Microbiology
Doctor 2018 | Medicine | JU

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

- Sporulation involves the production of many new structures, enzymes, and metabolites along with the disappearance of many vegetative cell components.
- The spore contains a complete copy of the chromosome, the bare minimum concentrations of essential proteins and ribosomes, and a high concentration of calcium bound to dipicolinic acid.
- The spore is a resting cell, highly resistant to desiccation, heat, and chemical agents. Can exist for centuries as viable spores.
- When returned to favorable nutritional conditions, the spore germinates to produce a single vegetative cell.
- The location of the spore within a cell can assist in identification of the bacterium.
- The ultra-structure and formation process of spores can vary from one species to another. (exact detailed structure is not exam material).

Information about sporulation process written in slides but not mentioned by the doctor (Details of the process are not our concern in this lecture):
Sporulation begins when a sporangium divides asymmetrically to produce two compartments: the mother cell and the forespore, which are separated by a septum. Next, the mother cell engulfs the forespore, and following membrane fission at the opposite pole of the sporangium, a double-membrane bound forespore is formed. Coat assembly begins just after the initiation of engulfment and continues throughout sporulation. The peptidoglycan cortex between the inner and outer forespore membranes is assembled during late sporulation. In the final step, the mother cell lyses to release a mature spore into the environment. Spores are capable of quickly germinating and resuming vegetative growth in response to nutrients.
Spore-forming bacteria are ubiquitous (existing or being everywhere) in nature. The resistance properties (highly resilient, surviving extremes of temperature, low-nutrient environment) of bacterial spores are the reason behind their widespread occurrence in foodstuff and soil.

- **Spore-Forming Gram-Positive rods:**
  - **Bacillus species:** The genus Bacillus includes large aerobic, gram-positive rods occurring in chains. Major pathogens include *Bacillus anthracis* and *Bacillus cereus*.
  - **Clostridium Species:** a genus of Gram-positive rods, are obligate anaerobes. Major pathogens include *Clostridium difficile*, *Clostridium perfringens*, *Clostridium tetani*, *Clostridium botulinum*.

<table>
<thead>
<tr>
<th>Bacillus anthracis</th>
<th>Characteristics:</th>
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<tbody>
<tr>
<td></td>
<td>1. Gram positive rods (long, serpentine chains).</td>
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<td></td>
<td>2. <strong>Size:</strong> large (1 × 3 to 8 μm) organism arranged as single or paired rods.</td>
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<td>3. Spores are not seen in clinical specimens.</td>
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- **Reservoir**
  - Animals, skin, soils.

- **Transmission**
  - Contact with infected animals or animal products (herbivores).
  - Inhalation (bioterrorism, biological warfare).

- **Routes of infection**
  - **Human B. anthracis disease is acquired by one of three routes:**
    1. Inoculation (Skin or cutaneous infections represent more than 95% of cases)
    2. Ingestion (gastrointestinal).
    3. Inhalation (lungs).

- **Why anthrax is a biological weapon?**
  - Anthrax spores are easily found in nature. Released quietly without anyone knowing, spores could be put into powders, food.
### Clinical case (Pathogenesis)

**Wool-Sorter’s disease**

Highlighted information is mentioned by the doctor during the lecture, it is good to know how these cases are diagnosed, their prognosis and possible treatments to be prescribed.

- Spores are inhaled by the individual, spores germinate in the tissue at the site of entry, and growth of the vegetative organisms (mediastinal lymph nodes) results in formation of a gelatinous edema and congestion (**mediastinal hemorrhagic lymphadenitis >> extra**).
- Almost all cases progress to shock and death within 3 days of initial symptoms unless anthrax is suspected and treatment is initiated immediately.

### Virulence factors

The major factors responsible for the virulence of *B. anthracis* are the:

1. **Capsule** (The capsule made of poly-D-glutamic acid inhibits phagocytosis of replicating cells).
2. **Edema toxin** is responsible for the fluid accumulation observed in anthrax.
3. **Lethal toxin** is cytotoxic (kills cells) and stimulates macrophages to release proinflammatory cytokines.
Typically, **cutaneous anthrax** starts with the development of a painless papule at the site of inoculation that rapidly progresses to an ulcer surrounded by vesicles and then to a **necrotic eschar**.

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

**EXTRA:** **anthrax is NOT contagious** (by coughing or sneezing) but cutaneous anthrax can be transmitted by direct contact with the drainage from an open sore (releasing spores).

**PREVENTIVE:** Preventive vaccine (toxoid) is available, given to individuals in high risk occupations (military).

**INTERESTING MEDICINE:** Some TV shows with episodes related to anthrax and bioterrorism (definitely not recommended by Dr. Anas):
- Criminal minds (season 4, episode 24 “Amplification”).
- Elementary (season 2, episode 20 “No lack of void”).
Bacillus cereus:

- **Reservoir**: Found in nature, ubiquitous organisms, present in virtually all environments.

- **Transmission**:
  1. Foodborne, intoxication.
  2. Major association with fried rice from Chinese restaurants.
  3. Associated with food kept warm.

