SHEET NO.

MICROBIOLOGY (Bacteriology)

DOCTOR 2019 | MEDICINE | JU

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SCIENTIFIC CORRECTION :

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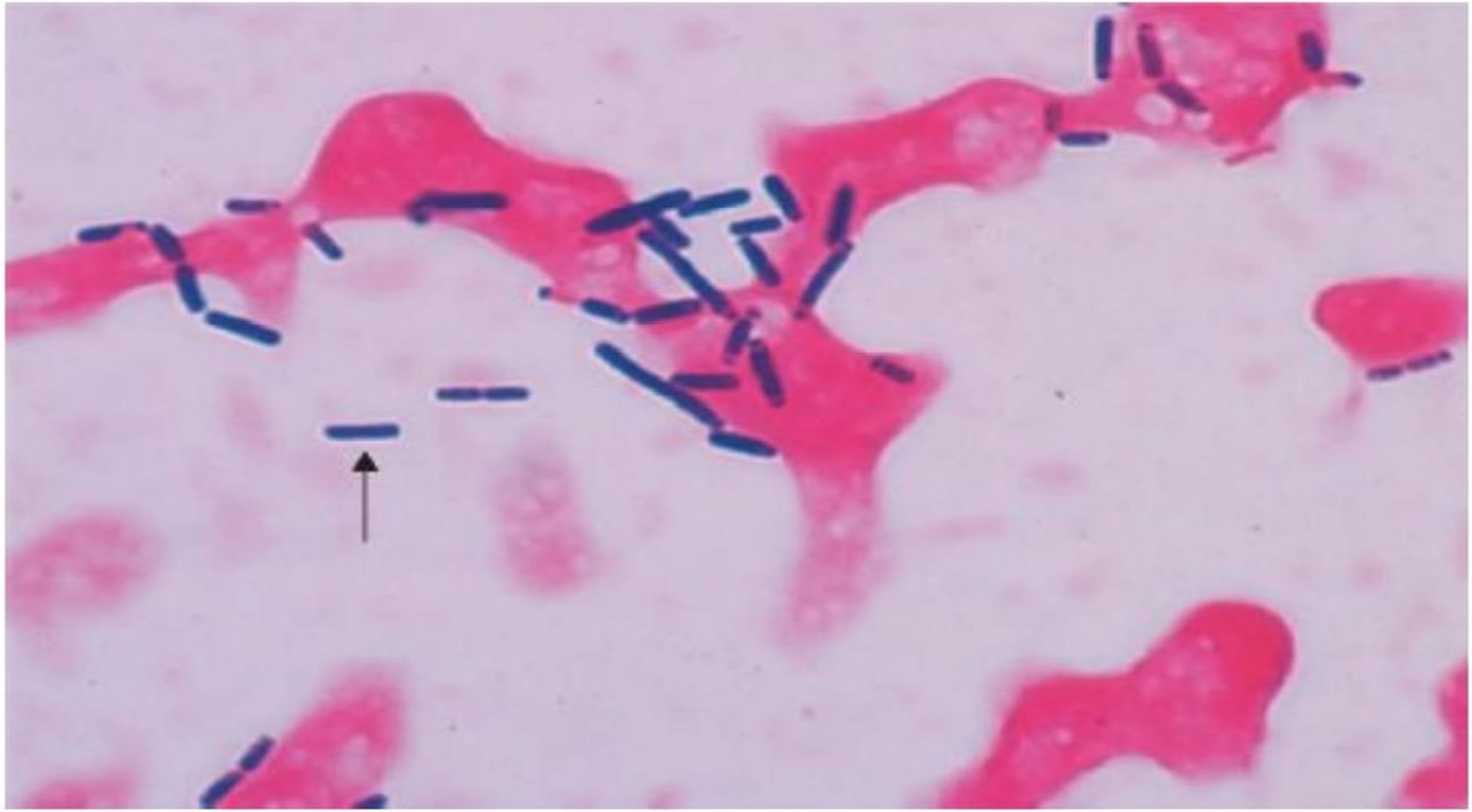


E 17–3 *Clostridium perfringens*—Gram stain. Arrow points

Treatment and Prevention:

- Penicillin G is the antibiotic of choice for Treatment and prevention.
  (their problem is not in antibiotics, but in their toxins.)
- 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.

Where a majority of water is released

- *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 (because of the abnormal spastic movement of colon and the small intestine) 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 (reheated rice will germinate the spores and then produce exotoxins which will cause the diarrhea, so if you want to reheat the rice, increase the temperature of heating to kill the bacteria.)

