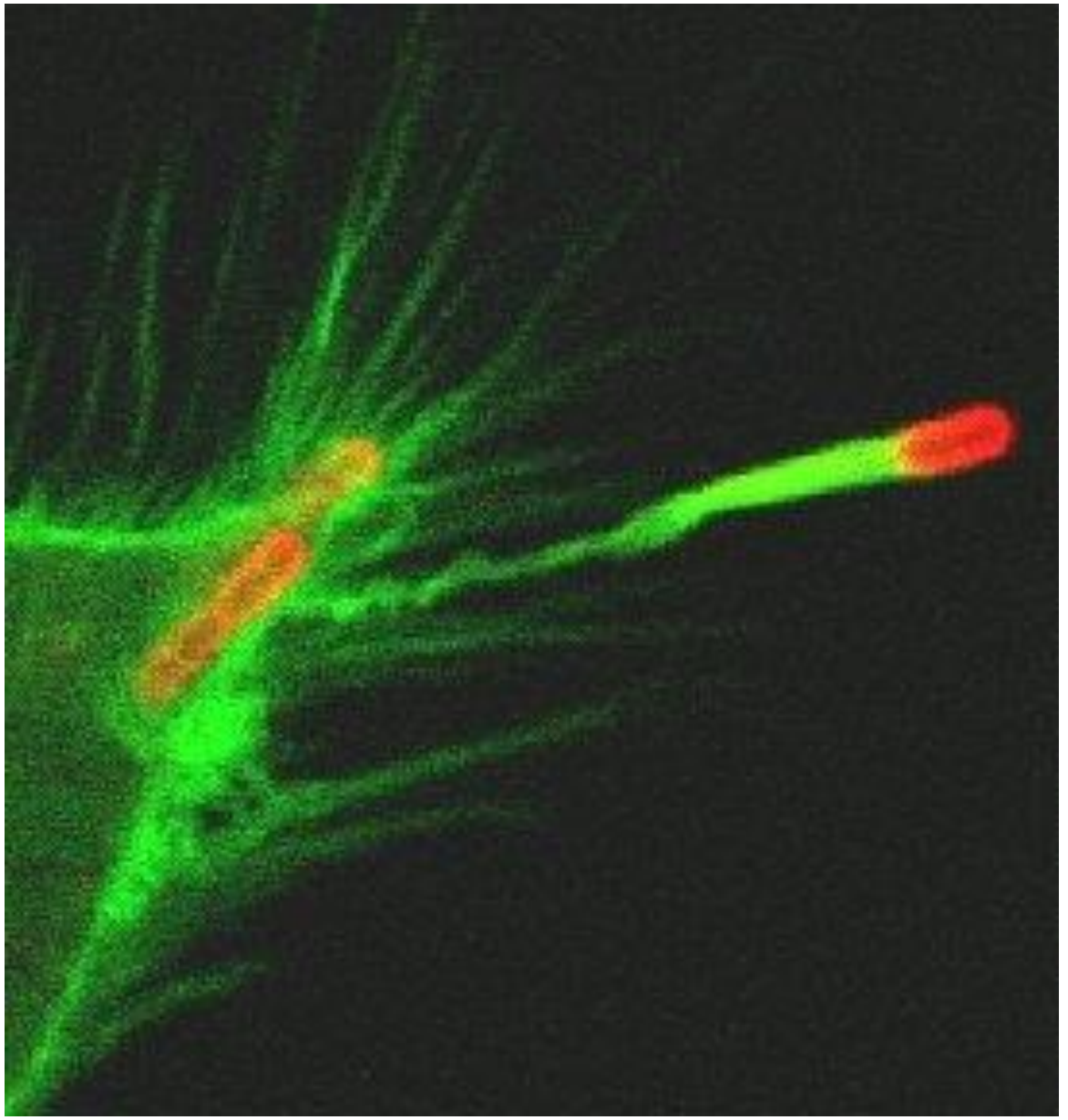


http://pages.jh.edu/~cmml/emph_ListSteps.html



http://www.sanger.ac.uk/news/view/2004-09-14-getting-a-grip-on-the-great-mimicker

Clinical Findings

- → Pregnancy infection : causes premature delivery (or abortion if its during early development), might also cause sepsis (very early on).
- → Newborn infection: picked up during delivery, presents with acute meningitis (through bacterimia) 1-4 weeks later. (very hard to detect based on the mother as she will have no symptoms or generalized flu like symptoms)
- → Adult infections: usually in immunocompromised adults can be either sepsis or meningitis.
- Gastroenteritis → can either be prodromal to sepsis or meningitis, however, self limited Gastroenteritis is also established caused by Listeria (watery diarrhea, fever, headache, myalgia, and abdominal cramps but little vomiting).
- Outbreaks are usually caused by contaminated dairy products, but undercooked meats such as chicken and hot dogs and ready-to-eat foods such as coleslaw have also been involved.

Laboratory Diagnosis

- Laboratory diagnosis is made primarily by Gram stain and culture (isolation of the organism, remember no exotoxin here).
- The appearance of gram-positive rods resembling diphtheroids, and the formation of small, gray colonies with a narrow zone of β-hemolysis on a blood agar plate suggest the presence of Listeria.
- The isolation of Listeria is confirmed by the presence of **motile organisms (tumbling motility)**, which differentiate them from the nonmotile corynebacteria.
- Identification of the organism as *L. monocytogenes* is made by sugar fermentation tests.

Treatment and prevention

- Treatment of invasive disease, such as meningitis and sepsis, consists of **trimethoprim-sulfamethoxazole**. Combinations, such as **ampicillin and gentamicin or ampicillin and trimethoprim-sulfamethoxazol**e, can also be used.
- Rare resistance, gastroenteritis is self limited and typically does not require treatment.
- Prevention:
- There is no vaccine, best practice is to limit exposure of pregnant women to to potential sources such as farm animals, unpasteurized milk products, and raw vegetables is recommended.
- Trimethoprim-sulfamethoxazole given to immunocompromised patients to prevent Pneumocystis pneumonia can also prevent listeriosis.

Diphteroids

- Diphtheroids are defined as aerobic, non-spore forming, pleomorphic Gram-positive bacilli which are more uniformly stained than *C. diphtheria*
- However they lack the metachromatic granules and are arranged in a palisade manner.
- They are usually commensals of the skin and mucous membranes.
- They differ from *C. diphtheriae* in biochemical reactions as well as in toxin production.
- Since, they are usually found as commensals on the skin, they are often considered as mere contaminants when isolated from clinical samples.
- However, there are increasing reports of these organisms being associated with various opportunistic infections (especially wounds).

End