- **Pathogenesis**:
  Two possible toxins >>> Two forms of food poisoning:
  1. **emetic form** of disease results from consumption of contaminated rice. An intoxication caused by ingestion of the enterotoxin (preformed toxin), not the bacteria. Thus, the incubation period after eating the contaminated rice is short (1 to 6 hours), and the duration of illness is also short (<24 hours).
  2. The **diarrheal form** of B. cereus food poisoning is a true infection resulting from ingestion of the bacteria in contaminated meat, vegetables, or sauces. With longer incubation period (toxin is produced in vivo).

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

- **Appearance under the LM**: Spores retain the **malachite green dye** in this special spore stain, and the vegetative cells are gray or colorless.

- **Treatment**: Self-limiting.
Clostridium genus features:
1. Gram-positive rods.
2. Spore-forming.
3. Obligate Anaerobes (cannot survive in the presence of O2, lack enzymes that get rid of ROS like catalase and superoxide dismutase).

Species of medical importance:
1. Clostridium difficile.
2. Clostridium perfringens.
3. Clostridium tetani.
4. Clostridium botulinum.

Clostridium difficile:

<table>
<thead>
<tr>
<th>Reservoir</th>
<th>Human colon/ gastrointestinal tract.</th>
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<tbody>
<tr>
<td>Pathogenesis</td>
<td>Hospitalized patients on antibiotics (The disease develops in people taking antibiotics, because the drugs alter the normal enteric flora, either permitting overgrowth of these relatively resistant organisms (part of the normal flora, endogenous) or making the patient more susceptible to exogenous acquisition of C. difficile).</td>
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<tr>
<td>Diseases</td>
<td>Antibiotic-associated diarrhea, colitis, or pseudomembranous colitis (yellow plaques on colon) <strong>Pseudomembranous colitis</strong> an inflammatory condition of the colon characterized by elevated yellow-white plaques that coalesce to form pseudo membranes on the mucosa</td>
</tr>
<tr>
<td>Treatment</td>
<td>Remarkable success with “fecal transplants” has been demonstrated, illustrating the fact that C. difficile does not become established when a healthy enteric population of bacteria is present.</td>
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### Clinical case

This old patient has a history of liver transplant which indicates a weakened immune system (immunocompromised) because of immunosuppressive drugs making him more susceptible to C. difficile infection, also the administration of antibiotic induced further disruption of the gut microbiota, with no healthy enough bacteria to protect the body from infection.  
**Diagnosis:** stool examination for toxin production.

### Clostridium perfringens:

| Distinguishing features | 1. Large (0.6 to 2.4 x 1.3 to 19.0 μm), rectangular, gram-positive rod.  
2. Spores rarely observed either in vivo or after in vitro cultivation, an important characteristic that differentiates this species from most other clostridia.  
3. Colonies of C. perfringens are also distinctive, with their rapid, spreading growth.  
4. **Double zone of hemolysis:** A presumptive identification of C. perfringens can be made by detection of  
   - zone of complete hemolysis (caused by the theta toxin)  
   - wider zone of partial hemolysis (caused by the alpha toxin), combined with the characteristic microscopic morphology. |

<p>| Transmission | Foodborne and contaminated wounds. |</p>
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<tr>
<th>Reservoir</th>
<th>Soil and human colon.</th>
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<tr>
<td></td>
<td>type A C. perfringens commonly <strong>inhabits the intestinal tract of humans and animals and is widely distributed in nature</strong>, particularly in <strong>soil and water contaminated with feces</strong>. Spores are formed under adverse environmental conditions and can survive for prolonged periods. Strains of types B through E do not survive in soil but colonize the intestinal tracts of animals and occasionally humans.</td>
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| Diseases   | 1. C. perfringens is responsible for a range of soft-tissue infections including **cellulitis** (shallow skin infection), **fasciitis or suppurative** (purulent exudate >> pus is discharged) **myositis** (deeper infection into muscle), and **myonecrosis with gas formation** (caused by the metabolic activity of the rapidly dividing bacteria) in the soft tissue (gas gangrene). The toxin involved in gas gangrene is known as **α-toxin**, which inserts into the plasma membrane of cells, producing gaps in the membrane that disrupt normal cellular function. 
|            | • Gas gangrene occurs when clostridia invade healthy muscle from adjacent traumatized muscle or soft tissue, originating from a wound contaminated with clostridia. |
|            | 2. **Clostridial food poisoning**, an intoxication characterized by 
|            | • a short incubation period (8 to 12 hours),
|            | • a clinical presentation that includes abdominal cramps.
|            | • a clinical course lasting less than 24 hours.
|            | • **Enterotoxin**: C. perfringens produces enterotoxin, primarily by type A strains, the enterotoxin is produced during the phase transition from vegetative cells to spores and is released in the alkaline environment of the small intestine when the cells undergo the terminal stages of spore formation (sporulation). |

| Treatment  | 1. **Gangrene**: debridement and excision, with amputation necessary in many cases. Water-soluble antibiotics (such as penicillin) alone are not effective because they do not penetrate ischemic muscles sufficiently to be effective. 
|            | 2. **Food poisoning**: Self-limiting. |
Infections caused by spore-forming bacteria have been associated with increasing illness and death among persons who inject drugs (PWID), the figure represents number of cases of botulism, tetanus, Clostridium novyi infection, and anthrax among persons who inject drugs.

