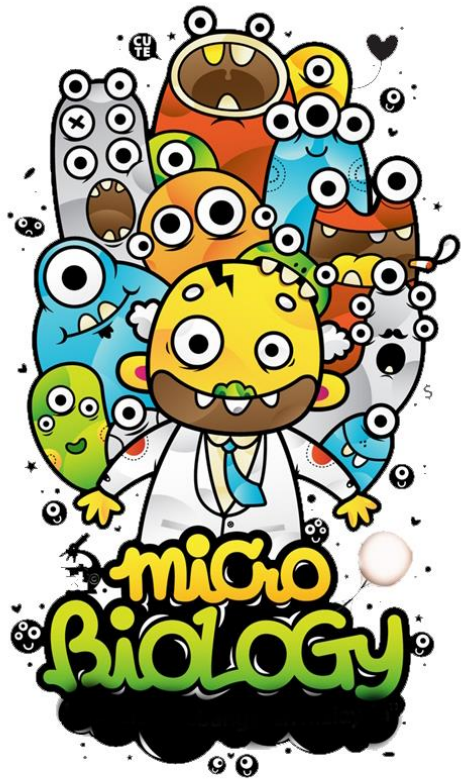

Subject: Gram Positive Rods
Lecture No.: 19
Done by: Zaid Al-fakhouri
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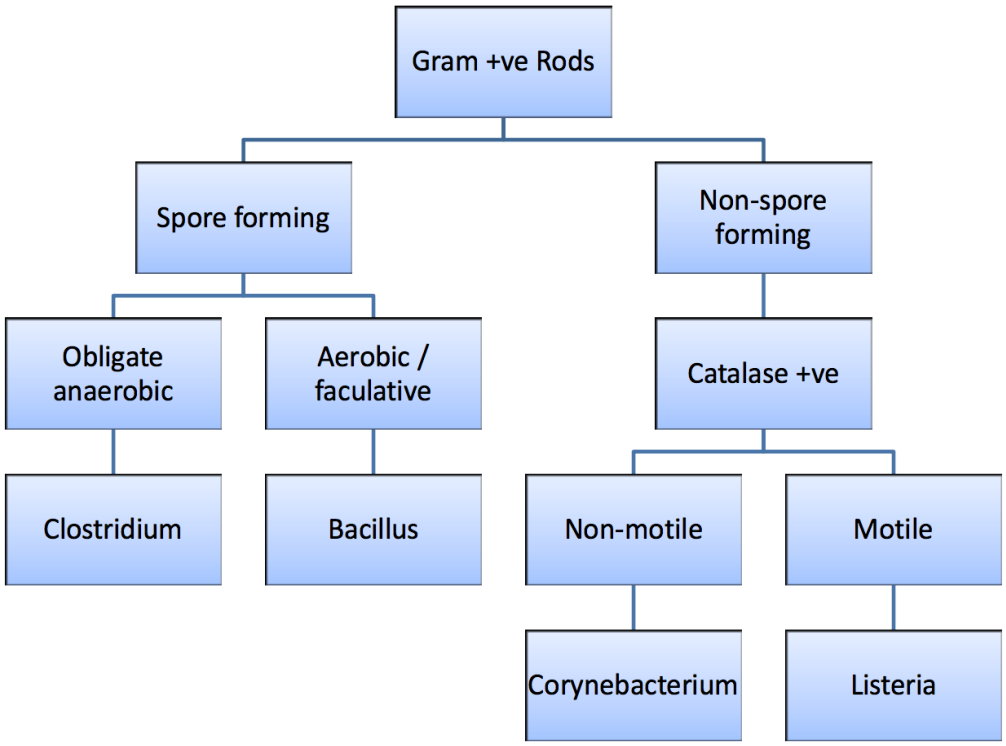
بسم الله الرحمن الرحيم

Gram Positive Rods

In studying Gram Positive Rods, we're mainly concerned with **four genera only** (the most medically important genera). The following diagram summarizes the classification of Gram Positive Rods.



-As can be seen from the diagram, we have two main Gram +ve Rods groups:

1- The spore forming Gram +ve Rods:

This group contains the main spore forming bacteria. These spore forming Gram +ve Rods are divided into two subgroups:

A- Obligate anaerobic bacteria: *Clostridium*.