•4. *Clostridium difficile*

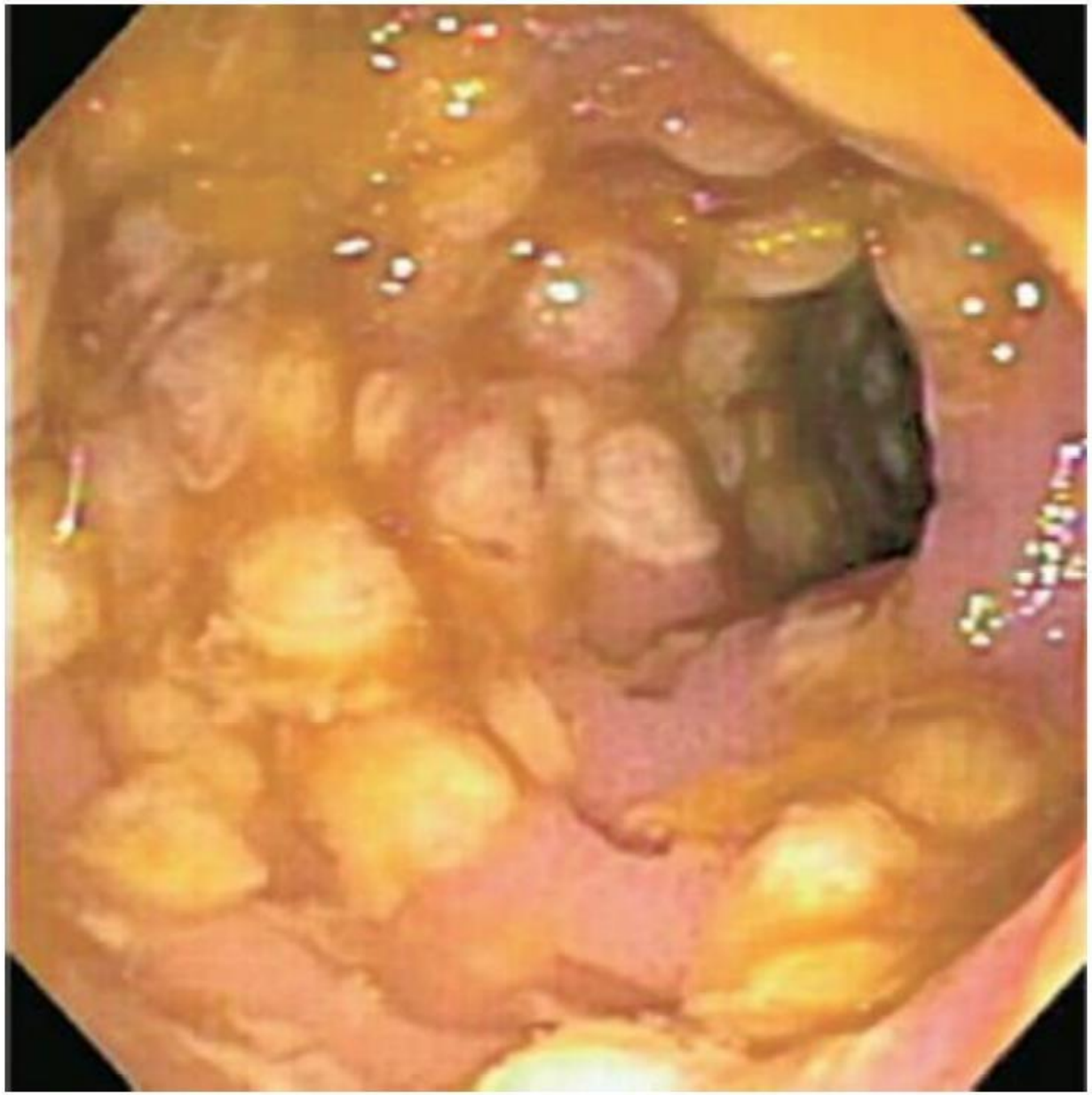
- Disease:

- *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. (and it is the first antibiotic used to destroy anaerobes)
- **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

