GIS

MICROBIOLOGY

Done by: Luna Hussein

Scientific Correction: Obada Froukh

Gramatical Correction: Bilal Odeh

Doctor: Nader Alaridah

"What lies behind us and what lies ahead of us are tiny matters compared to what lies within us"

***In this sheet, we'll be discussing two medically important spore-forming gram-positive species: Bacillus & Clostridium.**

- This group has four medically important genera. We'll go through two of them related to the GI system. The Bacillus & the clostridium species; they both form endospores, thus they're in the same group and cause food poisoning.
- Other important species (gram positive bacilli) that aren't spore formers:

*** Listeria Monocytogenes**: causes neonatal sepsis & neonatal meningitis (to be discussed in pediatrics)

***Corynebacterium d=Diphtheria**: To be discussed in the respiratory system.

***Important - Regarding Oxygen Requirements:**

- **Bacillus = aerobic**
- **Clostridium = anaerobic**

Recall that both of them form spores for survival, not for reproduction (Reproduction Spores= Fungi)

Bacillus cereus** causes ***food poisoning whereas **B. anthracis** causes ***anthrax***.

*Anthrax is found as one of these three clinical forms:

-**Cutaneous**: Starts as malignant pustule over the skin

-**Inhalational anthrax *(Woolsorter's disease)*:** classified as a bio-terrorist agent by the Centers for Disease Control and Prevention.

-**Gastrointestinal anthrax (very rare)**: in thecase of ingesting bacillus anthracis' spores.

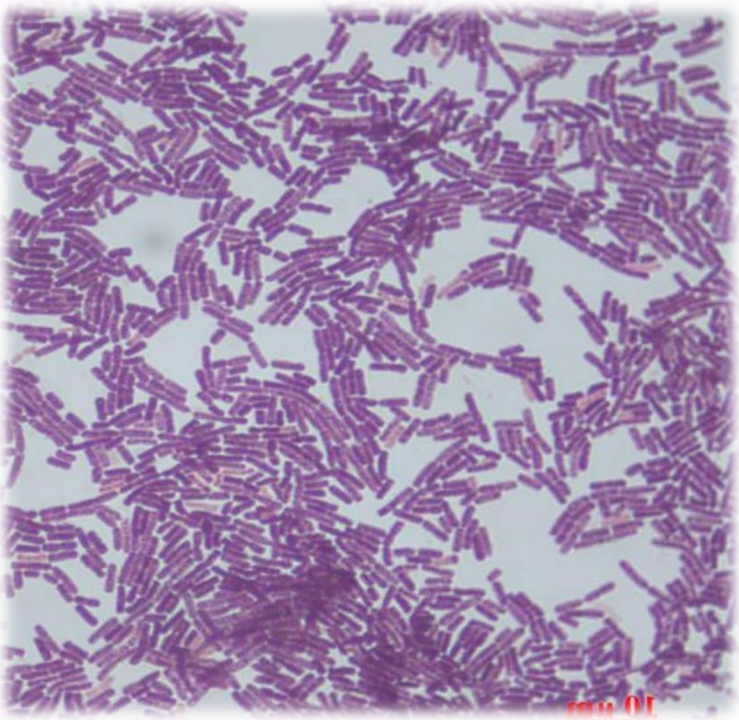
Bacillus Species

- The genus Bacillus includes large aerobic or facultative anaerobes, gram-positive, spore forming rods occurring in chains.
- Saprophytic, prevalent in soil, water, and air, such as Bacillus cereus and Bacillus subtilis.
- Some are insect pathogens, such as B thuringiensis.
- ***B anthracis*** and ***B. cereus*** are the principal pathogens of the genus.

*Morphology and identification

• A 3–4 µm, arranged in long chains; spores are located in the center of the motile bacilli.

• B. cereus can be differentiated from B. anthracis on the basis of colony morphology, β-hemolysis, motility, production of lecithenase and antimicrobial susceptibility patterns.



Bacillus cereus	Bacillus anthracis
Gram-Positive	Gram-Positive
Spore-Forming	Spore-Forming
Aerobic	Aerobic
motile	Non-motile
No Vaccine	Has a capsule consisting mainly of D- Glutamic acid. It also has a vaccine.
High Lecithinase activity	No or low lecithinase activity
Performs beta hemolysis	No beta hemolysis
Large feathery white colonies (typical for b.cereus)	Medusa-head" colonies (typical for b.anthracis)
resistant to Penicillins and Cephalosporins.	Sensitive to Penicillins and Cephalosporins.