B- Aerobic / Facultative anaerobic bacteria: *Bacillus*.

2- The non-spore forming Gram +ve Rods:

Divided into two subgroups:

A- Non-motile bacteria: *Corynebacterium*

B- Motile bacteria: *Listeria*

-Corynebacterium and Listeria are both **Catalase +ve** bacteria.

-A catalase test is made to distinguish these two pathogenic bacteria from other normal non-pathogenic (Gram +ve, catalase -ve) rods in the body such as *Lactobacillus**.
So if we get a positive catalase test then we either have Corynebacteria or Listeria. Otherwise, we have Catalase –ve bacteria.
To distinguish Corynebacteria from Listeria, we observe their motility. Corynebacteria is non-motile, while Listeria is motile.

* Lactobacillus is a very useful normal flora found in the GI tract and genital tract in the female. It's a Gram +ve, catalase –ve, rod shaped, non-spore forming bacteria.

Now we'll study each subgroup individually.

Listeria: *(Motile, non-spore forming bacteria)*

-The most important species is ***Listeria monocytogenes***.

-*Listeria monocytogenes* is widely spread in the environment. This pathogenic bacteria, although doesn't form capsules or spores, can resist harsh environmental conditions; it resists cold temperatures as low as 0°C, high salt concentrations, the extremes of pH and bile salts. Therefore, it's found almost everywhere.

-Listeria causes listeriosis. Listeriosis is usually a mild or subclinical (Asymptomatic or with very mild symptoms) infection in healthy adults, but often serious in fetuses, neonates and immunocompromised patients (Listeriosis mainly causes

meningitis, endocarditis and gastroenteritis in these groups).

Bacteria in general are attacked by the humoral immunity (antibodies). However, Listeria is attacked by the cell-mediated immunity because it is intracellular (it lives inside the cells, especially phagocytes (Monocytes and macrophages in particular)). So people with cell-mediated immunity problems are very susceptible to Listeria

- Fetuses are susceptible to Listeria because their cell-mediated immunity isn't well developed and because Listeria can cross the Placental wall.

-Listeria is one of the bacteria that can contaminate milk. Most cases of listeriosis are associated with ingesting contaminated dairy products (mainly), poultry and meat. Therefore, dairy products pasteurization (which kills vegetative bacteria) is the most important procedure in preventing Listeriosis, along with the proper washing, storage and cooking of foods.

-Listeria is a common cause of neonatal meningitis. As antibiotic administration during delivery is not useful in killing Listeria, preventing the pregnant woman from drinking unpasteurized milk or eating soft cheese is the best way to prevent neonatal meningitis (which has a high mortality rate), endocarditis and gastroenteritis (caused by Listeria).

Antibiotic treatment of Listeria must be used with patients at high risk (pregnant women) but not with normal individuals. Most Listeria strains are susceptible to **penicillins** and **macrolides** (Erythromycin, Azithromycin and Clarithromycin).

*Cell mediated immunity usually attacks viruses and fungi.
*The optimum temperature for pathogenic bacteria's growth is the physiologic pH (around 37°C).

To sum things up:

- The most important species is *Listeria monocytogenes.*
- Listeria can resist harsh environmental conditions.
- Listeria causes listeriosis, especially in people with cell-mediated immunity

problems.

- Most cases of listeriosis are associated with ingesting contaminated dairy products.
- Pasteurizing dairy products is a way of preventing Listeriosis.
- Most listeria strains are susceptible to **penicillins** and **macrolides**.

Corynebacterium: *(Non-motile, non-spore forming bacteria)*

All the species in the Corynebacterium genus are non-pathogenic except for *Corynebacterium diphtheriae* which is a highly infectious human pathogen. However, not all Corynebacterium diphtheriae strains are pathogenic, only the strains infected by a temperate (lysogenic) phage are. This is because for Corynebacterium diphtheriae to cause a disease, it must produce a toxin called (diphtheria toxin) which causes the harmful effects and diseases. The gene that is responsible for making this toxin is not found in the original chromosome of Corynebacterium diphtheriae. Instead, it acquires it from the genome of a phage. Therefore, only lysogenic strains (infected by a lysogenic phage) of Corynebacterium diphtheriae are pathogenic to humans.

