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Microbiology

Doctor 2017 | Medicine | JU

● Sheet

○ Slides

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DOCTOR

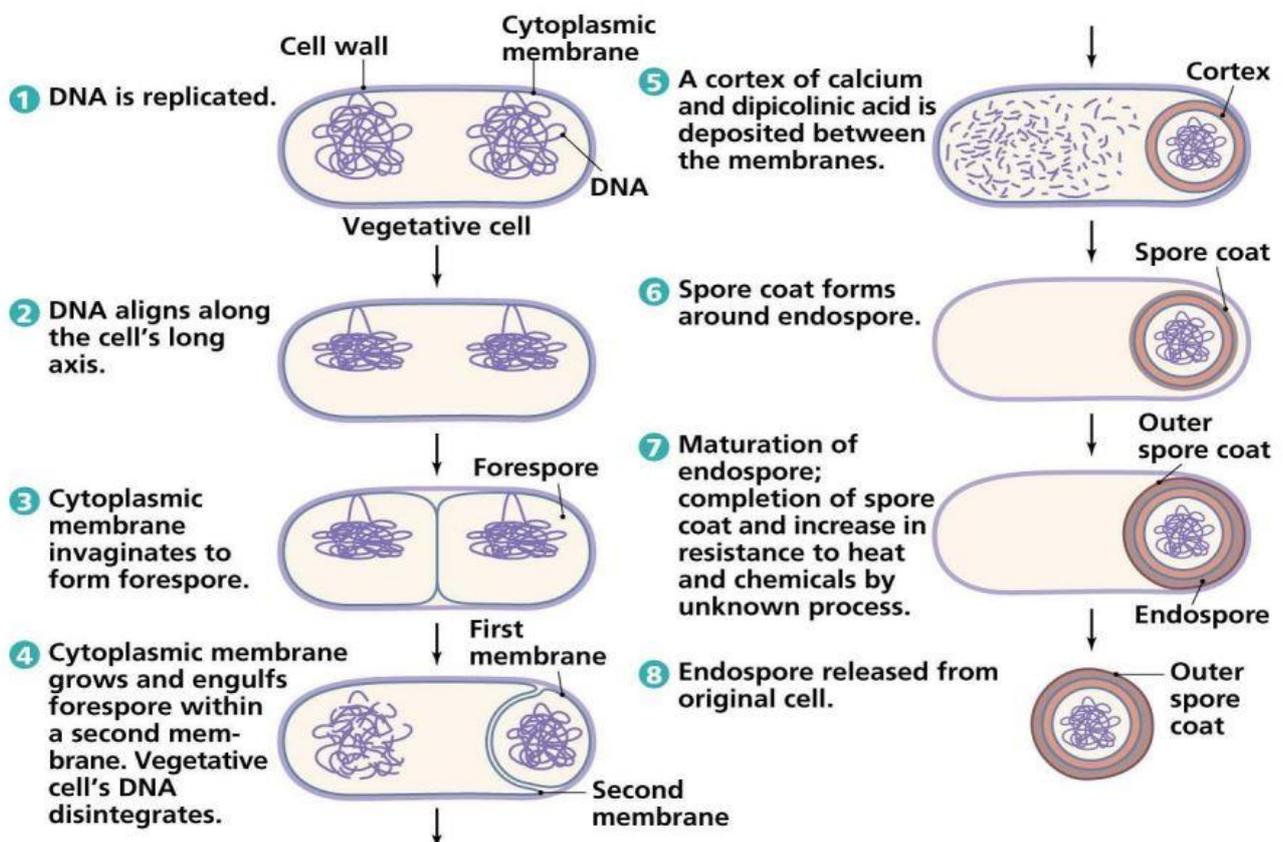
Dr. Anas

Endospores:

1. Some gram-positive (and very, very rarely gram-negative) bacteria can cope with environmental stresses (like depletion of nutrients, such as C, N, Phosphorus) by going through a process of differentiation called **sporulation**.
 - a. Sporulation is a process in which many new cell components are produced (structure, enzymes, metabolites, etc.), and some vegetative cell components are destroyed.
2. The **spore** is a resting cell that is highly resistant to desiccation⁽¹⁾, heat, and chemical agents, and it can survive and exist for centuries as viable spores.
 - a. When the environment becomes favorable again, it can **germinate** to return to its vegetative state
 - b. A spore contains a high concentration of calcium bound to dipicolinic acid (hardens spore and makes it hardy), as well as the minimum concentrations of essential molecules and a single copy of its somatic chromosome
 - c. The location of the spore within the cell, as well as the ultrastructure and formation process varies from species to species