*Bacillus cereus

• Gram-positive aerobic or facultative anaerobic, motile, spore-forming, rod-shaped bacterium that is widely distributed environmentally.

• B. cereus is associated mainly with food poisoning ***(very common & self-limiting)***.

• B. cereus has also been associated with localized and systemic infections, including endocarditis, eye infections, meningitis (Transplant patients), osteomyelitis, and pneumonia; the presence of a medical device or intravenous drug use predisposes to these infections. ***(Mainly in immunocompromised people)***

• ***Enterotoxins*** are usually produced by bacteria outside the host and therefore cause symptoms soon after ingestion of the toxins.

*Epidemiology

• The heat-resistant spores of B. cereus are widespread and contaminate rice and other cereals. The spores germinate if left at room temperature.

• A heat-labile toxin can also be produced which can survive “flash frying”.

• The natural environmental reservoir for B. cereus consists of decaying organic matter, fresh and marine waters, vegetables and fomites, and the intestinal tract of invertebrates, from which soil and food products may become contaminated, leading to the transient colonization of the human intestine. ***(ubiquitous=found almost everywhere)***

• Spores germinate when they come in contact with organic matter or within an insect or animal host.

*Pathogenesis

• Secreted toxins: hemolysins, distinct phospholipases, an emesis-inducing toxin, and three pore-forming enterotoxins: hemolysin BL (HBL), non-hemolytic enterotoxin (NHE), and cytotoxin K.

*Clinical findings:

- *For bacillus cereus to cause food poisoning, there must be enterotoxins.*

- There are two clinical syndromes produced by the toxins:

1- **Vomiting type –Heat stable toxin(*cerulide*)**: Incubation period ***0.5–6 hours***, diarrhea and cramps can occasionally occur. The

illness is usually **self-limiting** and over in 24 hours. Associated with (rice & cereals- flash frying Chinese food).

2 - **The Diarrheal Type-Heat labile toxin**: Incubation period ***6–15 hours*** followed by an illness similar to that seen with C. perfringens. The diarrhea and abdominal cramps may be associated with nausea (vomiting is rare) but are over in 24 hours. **Foods involved are: contaminated meat, vegetables and sauces.**

*** The clinical presentation depends on the produced toxin. We may have both toxins produced at the same time, but we usually have either not both.**

Very important*: Regarding the vomiting type, when the person eats the contaminated food, there'll be an already preformed toxin. Whereas in the diarrheal type, the toxin is being produced inside the colon after ingesting bacillus spores and the incubation period is slightly longer.*

*Diagnosis:

- Based on clinical grounds.
- Isolation of B. cereus from the suspect food (in the cases of outbreaks to prevent further spreading of the infection), as well as from the stool or vomitus of the patient.
  We usually take the sample from the food rather than the stool or vomitus of the patient because they're highly contaminated with other Bacteria.

- Culture and Gram stain of implicated material

*Sheep-blood Agar Plate:

Large feathery White Colonies, typical for Bacillus Cereus.

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*Treatment and Prevention:

-Regarding ***diarrheal diseases***, initially check the vital signs of the patient: respiratory rate, heart rate, temperature, blood pressure, dehydration signs. Then perform ***fluid & electrolyte replacement*** because our main concern is dehydration that might end up with acute renal failure.

* Food-poisoning is self-limiting; antimicrobial therapy is not normally required.

• B. cereus is resistant to a variety of antimicrobial agents, including Penicillins and Cephalosporins.

Clostridium Species

- Spores of clostridia are usually wider than the diameter of the rods in which they are formed. Most species of clostridia are **motile** and possess peritrichous flagella (we have an exception that will be discussed in this sheet).
- Clostridia are **anaerobes**; a few species are aero tolerant. In general, the clostridia grow well on the blood-enriched media or other media used to grow anaerobes.
- They are common in the environment. They are also considered as normal inhabitants of the human intestinal tract.

*Species of Medical Importance

- ***Clostridium Tetani*** **- tetanus, Rigid paralysis.**
- ***Clostridium Botulinum*****- botulism, flaccid paralysis.**
- ***Clostridium Perfringens*****- gas gangrene.**
- ***Clostridium Difficile*** **- pseudomembranous colitis.**

*__Rigid paralysis__: hypertonia in the muscle. There's a vaccine for tetanus. ***To be discussed in the CNS system.***

*__Flaccid paralysis:__ hypotonia in the muscle - the most common form is infant botulism.

*Clostridium botulinum

❖Distinguishing Features:

- **Anaerobic** ***Endospore-forming*** **gram-positive** bacilli - **motile**.

• **Botulism** is characterized by ***symmetrical***, ***descending***, ***flaccid paralysis*** of motor and autonomic nerves usually beginning with cranial nerves.