Corynebacterium diphtheriae is mainly transmitted by respiratory droplets and can cause upper respiratory tract infection (**diphtheria**), mainly in the pharynx. Therefore, diphtheria infection is a special type of pharyngitis (strep throat). Yet, diphtheria is more sever than strep throat and may be life threatening, especially in infants and elderly people. Hence, diphtheria is never called pharyngitis in common practice because its more sever than pharyngitis.

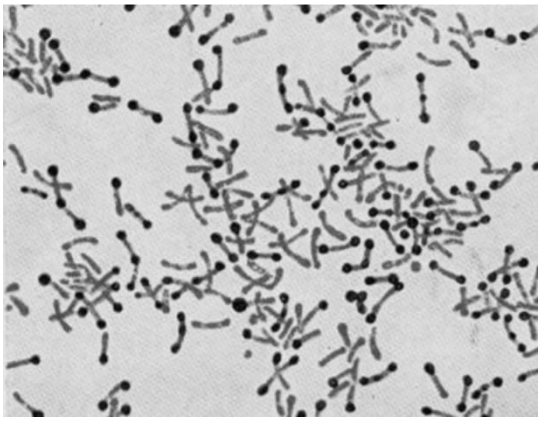
What does Corynebacterium diphtheriae exactly do to cause diphtheria?
- Corynebacterium diphtheriae enters the pharynx and causes pharyngitis. Then, because Corynebacterium diphtheriae lacks virulence factors that enable it to enter the bloodstream, only the diphtheria toxin enters the blood. Entry of the toxin into the blood makes the disease a serious one.
After entering the blood, the toxin reaches many tissues including the heart, motor neurons and adrenal glands. There, the toxin enters the host cells and inhibits the protein biosynthesis in them leading to host cells necrosis. Most deaths caused by diphtheria toxin are due to cardiomyopathy (a damage in the

heart muscle).

* Corynebacterium diphtheriae usually infects infants and elderly people and has a high mortality rate in these two age groups.

*The non-pathogenic species of Corynebacterium (called **Diptheroids**) are usually found as normal flora in the body, especially in the upper respiratory tract.

-Corynebacterium are irregularly shaped bacilli that stain unevenly (look like Chinese letters under the microscope). This is because Corynebacterium produce high amounts of polyphosphate granules. Regions with polyphosphate granules look darker than other regions of Corynebacterium.



-Treatment of diphtheria involves the usage of antibiotics in combination with antitoxins. Antibiotics will kill the bacteria, but they won't affect the toxin that has already reached tissues. Therefore, using antitoxins is a must in treating diphtheria.
The used antibiotics are mainly **penicillins** and **macrolides**, while the used antitoxin is the **antitoxin serum**. Antitoxin serum consists of the antibodies that attack the diphtheria toxin and neutralize it. Antitoxin serum is either produced in horses or in humans.

-Luckily, this bacterium has an effective vaccine. Vaccines are used to produce antibodies that can attack the toxin. Therefore, this vaccine is a **toxoid**. The toxoid is the same toxin produced by the bacteria but its heated or chemically treated to be neutralized and lose its toxicity while preserving its antigenicity. So when the

human is vaccinated, the body will produce antibodies against the toxin itself. So in the future, if the patient gets infected, the immune system will neutralize the toxin before entering the host cells.

-The diphtheria vaccine is part of the trivalent bacterial vaccine "**DTaP vaccine**" given to children at 2, 3,4 and 18 months of age as a part of the national immunization program in many countries including Jordan.
DTaP is a vaccine for three deadly diseases: Diphtheria, Tetanus and Pertussis. This vaccine protects patients from these three diseases for around ten years. That's why adults may be prone to one of these diseases even if they took these vaccines in their childhood. It is recommended that adults take a booster shot (An additional dose of the vaccine) in order to be protected.

*C. diphtheria is best grown on blood tellurite agar.

