MICROBIOLOGY

بسم الله الرحمن الرحيم

MID | Lecture 2

Spore forming G+ bacteria

﴿ وَإِن تَتَوَلَّوْا يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَالَكُم ﴾

اللهم استعملنا ولا تستبدلنا

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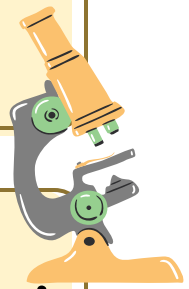

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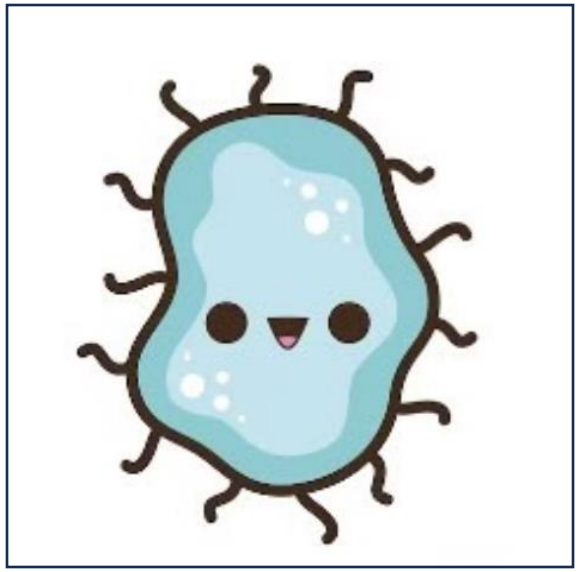


(اللهم يامعلم إبراهيم علمني ، ويامفهم سليمان فهمني ، ويامصبر ايوب صبرني ، ويامؤتي لقمان الحكمه
آتني الحكمه وفضل الخطاب ، اللهم علمني ما ينفعني وانفعني بما علمتني)

REMEMBER FROM GENERAL MICROBIOLOGY

- Remember:
- “When discussing bacterial spores, it’s important to mention that they are highly resistant to harsh environmental condition, making them ubiquitous in the environment(soil ,air, water), specifically Clostridium species which can be part of the normal flora of humans or animals.

Spore-Forming Gram-Positive Bacilli: Bacillus and Clostridium Species

Regarding gram-positive bacilli, there are four genera of major medical importance: Bacillus (mainly aerobic) and Clostridium (mainly anaerobic), both of which are spore-forming bacteria. The other two are Corynebacterium diphtheriae (which causes pseudomembrane formation in the throat) and Listeria monocytogenes (primarily associated with CNS involvement, but also capable of causing food poisoning from unpasteurized milk); both are **non-spore-forming** gram-positive bacilli.

By : Nader Alaridah MD , PhD

Bacillus Species

- The genus Bacillus includes large aerobic or facultatively anaerobic, gram-positive , spore forming rods occurring in chains.

- Saprophytic(meaning they are mostly found where there is decomposed organic matter so they are ubiquitous(, prevalent in soil, water, and air, such as Bacillus cereus and Bacillus subtilis (is a model organism in bacteriology, particularly used for studying chromosomal multiplication in bacteria and for its role in antibiotic production as well as probiotic production, but overall it does not seem to cause significant human disease except in immune compromised individuals).

❖ Some are insect pathogens, such as B thuringiensis which is found in insecticides.

❖ **B anthracis, which causes anthrax, B. cereus are the principal pathogens of the genus.**

- B.anthrax the causative agent of anthrax, and it has four clinical syndromes:
- 1-gastrointestinal anthrax (the rarest form)
- 2-inhalation anthrax(or woolsorter disease)
- 3-cutaneous anthrax (the most common form)
- 4-if it accessed the blood it could cause meningitis.