*spores are extremely difficult to kill, and are the hardest pathogens to disinfect

*Called endospores, because it's formed inside the cell



Spore Forming Process:

1. **Sporangium** divides asymmetrically into **mother cell** and **forespore**, which are separated via a **septum**
2. Mother cell engulfs the forespore and forms double-membrane bound forespore (the calcium and dipicolinic acid are deposited between these membranes) (Dipicolinic acid is exclusive to spores)
3. A protein coat is added after the forespore is engulfed, and continues throughout sporulation
4. The peptidoglycan cortex between the inner and outer forespore membranes is assembled during late sporulation
5. The mother cell lyses, releasing a mature spore into the environment

*the spore can quickly germinate after being released

*Be aware that this process is quite different than the one in 1st pic (1-8 steps), because we have different kinds of species, so understand both processes!

Spore Forming Gram-Positive Rods:

There are two species of rods that form spores; **bacillus** and **clostridium**

10:00

- Bacillus: large, **aerobic** (can be facultative aerobes), chain forming
- Clostridium: **obligate anaerobe**

Both species are **ubiquitous**, meaning they are easily found in nature.

1st, Major **Bacillus** pathogens include:

1- **Bacillus Anthracis:**

- large (1 by 3-8 μm)
- arranged as single or paired Gram positive rods or as long, serpentine chains.
- spores not seen in clinical specimens.
- causes the disease anthrax, which is mainly an herbivore disease; humans are infected through exposure to contaminated animals or animal products. Exposure can also be part of biological warfare, but human contact is uncommon.
- highly virulent and deadly.
- is acquired via **inoculation** (skin infections make up 95% of cases), **ingestion**, and **inhalation**.

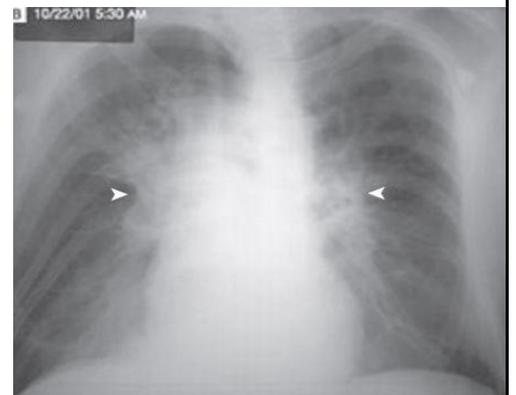
- secretes two major toxins; edema factor (EF) and lethal factor (LF), which are nontoxic individually but are toxic when combined with protective antigen (PA), forming edema toxin (PA plus EF) and lethal toxin (PA plus EF).
- B. anthracis is covered in a capsule which is made of polypeptides (poly-D-glutamic acid) NOT polysaccharide as usual, which inhibits phagocytosis.
- Edema toxin causes accumulation of fluids, while lethal toxin is cytotoxic and stimulates macrophages to release proinflammatory cytokines.
- Spores germinate at the site of entry and growth of the vegetative organisms, results in ulcers, gelatinous edema, necrotic skin (eschar), and congestion.
- Cases result in shock and death within 3 days of symptoms, if not treated.

Cutaneous anthrax goes through the following stages:

1. Development of Painless papule at site of inoculation.
2. Rapidly progresses to an ulcer surrounded by vesicles.
3. Ends in necrotic eschar. (*eschar is a necrotic skin that can't be regenerated*)



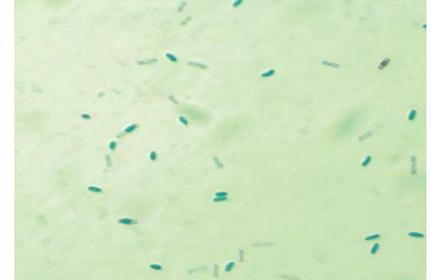
Inhalation anthrax can be associated with a prolonged latent period (2 months or more), during which the infected patient remains asymptomatic (Without any symptoms). Spores phagocytosed in the lungs; and transported by the lymphatic drainage to the mediastinal lymph nodes, where germination occurs. Symptoms begin with hemorrhagic necrosis and edema of the mediastinum, and progress to sepsis, where the infection can spread to other organs, so (GI ulcerations, meningitis) can take place.