To sum things up:

- The pathogenic species of Corynebacterium is the *Corynebacterium diphtheriae.*
- The non-pathogenic species of Corynebacterium (**Diptheroids**) are found as normal flora in the upper respiratory tract.
- *Corynebacterium diphtheriae* mainly causes upper respiratory tract infection (**Diphtheria**) with systemic complications affecting the heart, motor neurons and adrenal glands. These are mainly caused by the diphtheria toxin.
- Diphtheria is treated by using antibiotics (penicillins and macrolides) and antitoxin serum.
- Diphtheria vaccine (a toxoid) is used to protect children mainly from diphtheria. It's part of the DTaP vaccine.
- Corynebacterium are irregularly shaped bacilli that stain unevenly.

Bacillus: *(Aerobic/ facultative anaerobic, Spore forming bacteria)*

Gram +ve bacilli that can form spores under harsh environmental conditions.

Spores are highly resistant to dryness, temperature and chemical antimicrobials.

This helps the bacteria to survive for a long time. By definition, endospores are resistant to antibiotics. However, liquid chemical sterilants can cause distractions to these endospores. If these endospores germinate and become vegetative again, they will be susceptible to antibiotics.

Question: Why are endospores resistant to antibiotics?
Answer: Because they are metabolically inactive. Antibiotics mechanisms of action include interfering with one or more synthetic steps. Since endospores don't synthesize anything, so there will not be anything to be inhibited.

Question: Why can some disinfectants affect endospores?
Answer: Disinfectants are less selective and some of them (High level disinfectants) can cause direct distraction to the cellular components.

-All spore forming bacteria (including bacilli) are widely spread in the environment because they are highly resistant to harsh conditions. Only few bacilli species are pathogenic to humans.
Most medically important are ***B. cereus*** and ***B. anthracis***.

B. cereus and ***B. anthracis*** are found in the environment as spores. They don't produce negative effects on humans unless they germinate. This is because most of the spore-forming bacteria infect humans with diseases mediated by toxins. Spores are metabolically inactive and incapable of producing toxins, so germination is needed in order to produce toxins.

1- Bacillus cereus:

Bacillus cereus is a common food contaminant (**rice**, meat, fish and dairy

products), in which it can survive, vegetate and produce a heat-stable **enterotoxin**. Ingestion of toxin-contaminated food results in food poisoning that usually causes vomiting and diarrhea.

-Its considered as a mild food contaminant and its self-limiting. Therefore, antibiotic treatment is not needed in this case because the toxin is already present in the body and won't be affected by the antibiotic. Since this is a minor infection, antitoxins also aren't needed because they have many side effects. Resting and fluid replacement (to compensate for fluids lost in vomiting and diarrhea) are enough to overcome such infection.

Question: Since B. cereus is wide spread, why is it mainly effective in contaminating food?
Answer: High temperatures used in cooking will not kill the endospore. However, cooking temperatures will cause their germination. This mainly happens in restaurants when they cook rice. After cooking it, its left for a long time before being served. During this time, germinated bacteria produce their enterotoxins. By the time of serving, enterotoxins will have accumulated in the food and will cause infections when ingested. After hours, B. cereus will resolve by itself.

A student's question: Will the body produce antibodies for these enterotoxins?
Answer: The toxins must reach the blood or the lymphatic system to have a proper immune response. B. cereus's sight of action is the GI tract so the toxins will not induce antibodies formation.

*B. cereus incubation period can take up to 16 hours, so symptoms may appear after 1-2 hours of ingesting the contaminated food.

2- Bacillus anthracis:

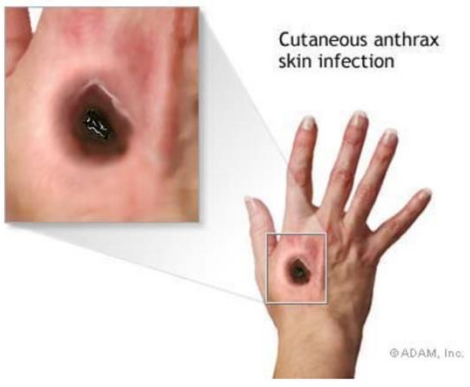
-Bacillus anthracis is found in many animals including goats, sheep and cattle. It causes a disease called **anthrax.** Anthrax is a zoonotic disease transmitted from animals to humans who handle animal wool or hair. It mainly manifests as either cutaneous anthrax or pulmonary anthrax.