• **Habitat:** Since it is found in soil, it may contaminate vegetables cultivated in or on the soil. It also colonizes the gastro-intestinal tract of fish, birds and mammals .

*Pathogenesis:

❖Botulinum toxin: Highly toxic neurotoxin - coded for by a prophage.

- One of the most potent toxins on Earth. One microgram per kilogram is a lethal dose! For a man weighing 70 kg, 70 micrograms would be enough to kill him/her.

- There exist seven serotypes (A-G) based on the antigenicity of the botulinum toxin produced. ***A, B & E*** are most commonly associated with human infections. Thus, we give patients **Botulism Immune Globulin** Intravenous (Human) (BIG-IV), that contains the three common serotypes ***(trivalent)***.

*Mechanism of action

• The most common offenders are spiced, smoked, vacuum packed, or canned alkaline foods that are eaten without cooking. In such foods, spores of C. botulinum germinate; that is, under anaerobic conditions, vegetative forms grow and produce toxin.

• **Absorbed by the gut** and carried by blood to peripheral nerve synapses.

• **Blocks release of acetylcholine** at the myo-neuronal junction resulting in reversible ***flaccid paralysis.***

*Botulism

There are four clinical categories of botulism:

1) Foodborne botulism (ADULT BOTULISM)
2) Wound botulism (contaminated wounds).
3) Infant botulism.
4) Inadvertent, following botulinum IM toxin injection (a wrong injection may result with botulism - recall that the botulinum toxin is used for cosmetic purposes).

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*Clinical Findings

* Initial symptoms may include **nausea, vomiting, abdominal cramps** or diarrhea that begin 18–36 hours after ingestion of the toxic food.

***Dry mouth**, blurred vision, and **diplopia** are usually the earliest neurologic symptoms. They are followed by **inability to swallow** and speech difficulty. In severe cases, extensive respiratory muscle paralysis leads to ventilator failure.

*The infants in the first months of life develop **poor feeding**, weakness, and signs of paralysis ***(floppy baby)***. Infant botulism may be one of the causes of **sudden infant death syndrome**, especially in babies younger than 1-year old.

Very Important:

*In foodborne botulism (adult botulism), the toxin is already preformed outside of the body.

*In infant botulism, the baby usually eats honey that contains spores which will germinate later in the guts and liberate their toxins there.

*Infant Botulism is the most common form of botulism.

*Diagnosis

- Clinical grounds
- Toxins can often be demonstrated in serum, gastric secretions, or stool from the patient, and toxins may be found in leftover food using **ELISAs** and **PCR**.
- **Mouse bioassay** is the test of choice for the confirmation of botulism.

***Mouse lethality assay is the gold standard and the definitive diagnosis that is performed in reference laboratories. You inject a mouse intraperitoneally with the susceptible sample, then the mouse will die immediately. Then you inject another mouse but you give it the antitoxin afterwards and detect the neutralization of the toxin.**
This is the final identification test for botulinum toxin.

*Treatment

-Very important: Do not give antibiotics! You will worsen the situation by putting the bacteria under stress, producing more toxins.

- Supportive treatment, especially **adequate mechanical ventilation**, is of prime importance in the management of severe botulism.
- Surgical debridement in wound botulism.
- **Antitoxin administration**. A trivalent (A, B, E) anti-toxin must be promptly administered intravenously with supportive care.
- Although most infants with botulism recover with supportive care alone, **antitoxin therapy is recommended.**

*Prevention and Control

- **Canned food** must be sufficiently heated to ensure destruction of spores. (نلاحظ انتفاخ المعلّبات)
- The risk from home-canned foods can be reduced if the food is **boiled for more than 20 minutes** at a very high temperature **(more than 100°C**) to ensure the destruction of spores before consumption.
- **No honey for first year infants** *(Honey is the most common vehicle for food botulism).*

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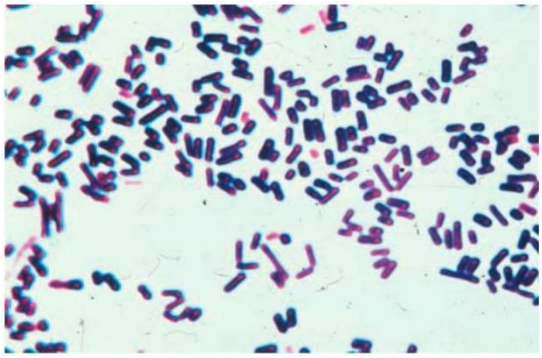
*Clostridia that produce invasive infections