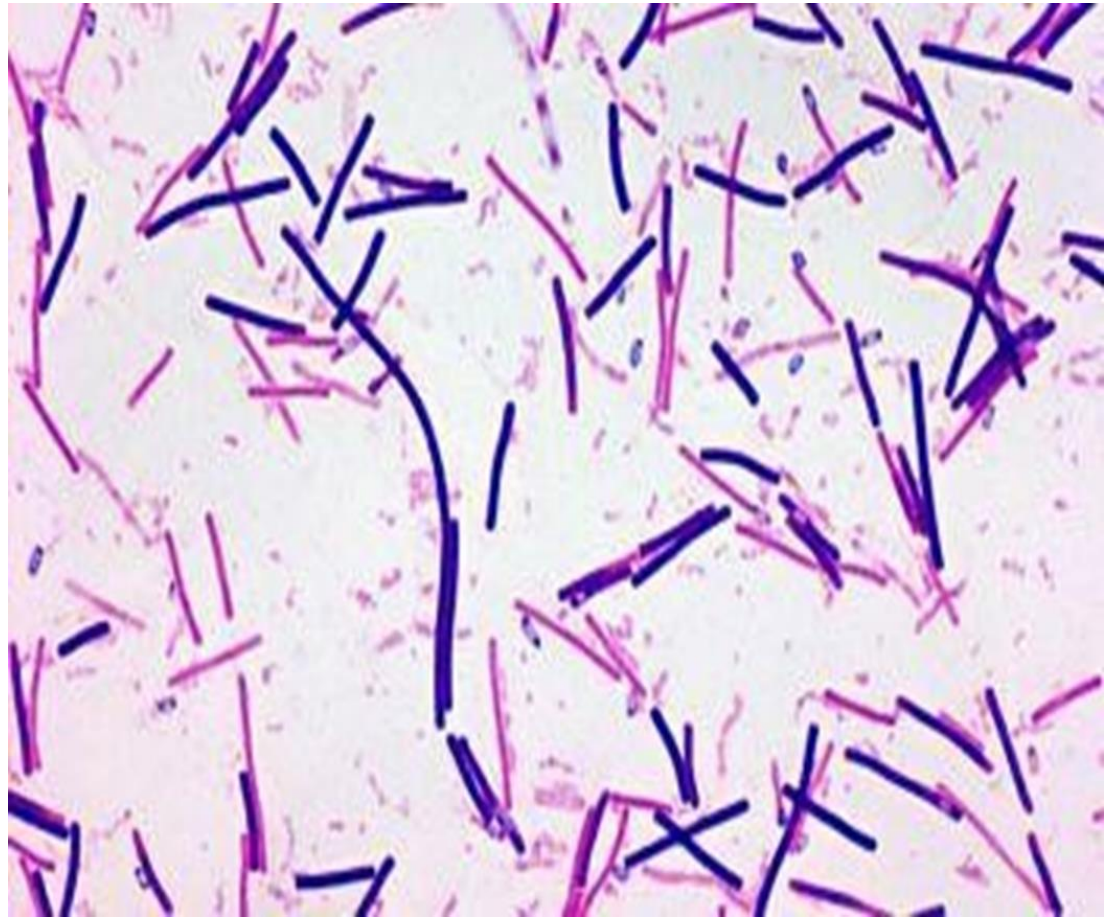
•Clinical Findings

- 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

It is self-resolved once the rebalance of normal flora is established

•Laboratory Diagnosis

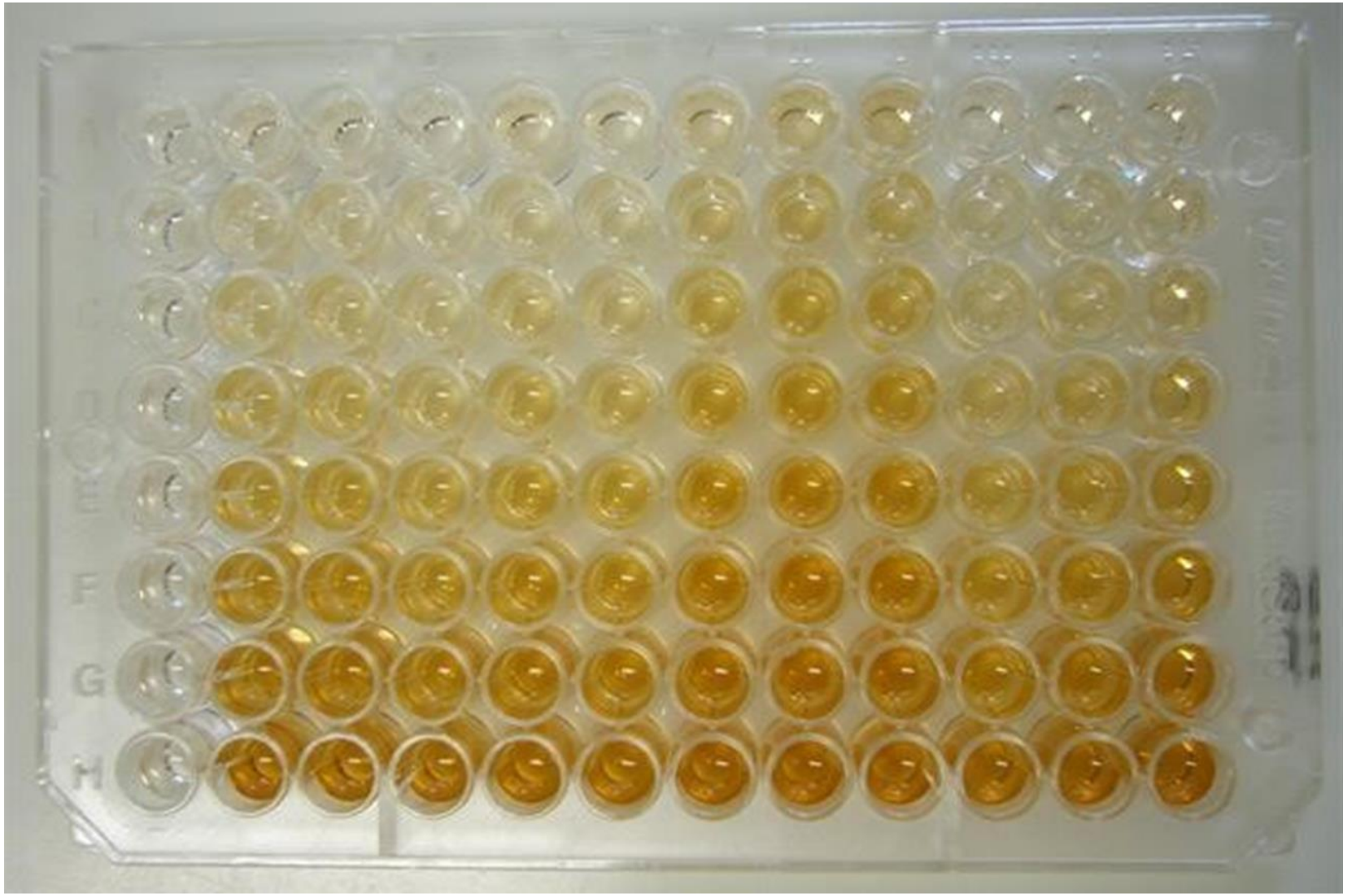
- As mentioned the basis of Dx is to find (filtrate) the exotoxin in stool.
- We cannot only depend on culture of *C. difficile* (due to the fact that some people are asymptomatic carriers and any diarrhea or GI symptom may not necessarily be caused by it)
- It would take time to link the culture to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

ELIZA test



•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)
  (vancomycin resistant enterococci)

- 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

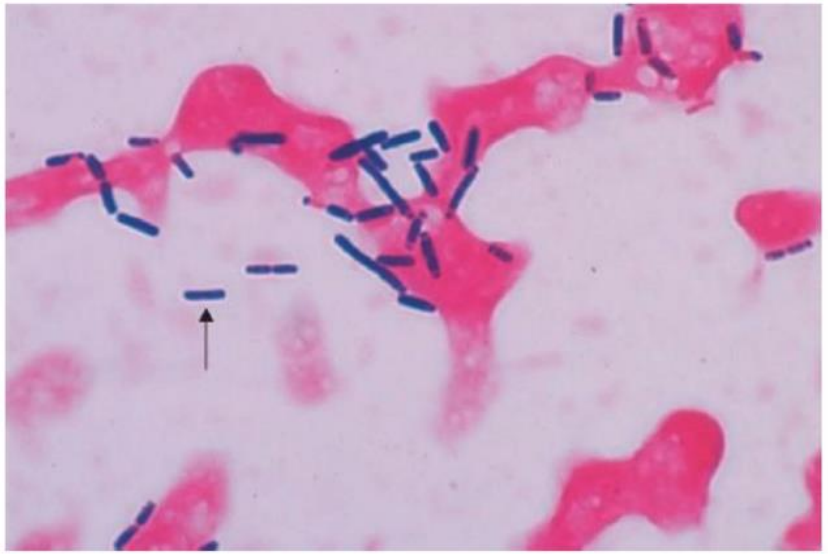
- Fecal bacteriotherapy approach can be used to replace the normal flora (we take feces from a normal patient and we seed it in an abnormal patient colon so he will carry all the normal bacteria like a transplant). Very high cure rates are claimed for this approach but safety is a major concern it is not always safe.