-Bacillus anthracis produces its negative effects through three main toxins (factors):

1- **Lethal factor**: Causes tissue damage (cells necrosis).
2- **Edema factor**: Causes edema and increases secretions in the affected area.
3- **Protective factor (antigen)**: Doesn't work directly on the human tissues. Instead, it protects the other two factors (Lethal and edema factors) from the human proteases that might affect them. Also, it induces protective antibodies when used as a vaccine for bacillus.

These three factors mainly cause cells **necrosis** and tissue damage. This involves the blood vessels and causes **hemorrhage** accompanied by **edema**.

Cutaneous anthrax: When spores become in contact with human skin, they vegetate and cause cutaneous anthrax. It is manifested by swollen, necrotic and hemorrhagic lesions (malignant pustules). They look black because of the oxidized, discolored blood since lesions are hemorrhagic. Although it causes malignant pustules(widely spread over the body),



cutaneous anthrax is not fatal (mainly with proper treatment).

Pulmonary anthrax: When spores are inhaled, they travel to lymph nodes (mediastinal lymph nodes) in the chest (near the lungs) where they vegetate and produce toxins. Hemorrhagic fluid accumulates in the lungs and affects respiration and gas exchange, and because the blood vessels rupture (tissue damage) the bacteria (and its toxins) can disseminate into the blood causing septic shocks and septicemia (becomes a systemic infection). It is highly fatal even with antibiotic treatment if the symptoms have already appeared.

*Spores of B. anthracis are also used as warfare agents

Clostridia: *(Obligate anaerobic, spore-forming bacteria)*

- They are widely spread in the environment
- They produce wide range of toxins specific to each Clostridia species. Each species produces one or more types of toxins.
- We are interested in studying 4 main clostridia species.

1-Clostridium tetani:

- It causes tetanus.
- It is mainly found in cultivated soil and GIT of animals. It is found in the environment as spores, so it can resist harsh environmental conditions.
- These spores enter the body through accidental puncture wounds by items contaminated with dust. Because all clostridia are obligate anaerobes, they are transmitted through puncture wounds not surface wounds. After contamination of deep wounds (by dust for example), these spores germinate and release a powerful neurotoxin (**tetanospasmin**) that enters motor nerve endings and **travels in axons of peripheral nerves** to CNS

(doesn't travel in blood), causing tetanus.

-Travelling in axons is slower than travelling in blood, that's why tetanus has a long incubation period **(4-10 days).**

The incubation period depends on the site of injury and how far it is from the CNS (the farther from the CNS, the longer the incubation period).

* (Tetanospasmin directly affects the CNS but not the peripheral nerves, i.e. it doesn't produce its effects at the site of injury)

-Tetanospasmin inhibits the inhibitory neurons of the CNS (especially the ones that inhibit motor neurons). These neurons prevent muscles from contracting for a long time. Thus, inhibiting them will inhibit muscle relaxation and will keep the muscles contracted. This contraction will cause the loss of the muscle mobility, resulting in **spastic paralysis** that begins in the jaw and then progresses to the rest of the body. When it reaches the diaphragm, it causes respiratory arrest and might be fatal if not treated.

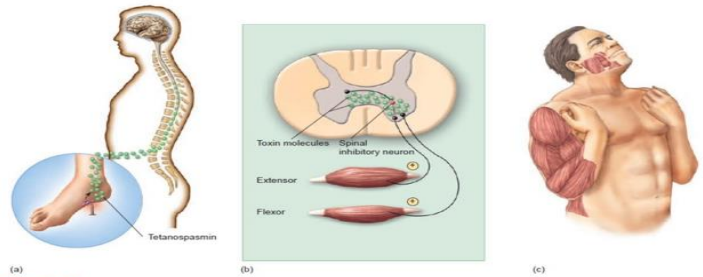


Figure 19.22 The events in tetanus. **(a)** After traumatic injury, bacteria infecting the local tissues secrete tetanospasmin, which is absorbed by the peripheral axons and is carried to the target neurons in the spinal column. **(b)** In the spinal cord, the toxin attaches to the junctions of regulatory neurons that inhibit inappropriate contraction. Released from inhibition, the muscles, even opposing members of a muscle group, receive constant stimuli and contract uncontrollably. **(c)** Muscles contract spasmodically, without regard to regulatory mechanisms or conscious control. Note the clenched jaw typical of *risus sardonicus*.