2- Bacillus Cereus:

- Responsible for food poisoning. (*Remember that S. aureus can also cause it*)
- Can cause vomiting (emetic form) or diarrhea (diarrheal form), so it's less fatal.
- The emetic form is considered an intoxication by ingestion of the enterotoxin, not the bacteria. (E.g: consumption of a rice contaminated with enterotoxin)

- The emetic form has a short **incubation** (1-6 hours) and duration of illness (<24 hours)
- In diarrheal form, you ingest spores, which then produce the enterotoxin within the intestine and give rise to diarrhea.
- So the diarrheal form is true infection resulting from ingestion of the bacteria in contaminated meat, vegetables, or sauces. With longer incubation period.
- B. cereus can cause ocular infection as a result of eye-trauma with a soil contaminated object



As seen under the microscope, the spores retain a special **malachite green dye**, while the vegetative cells are gray/colorless (because they're washed out).

Now, let's talk about major Clostridium pathogens:

1- Clostridium Difficile:

20:00

- Large (0.5-1.9 by 3-17 μm)
- **Obligate anaerobe** (so The vegetative cells die rapidly when exposed to oxygen)
- freely forms spores in vivo and in culture.
- Transmitted **feco-orally**, usually in a hospital environment (So it's a nosocomial infection), and some people are colonized with clostridium difficile.
- In any case of clostridium difficile, you'll take antibiotics for a prolonged period, so you'll damage your microbiota. So remember that clostridium will over populate and start producing toxins.
- Is part of the intestinal flora in small numbers of healthy people and hospitalized patients.
- Produces an enterotoxin (A) and a cytotoxin (B), which targets the colon/intestine , and cause diarrhea and inflammation
- Can be mild diarrhea or life threatening colon inflammation (pseudomembranous colitis)
- Pseudomembranous colitis is an inflammatory condition of the colon characterized by elevated yellow-white plaques that coalesce to form pseudomembranes on the mucosa.

Commonly develops in people taking antibiotics, because it alters the normal flora, allowing C. difficile to overgrow, or it makes the patient more susceptible to **exogenous acquisition**.

*an effective treatment for this bacteria is a fecal transplant, which shows C. difficile cannot grow when there is a normal/healthy level of enteric bacteria.

2- Clostridium Perfringens:

- Large (0.6-2.4 by 1.3-19 μ m)
- Rectangular and gram positive rods.
- In some cases they are Gram variable, because they are gram indeterminants which means that they don't always stain properly with gram stain. Also, sometimes in older cultures of bacteria, the gram stain starts to be lost from the bacteria, so some bacteria will start appearing as gram negative, although in reality it's a gram positive bacteria.
- **Anaerobic**
- Spores are rarely observed either in vivo or after in vitro cultivation.
- Colonies grow **rapidly** and **spread out**
- Type A C. perfringens is **ubiquitous** and is found in human and animal intestinal tracts (commonly found in water or soil that has feces). Spores are formed under adverse environmental conditions and can survive for long periods
- Types B-E is mainly found in animals and humans. (*because they do not survive in soil*)
- In a blood agar plate, colonies will have **a halo of complete hemolysis** (caused by theta toxin), surrounded by a **zone of partial hemolysis** (caused by alpha toxin)
- Causes soft tissue infections such as **cellulitis**, **fasciitis/suppurative myositis**, and **myonecrosis**. (*last 2 means, inflammation of the muscle then death of the muscle*)
- In cases of myonecrosis, gas is formed due to the metabolic activity of the rapidly dividing bacteria (known as **gas gangrene**⁽²⁾)
- The toxin responsible for gas gangrenes is **α -toxin**, and causes gaps in the plasma membrane of cells which disrupts function
- Food poisoning is considered an intoxication, characterized by: **(1)** a short incubation period (8 to 12 hours), **(2)** a clinical presentation that includes abdominal cramps. **(3)** a clinical course lasting less than 24 hours.
- Type A strains also release an enterotoxin when transitioning from their vegetative state to spores in the small intestine(alkaline environment), when the cells undergo the terminal stages of spore formation (sporulation).
- Treatment usually involves **debridement and excision**. (cutting away dead tissue) because many water-soluble antibiotics cannot penetrate ischemic muscle easily,

because the tissue is already dead and the circulation is very poor, so soluble antibiotics (such as penicillin) alone are not effective .