**Clostridium tetani:**

| Distinguishing features | 1. large (0.5 to 2 × 2 to 18 μm).
| | 2. motile.
| | 3. spore-forming rod.
| | 4. The organism produces round, terminal spores that give it the appearance of a drumstick. |

| Reservoir | C. tetani is ubiquitous. It is found in fertile soil and transiently colonizes the GI tracts of many animals, including humans. |

| Transmission | Dirty puncture wounds/trauma. |

| Pathogenesis | I will briefly mention the steps so you can get the whole idea: |
| | 1. Spores germinate in the tissues, producing tetanus toxin (tetanosasmin). |
| | 2. Carried intra-axonally to CNS. |
| | 3. Binds to certain receptors. |
| | 4. Blocks release of inhibitory mediators (GABA) at spinal synapse. |
| | 5. Excitatory neurons are unopposed ➔ extreme muscle spasm (rigid paralysis). |
| | As written in slides: Tetanosasmin inactivates proteins that regulate release of the inhibitory neurotransmitters glycine and gammaaminobutyric acid (GABA). This leads to unregulated excitatory synaptic activity in the motor neurons, resulting in spastic paralysis. |
| | Disease is relatively rare because of the high incidence of vaccine-induced immunity. |
| Toxins | C. tetani produces two toxins:  
1. An oxygen-labile hemolysin (tetanolysin)  
|---|---|
| Clinical case | This old unvaccinated man was infected by clostridium tetani from a contaminated wound which resulted in him losing the ability of breathing, ventilation machine is required to provide oxygen.  
EXTRA: prevention involves vaccination that is a Toxoid, it is important because disinfectants have poor sporicidal action. |

Tetanus results in continuously firing nerves, over and over, leading to spasming muscles. The stronger muscles of the body dominate in this response. Extensor muscles of the back arch backwards and lock (spastic paralysis).

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

Clostridium botulinum:

| Distinguishing features | 1. Heterogeneous collection of large (0.6 to 1.4 × 3.0 to 20.2 μm), fastidious.  
2. Gram positive spore-forming rods.  
3. Anaerobic rods. |
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<td>Reservoir</td>
<td>C. botulinum is commonly isolated in soil and water samples throughout the world.</td>
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**Transmission**

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

**Pathogenesis**

1. Spores survive in soil, germinate in anaerobic conditions.
2. Botulinum toxin is released.
3. Toxin is absorbed by the gut and carried by blood to the PNS.
4. **Blocks the release of Ach at the neuromuscular junction resulting in flaccid paralysis.**

**Toxin**

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

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

**Clinical case**

- The source of foodborne botulism is canned food (anaerobic environment suitable for Clostridium).
- Descending flaccid paralysis (muscles cannot contract at all) is characteristic for botulism, this can cause serious problems since breathing is controlled by the muscle of diaphragm.

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![Clinical Case 30-4 Foodborne Botulism with Commercial Carrot Juice](image)
Infant VS adult botulism:

**Infant botulism (Floppy baby syndrome):**
- Spores ingested, house hold dust, honey.
- Toxins produced in gut (in vivo).
- Associated with consumption of foods (e.g., honey, infant milk powder) contaminated with botulinum spores and ingestion of spore-contaminated soil and dust.
- Caused by neurotoxin produced in vivo by C. botulinum colonizing the GI tracts of infants.

**Adult botulism:**
- Preformed toxin ingested
- Poorly canned food.

**QUIZ:**

1. An eschar is associated with which of the following types of anthrax?
   
   A. cutaneous anthrax.
   
   B. inhalation anthrax.
   
   C. both cutaneous and gastrointestinal anthrax.
   
   D. gastrointestinal anthrax.
2. Choose the answer that best describes the morphology/biochemistry of C. difficile?

A. gram-positive, anaerobic, spore-forming, rod-shaped bacterium
B. gram-negative, anaerobic, spore-forming, rod-shaped bacterium
C. gram-negative, anaerobic, spore-forming, cocci-shaped bacterium
D. gram-positive, aerobic, spore-forming, rod-shaped bacterium

3. A 70-year-old man is hospitalized for an infection and treated with clindamycin. The patient improves and returns to his nursing home. Two weeks later he is rushed to the emergency room with fever and loose, mucoid green stools. The diarrhea is voluminous, and he is having severe abdominal pain. Sigmoidoscopy of his colon reveals yellow-white plaques. What is the most likely event/factor that contributed to this patient’s current illness?

A. Drinking unpasteurized milk.
B. Eating contaminated cold cuts.
C. Advanced age.
D. Administration of antibiotics.

4. One way parents can help prevent botulism in a baby is to:

A. Have the child vaccinated.
B. Not give honey to a child under a year.
C. Stay away from swimming pools.
D. All of the above.
ANSWERS:
1. A
2. A
3. D
4. B

Good luck ❤️