•Prevention

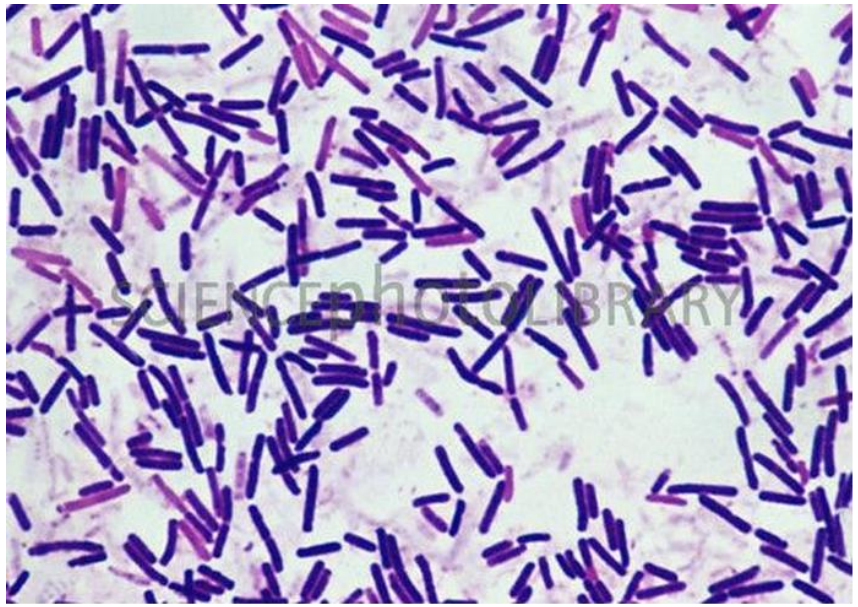
- We don’t have vaccines or antimicrobial to prevent
- Antibiotic control: especially in the hospital setting is becoming the standard approach to control C diff.

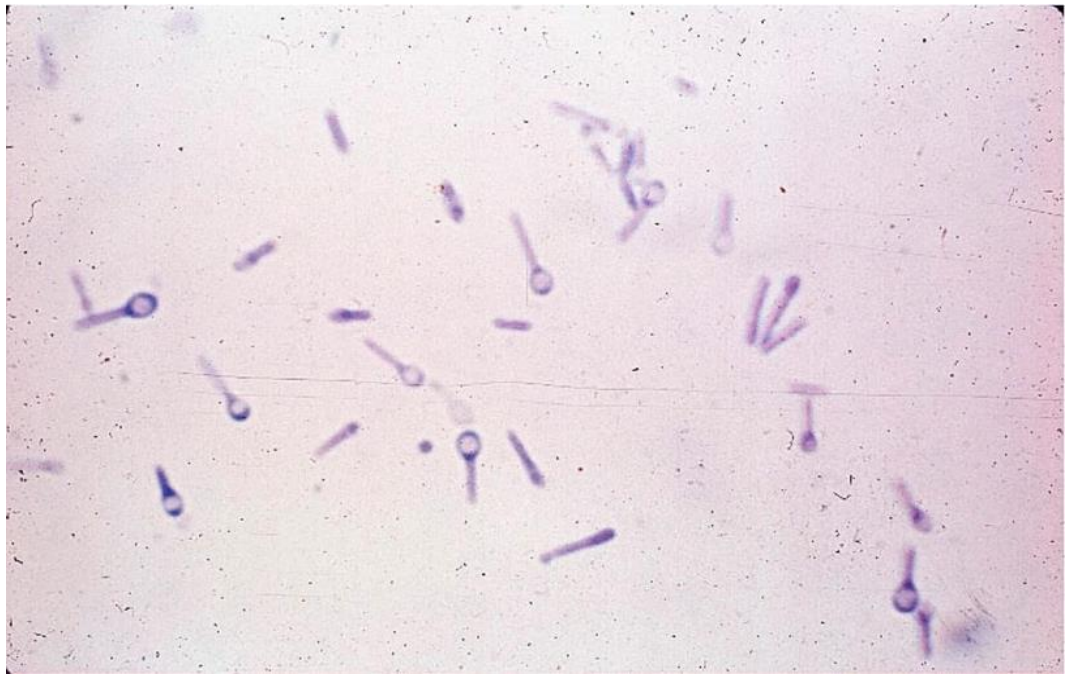
➢Infection control procedures (we should not have 3% to 30%) rigorous handwashing.

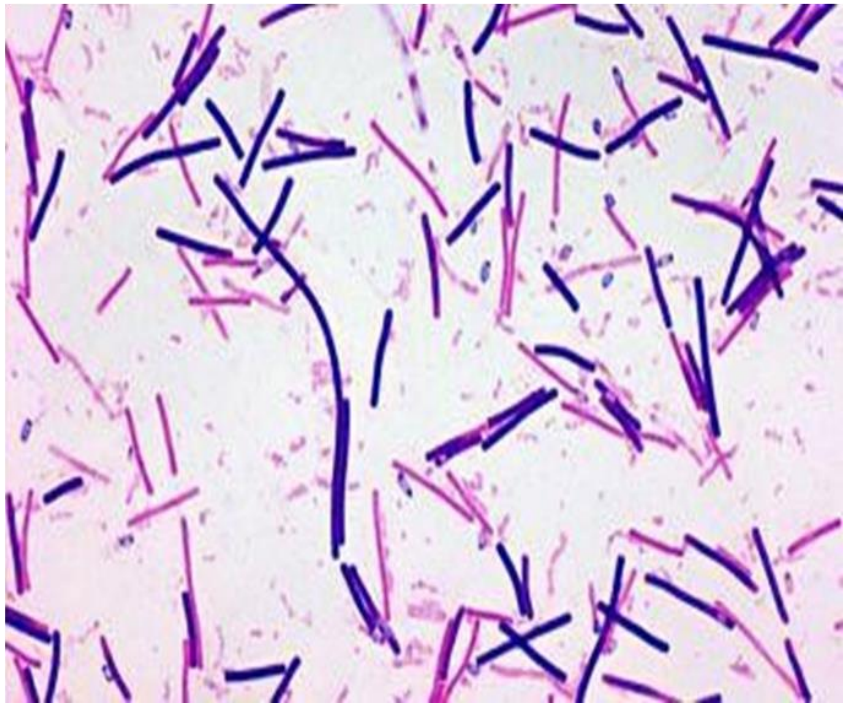
- Probiotics such as Lactobacillus, Bifidobacterium, or the yeast
- Saccharomyces may be useful to prevent pseudomembranous colitis.



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 (diphtheroid) are implicated in opportunistic infections.
    - [we see diphtheria but not cause disease bacteria , so it present as opportunistic infections.]

This whitsh gray pesudomembrane , don’t touch or remove it→ cause spasm and may kill the patient due to the spasm of larynx.
Very red= very high inflamed caused bu toxin+ endotoxin.