- Applying a high concentration of oxygen to the infected area has also been used as a treatment method



Patient with gas gangrene

3-Clostridium Tetani:

30:00

- Large (0.5-2 by 2-18 micrometers) and **motile**, spore-forming rod.
- Form round, **terminal spores** giving it a **drumstick** appearance
- Ubiquitous, and is found in soil and animal GI tracts, including humans
- Produces two toxins; **tetanolysin** and **tetanospasmin**
- Tetanolysin is an oxygen-labile hemolysin
- Tetanospasmin is a plasmid-encoded, heat-labile neurotoxin that **targets neuromuscular junctions** by inactivating proteins that regulate the release of the inhibitory neurotransmitters glycine and GABA, so there will be a continuous excitation, causing **spastic paralysis** and **generalized tetanus** in advanced cases
- Spastic paralysis is due to **unregulated excitatory activity** in the neuromuscular junctions
- Usually enters the body through a break in the skin, the closer the site of infection is to the CNS the faster the symptoms develop
- Disease is rare due to vaccine

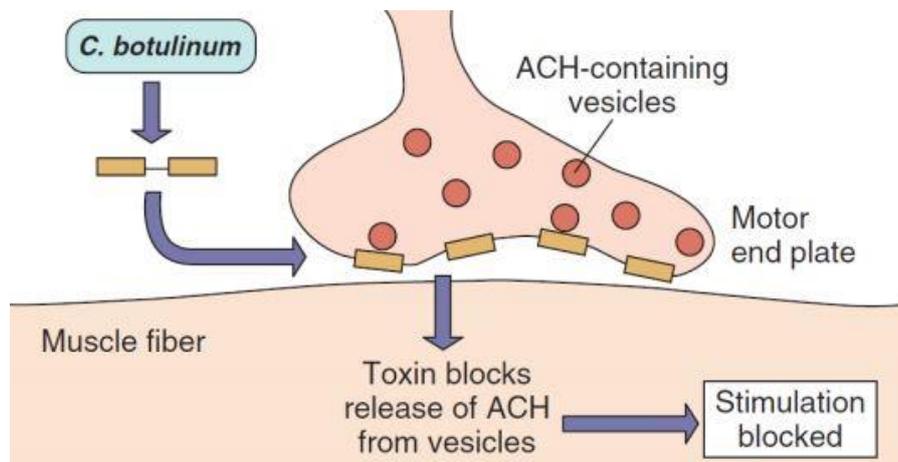
The presenting sign of infection is usually the characteristic **sardonic smile** seen in patients (trismus or **lockjaw**)



4-Clostridium Botulinum:

- Large (0.6-1.4 by 3-20.2 μm), fastidious, spore-forming, anaerobic rods.
- commonly isolated in soil and water samples throughout the world.
- Most infection cases are associated with **foodborne botulism**. (most are associated with consumption of home-canned foods)
- Patients usually become generalized **weak and dizzy** 1-3 days after ingestion, so you won't even be able to open your eyes in some cases.

- With progression, peripheral muscles begin to weaken (**flaccid paralysis**), and finally death is caused by **respiratory failure**
- **Infant botulism** Associated with consumption of foods (e.g., honey, infant milk powder) contaminated with botulinum spores and ingestion of spore-contaminated soil and dust. In contrast with foodborne botulism, this disease is caused by neurotoxin produced in vivo by *C. botulinum* colonizing the GI tracts of infants.
- The neurotoxin inactivates proteins that regulate **acetylcholine** release at neuromuscular junctions, so decrease in stimulation, causing flaccid paralysis
- Seven different strains (A-G) with human disease associated with **A, B, E, and F**



Non-Spore Forming Anaerobic Bacteria:

- Gram-positive cocci usually colonize the oral cavity, GI tract, genitourinary tract, and skin; cause infections when they move to normally sterile sites
- Cocci tend to have a predisposition to infect certain sites
- E.g.- peptostreptococcus have been recovered from subcutaneous soft tissue abscesses (where there's no oxygen concentration) and diabetes related foot ulcers than from intra-abdominal infections, and are usually associated with chronic infections that are **synergistic** (work together with other pathogens during an infection, like working with staph/streptococci, etc)
- Remember that in diabetics (chronic inflammation), there's a problem in motor neuron and sensation, due to suppressed immunity, so that will cause foot ulcers.