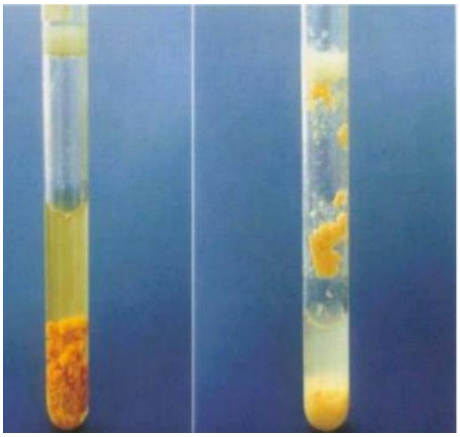
• Many different toxin-producing clostridia can produce invasive infections (including myonecrosis and gas gangrene) if introduced into damaged tissue. About 30 species of clostridia may produce such an effect, but the most common in invasive disease is **C. perfringens** (90%).

-An enterotoxin of C. perfringens is a common cause of food poisoning.



*Distinguishing Features

• Large gram-positive, **spore-forming rods** (*spores rare in tissue*)
***_**Non-motile (this is the exception)- No peritrichous flagella**_



• **Anaerobic:** **"stormy fermentation"** in milk media
***This is a characteristic of C. **perfringens - coagulation of milk in litmus milk test, in addition to gas formation.**

•**Double zone of hemolysis:** **Around the colony, we have a zone of beta hemolysis & another one of alpha hemolysis.**

• Reservoir - soil and human colon.
• Transmission - foodborne and traumatic implantation (on wounds during car accidents for example, causing gas gangrene).
* Gas Production.

*Pathogenesis

• In invasive clostridial infections, spores reach tissue either by contamination of traumatized areas ***(soil, feces)*** or from ***the intestinal tract.*** The spores germinate at low oxidation reduction potential; vegetative cells multiply, ferment carbohydrates present in tissue, and produce gas.

• Toxins have lethal, necrotizing, and hemolytic properties. The ***alpha (lecithinase)***, the ***theta (necrotizing)*** and the ***epsilon (edematous)*** toxins. Some strains of C. perfringens produce a powerful **enterotoxin** (in the GI tract) as well.

*Clinical Findings

- From a ***contaminated wound*** (eg, a compound fracture, postpartum uterus), the infection spreads in 1–3 days to produce crepitation in the subcutaneous tissue and muscle, foul-smelling discharge, rapidly progressing necrosis, fever, hemolysis, toxemia, shock, and death.

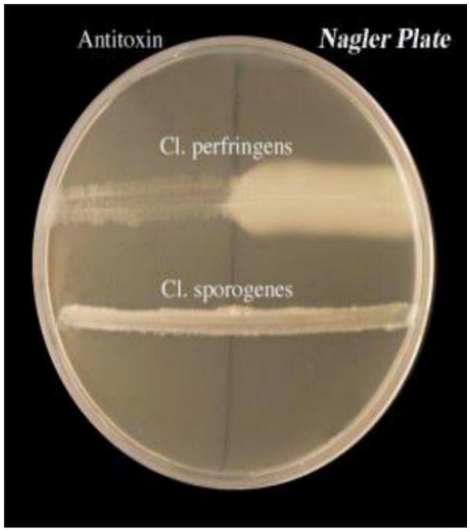
- C. perfringens food poisoning usually follows the ingestion of large numbers of clostridia that have grown in **warmed meat dishes**. The toxin forms when the organisms ***sporulate in the gut***, with the onset of diarrhea — usually without vomiting or fever — in 7–30 hours. The illness lasts only 1–2 days; usually self-limiting.

- C. perfringens causes **Endometritis -** inflammation of the inner lining of the uterus (endometrium) because it exists in the vagina of the female. Thus, it may cause post-partum syndrome or septic shock.

*Diagnostic Laboratory Tests

- Gram-stained smears of specimens from wounds, pus, and tissue.

- Culture material into **thioglycolate medium** (*a universal agar for anaerobes)* and onto blood agar plates incubated anaerobically. The growth from one of the media is transferred into milk.



** C. perfringens rarely produces spores when cultured on agar in the laboratory. If we grow them in a lab or in tissues, they don't show spores. But they're still considered **spore-forming**.

- Final identification rests on toxin production and neutralization by specific antitoxin. e.g

Nagler test - through which we put an antitoxin that's specific to C. perfringens toxin on the plate, if there's diffusion of the anti-toxin towards a strain producing toxin, this indicates a positive test.