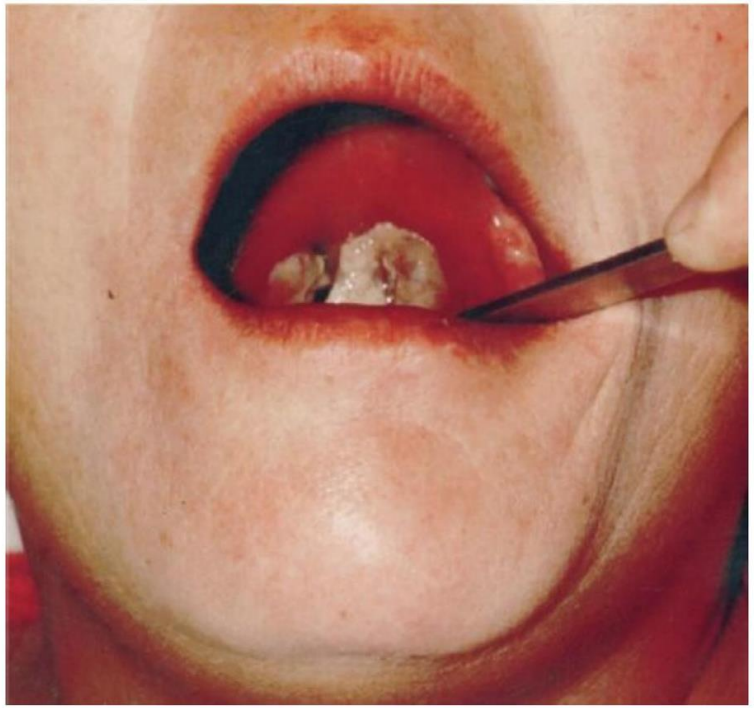
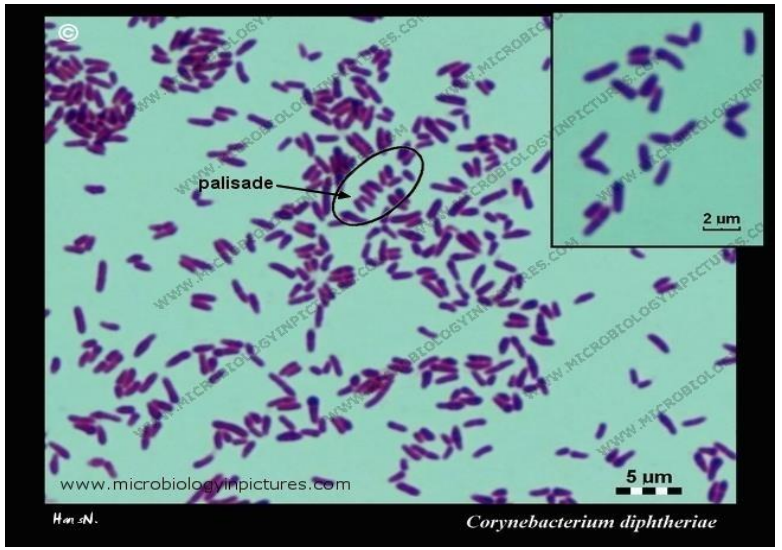


FIGURE 17–7 Diphtheria. Note whitish-gray pseudomembrane covering posterior pharynx and marked inflammation of palate and pharynx. Caused by diphtheria toxin, an exotoxin that inhibits protein synthesis by inhibiting elongation factor-2. (Courtesy of Dr. Peter Strebel.)

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 highenergy phosphate bonds.
- The granules stain metachromatically (i.e., a dye that stains the rest of the cell blue will stain the granules red).

Granules give them club shape



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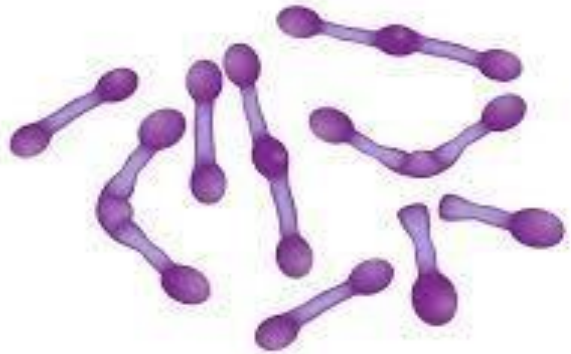
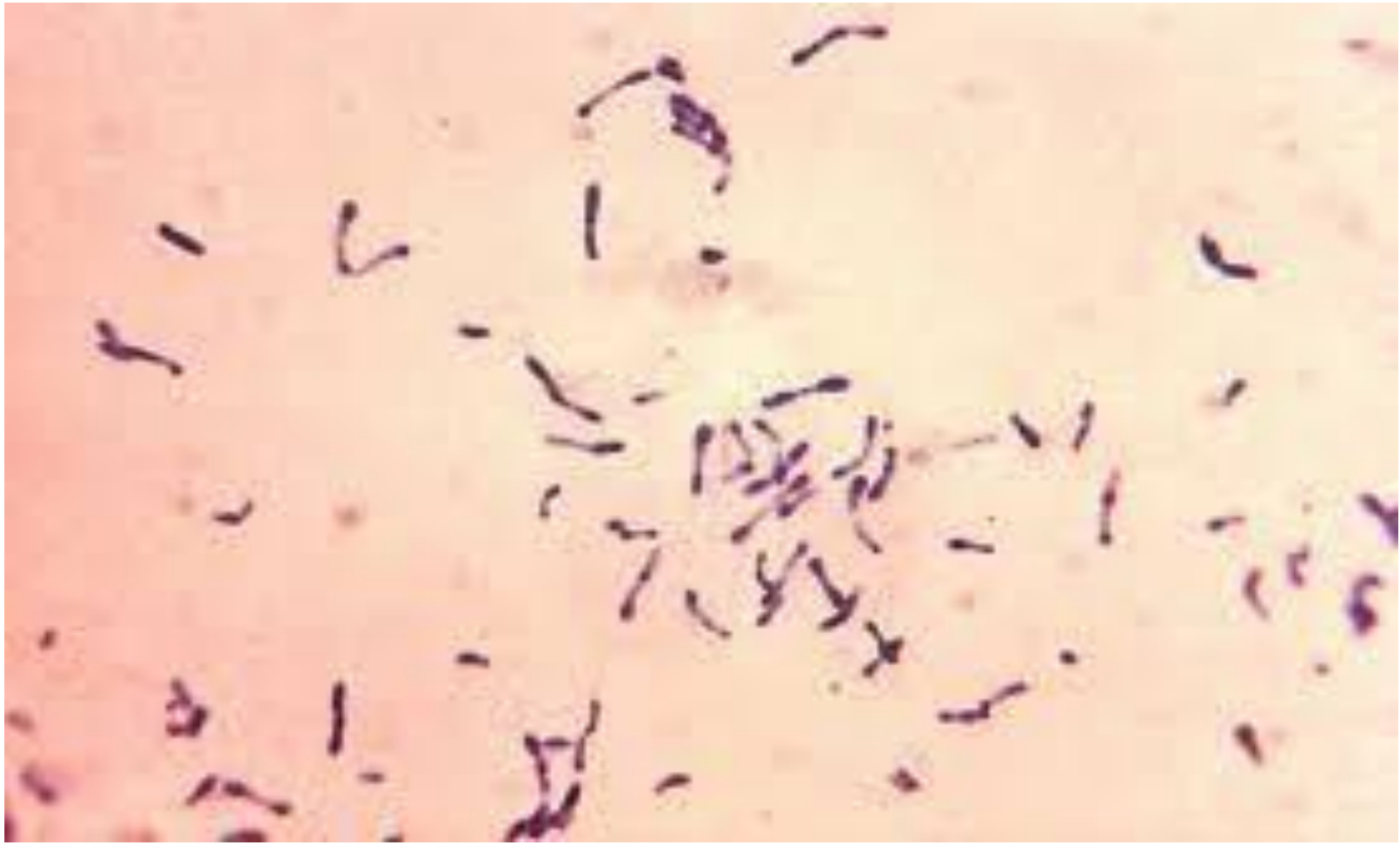
Club Shaped