- Luckily, this toxin has a vaccine which is a part of the D**T**P vaccine. Again, this vaccine is a **toxoid** that protects the child for 10 years only. Thus, a reliable treatment for adults is needed. This treatment involves the usage of **post-exposure vaccination**. Usually vaccines are only effective if given before exposure, but infections with long incubation periods (ex. Tetanus) can be treated using post-exposure vaccination. If we give the vaccine directly after exposure, it will be highly effective as the vaccine will produce its effects before the immune system responds to the toxin, due to its long

incubation period.

If the vaccine was not directly given to the patient, using **the antitoxin serum** along with the toxoid becomes crucial. (Recall that the antitoxin serum consists of antibodies that can attack the toxin).
Also, using **antibiotics** (penicillin or tetracycline is important to kill the bacteria).

-Using the antitoxin serum and antibiotics without the toxoid is not effective. The toxoid must be given to kill the bacteria because they are spores, and antibiotics will only kill those that have germinated.
So, treatment of tetanus involves the usage of antibiotics and the toxoid directly after exposure. If not given directly, antitoxin serum must also be used.

2-Clostridium botulinum:

- It is widespread in the environment and can be found in soil, water and GIT of animals, but mainly in improperly prepared canned food.
- It causes **Botulism.** Botulism is an intoxication with **botulinum toxin** caused by:
  A- **Food-borne botulism**: Ingestion of preformed toxin in food (bacteria have already germinated and produced this toxin) causes food-borne botulism. This is the most common form of botulism, especially in canned food because food is preserved with nitrogen instead of oxygen, which allows the growth of anaerobic bacteria.
  B- **Wound botulism**: Contamination of deep wounds by botulinum toxin.
  C- **Infant botulism**: If infants ingest spore-containing food, these spores can germinate in their GIT and produce the botulinum toxin. On the other hand, clostridium botulinum can't survive in the GIT of adults due to the presence of the normal flora (infants have less amounts of normal flora).

- Onset of botulism symptoms takes 12 to 72 hours, depending on the size of the toxin dose.

- Clostridium botulinum produces botulinum toxin which is the most poisonous material known to humans (1 gram of this toxin is enough to kill two million adults or four million children, so it is very toxic).
  After ingesting contaminated food, botulinum toxin travels through the blood (unlike tetanospasmin) to the neuromuscular junctions and produces its effects there. Botulinum toxin prevents the release of acetylcholine in neuromuscular junctions and prevents muscle contraction, leading to **flaccid paralysis**. If untreated, it causes death due to respiratory arrest.

  *Notice that both tetanospasmin and botulinum toxins cause death by causing respiratory arrest either by spastic paralysis or flaccid paralysis.

- Antibiotic treatment is only effective with infant botulism or wound botulism, because bacteria produce the toxin inside the body. However, in food-borne botulism (the most common type), bacteria have already produced the toxin in food, so antibiotics are completely useless. Thus, treating food-borne botulism requires using <u>horse antitoxin serum only</u>.

  Although a vaccine is available for this disease, it is not commonly used because it is associated with significant side effects. It might only be used to protect people with high risk to acquire this disease.

  <u>Question:</u> Why isn't there a human antitoxin serum for botulinum?
  <u>Answer:</u> Because to produce human antitoxin serum we have to give human antitoxoid to humans, but as we said, this antitoxoid has serious side effects, so we don't give it to humans. Therefore, we only have horse antitoxin serum.
  -Horse antitoxin serum is the only treatment for botulism (especially food-borne botulism), but it must be accompanied by supportive care, especially respiratory and cardiac supportive care.
  When patients become symptomatic, we give them antitoxin serum and a mechanical ventilator to help them breathe.

3-Clostridium perfringens

4-Clostridium difficile

Will be discussed in the next lecture.

"وَفِي أَنفُسِكُمْ أَفَلَا تُبْصِرُون"