*****Recall that it's not enough to detect the presence of bacteria. We have to check whether there's production of a toxin or not.**

*Treatment and Prevention

• Prompt and extensive ***surgical debridement of the involved area and excision of all devitalized tissue***, in which the organisms are prone to grow. (in case of gas gangrene – necrotizing fasciitis)

• Administration of antimicrobial drugs, particularly penicillin, is begun at the same time. Hyperbaric oxygen may be helpful; it is said to "detoxify" patients rapidly. ***(we use antimicrobials in the case of gas gangrene – necrotizing fasciitis).***

• Antitoxins are available against the toxins of C. perfringens, usually in the form of concentrated immunoglobulins. Antitoxins should not be relied on.

• Food poisoning caused by C. perfringens' enterotoxin usually requires ***symptomatic care*** **only** **(Fluid & Electrolyte replacement).**

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*Clostridium Difficile Infection (CDI)

-Epidemiology:

• Ubiquitous in the environment and colonizes the intestine of 50% of healthy neonates and 4% of healthy adults.

• A major cause of **healthcare - associated infections**; patients taking antibiotics, e.g. ***Cephalosporins, Clindamycin*** are at an increased risk of developing C. difficile antibiotic associated diarrhea.

Note: ***Antibiotic associated diarrhea is different from the transient diarrheal effect of antibiotics.

• This is due to suppression of the normal bowel flora and subsequent overgrowth of C. difficile. Infection may be **endogenous** *(taking into consideration that C. difficile colonizes the intestine)* or **exogenous** (*through ingestion of environmental spores*).

***The most common cause of nosocomial diarrhea is C. difficile.**

*Pathogenesis

➢Produces two major toxins: **Toxin A** (enterotoxin) and **Toxin B** (cytotoxin).

• Toxin A induces cytokine production with hypersecretion of fluid.

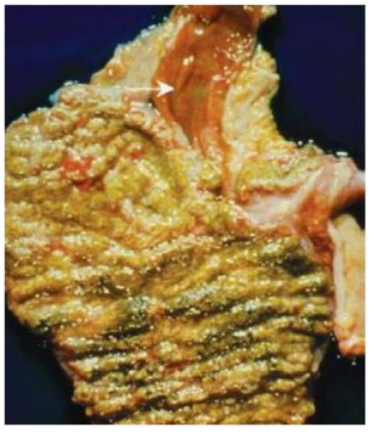
• Toxin B induces depolymerization of actin with loss of cytoskeleton. Adhesin factor and hyaluronidase production are also associated virulence factors.

• **Hypervirulent, hypertoxin producing strains are now recognised (e.g. ribotype 027, 078). – Very Important**

*Disease

➢**Antibiotic associated diarrhea -** Mild to moderate

➢**Pseudomembranous colitis (PMC), fulminant colitis -** Severe, considered a medical emergency because it may progress to toxic mega colon that needs surgical intervention.
You can observe the whitish membrane covering the colon in the picture. Micro-abscesses may also be observed.



*Diagnosis

❖The diagnosis of CDI is based on a combination of clinical criteria:

• (1) **Diarrhea** (≥3 unformed stools per 24 h for ≥2 days) with no other recognized cause.

• (2) **Toxin** A or B detected in the stool (e.g. ELISA, latex agglutination, and polymerase chain reaction (PCR)) or culture of C. difficile on selective agar.

• (3) **Pseudo membranes** seen in the **colon**.
❖PMC is a more advanced form of CDI and is visualized at endoscopy in only ~50% of patients with diarrhea who have a positive stool culture and toxin assay for **C. difficile**.

*Treatment and Prevention

- Discontinue other antibiotic therapy. If there's another infection that requires antibiotics, switch to a narrow spectrum one.

- **Oral** administration of **Vancomycin** or **Metronidazole (anti-parasitic or for anaerobic bacteria)** is recommended for CDI treatment.

***Very Important:** C. difficile Infection **is the only medical indication for oral Vancomycin. Limited use (consider resistance) for severe intractable (unstoppable) diarrhea.**
(Oral not IV.)

- Caution in overprescribing broad-spectrum antibiotics (limited-spectrum drugs should be considered first).

- In the nursing home setting, patients who are symptomatic should be isolated.

- Autoclave **(temperature of 121 °C & pressure)** bed pans to ensure the destruction of spores. Recall that the C. difficile infection is very bad, the spores might contaminate everything around e.g. fomites, surfaces, curtains. etc.

- Fecal Transplantation: Transmission of feces from a healthy person to a diseased one, by which the healthy microbiome will constitute the new microbiome of the diseased person. As a result, C.difficile isn't active anymore.

The End

May God Bless You All