Corynebacterium diphtheriae



https://www.google.ie/imgres?imgurl=https%3A%2F%2Fupload.wikimedia.org%2Fwikipedia%2Fcomm

G+Ve rods

- **Transmission**
- • **Humans** are the only natural host of C. diphtheriae
- • Both toxigenic and nontoxigenic organisms reside in the upper respiratory tract [pharynx] and are transmitted by airborne droplets [cough] (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. [that's why produce whitsh gray mem.]
- 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. → destroy the 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=no function)

•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. → children may have damage their heart [due to action of exotoxin]
- (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** (special media ,**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. [very important]
- 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).

black colonies seen in [tellurite plate.]

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

on **Loeffler's medium** (**cream** colored colonies)

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 [because alots of diphtheroid], the finding of many tapered, pleomorphic Grampositive 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)→ if you give someone antitoxin and they don't have atoxin, it's not hurt.
- The need for **immediate** treatment with antitoxin is due to the toxin's RAPID and IRREVERSIBLE action on cells→ can not back , 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→ can use in this case as antigen .
- 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

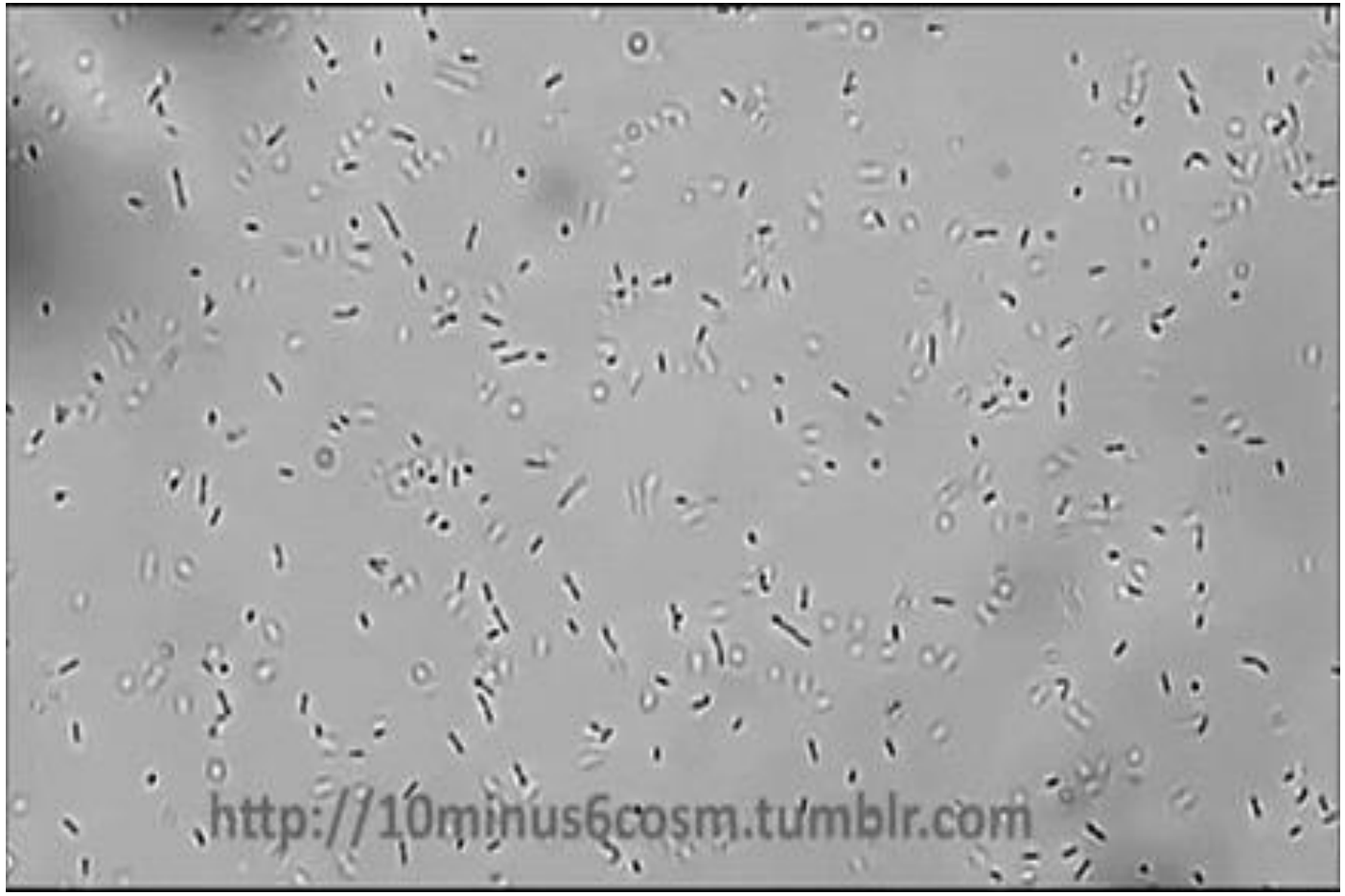
Non-spore formers

- Diseases :
- *L. monocytogenes* has similar spectrum of illness to other respiratory pathogens, namely **sepsis** and **meningitis [by invasion in blood in GIT]**.
- However, it is known to be dangerous and targeting **newborn**, **pregnant** women and **immunocompromised** patients
- It is mainly transmitted on contaminated **food** (vegetables ,daily product[non-pasteurized] 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 Lshaped formations similar to corynebacteria. / relaxed shape
- 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."**

tumbling movement



•Pathogenesis

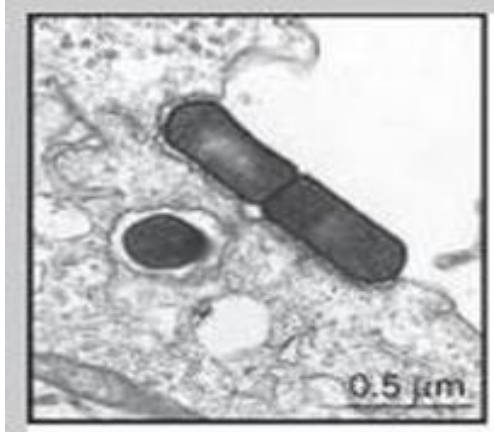
- Listeria infections occur primarily in two clinical settings:
- (1) in **the fetus** or in a newborn as a result of transmission across the placenta or during delivery
- (2) in **pregnant** women and immunosuppressed adults, especially renal transplant patients.
- (Note that pregnant women have reduced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

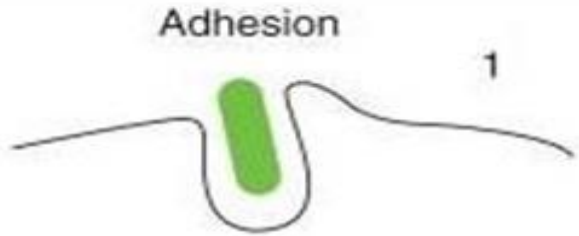
- Contact with domestic farm animals and their feces is also an important source.
- In the United States, listeriosis is primarily a food-borne disease associated with eating unpasteurized cheese and delicatessen meats.
- Following ingestion, the bacteria appear in the colon and then can colonize the 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:

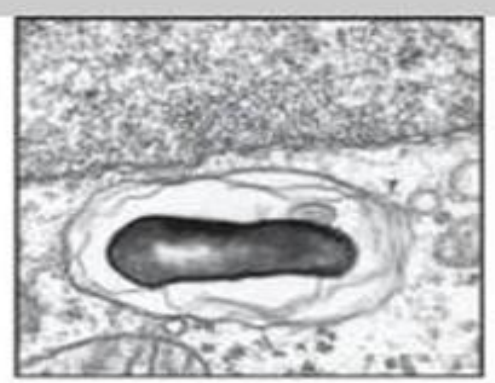
As an **intracellular pathogen**,

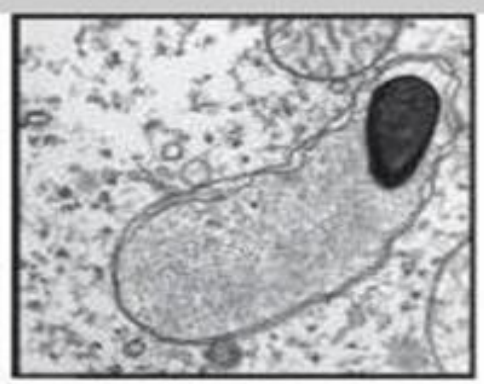
- listeria virulence depends on its ability to escape the defenses of the cell, thrive intracellularly and jumping physically from cell to cell→then invade other cells.
-Invasion of cells is mediated by bacterial factor internalin (Listeria side) + Ecadherin (Human cell surface). [no need to go through endocytosis.]

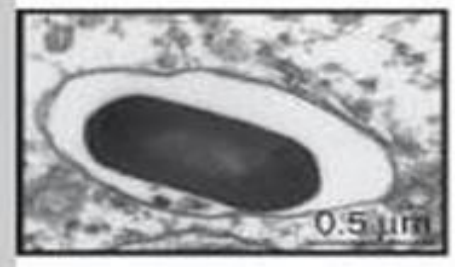
- The ability of Listeria to pass the placenta, enter the meninges, and invade the gastrointestinal tract depends on the interaction of internalin and Ecadherin on those tissues.
- Upon entering the cell, the organism produces **listeriolysin**,(this enzyme allows it to break away from the phagosome 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.

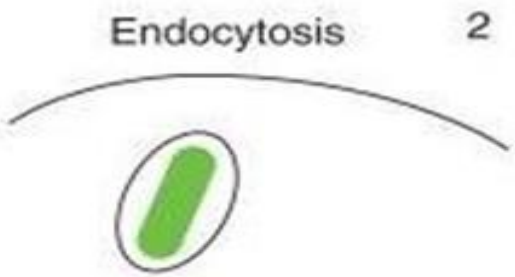


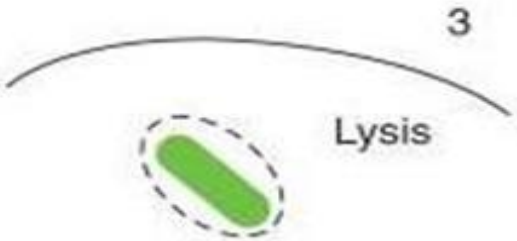


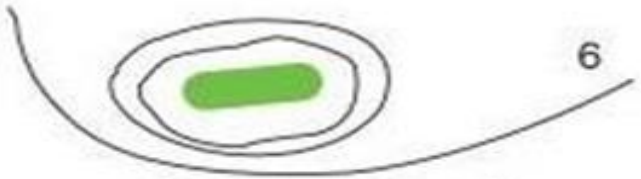


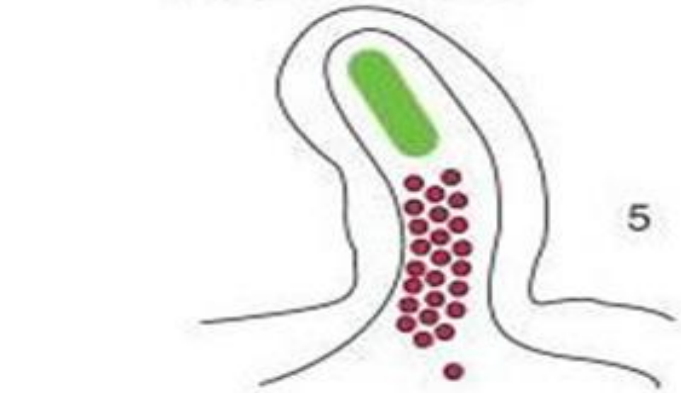


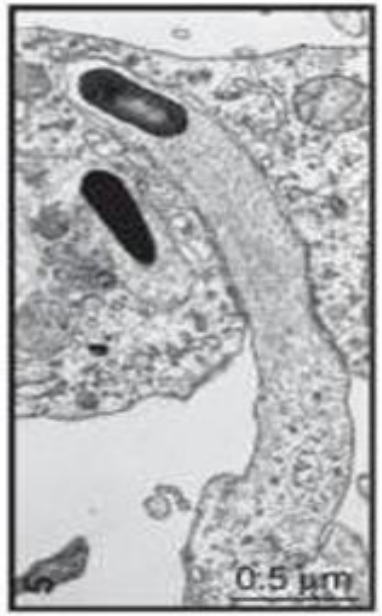


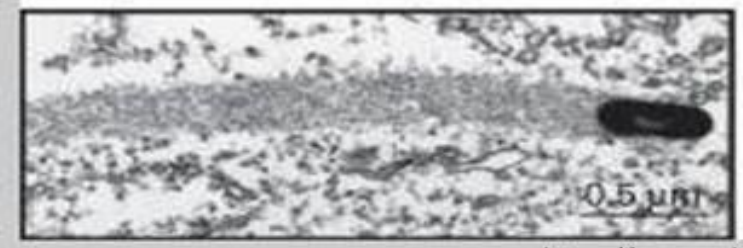












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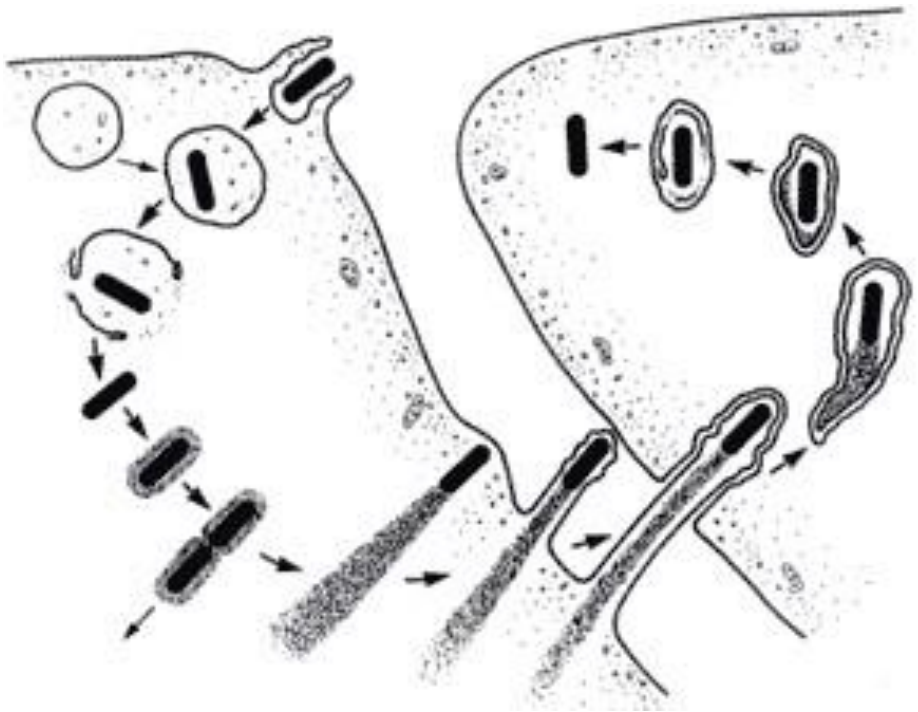

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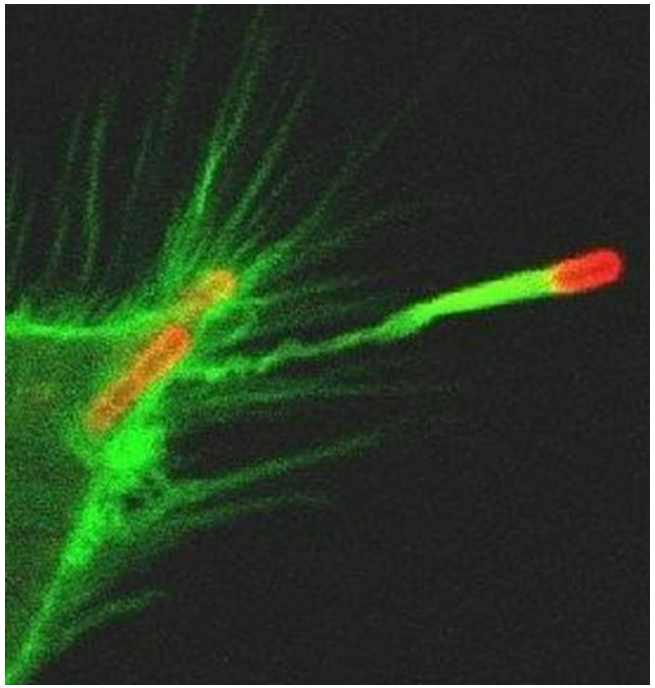

•Clinical Findings

- → 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 βhemolysis 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-sulfamethoxazole**, 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 and mucus mem. In the mouth, 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.