Bacillus cereus

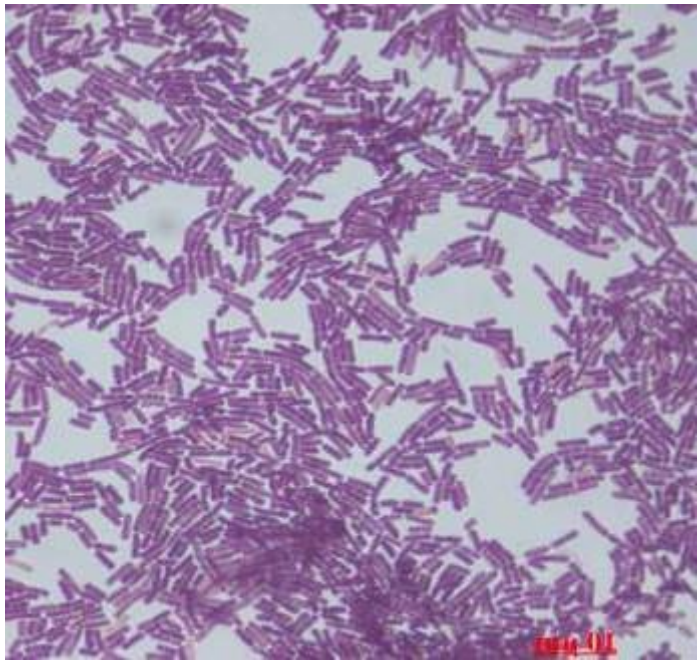
- Gram-positive aerobic or facultatively anaerobic(in the absence of oxygen), motile, spore-forming, rod- shaped bacterium that is widely distributed environmentally.

- *B. cereus* is associated mainly **with food poisoning**.

- B cereus has also been associated with localized(specially localized eye infection called endophthalmitis after ocular trauma involving soil or contaminated objects by the spores)and systemic infections, in immunocompromised patients it could lead to serious infections including endocarditis, meningitis (Transplant patients), osteomyelitis, and pneumonia; the presence of a medical device or intravenous drug use predisposes to these infections, and catheter associated septicemia.
- Enterotoxins are usually produced by bacteria outside the host and therefore cause symptoms soon after ingestion of *B. cereus*.

Morphology and identification

- A 3–4 µ m, 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: B.cereus produces large, wildly spread feathery colonies upon blood agar or in exclusive agar as MYP agar (Mannitol yolk polymyxin agar),while B.anthrix produces dry waxy colonies with a specific pathognomic appearance called **Medusa head colonies** .

B. anthracis

(رَبِّ إِنِّي لِمَا أَنزَلْتَ إِلَيَّ مِنْ خَيْرٍ فَقِيرٌ)

- Motility: B.cereus are motile while B.anthrax are not.
- Produce licithenase :B.cereus are positive for licithenase activity(a phospholipase c) meanwhile B.anthrax are not.
- β-hemolysis: B.cereus shows Beta-hemolysis, While B.anthrax lacks it.
- Antimicrobial susceptibility patterns:**B.cereus are inherently resistant to penicillin and cephalosporins** ,while B.anthrax could be sensitive to them(that is why the main stream of treatment for gastroenteritis is fluid and electrolytes replacement and no need for antibiotics treatment since they are inherently resistant to penicillin and cephalosporin).

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-stable toxin (cerulide) can also be produced which can survive "flash frying" and in open buffet and Chinese food where the rice is kept warm for long periods.
- 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 .

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

Pathogenesis

Food poisoning by B.cereus whether it is vomiting type or diarrheal type, both are **exotoxin mediated**, and the toxins that involved in that are:

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

Food poisoning

❖Food poisoning caused by B cereus has two distinct forms :

- The emetic type(vomiting type), which is associated with fried **rice**(flash frying) & cereals, it is **intoxication** that results from ingestion of a preformed, heat-stable toxin produced when cooked rice is left at room temperature, enabling spore germination and subsequent toxin production upon reheating.

- The diarrheal type, which is associated with meat dishes ,sauces and vegetables, results from ingestion of **food contaminated with the spores,** which germinate in the gastrointestinal tract and produce enterotoxins locally, leading to symptoms.

❖The enterotoxin may be preformed in the food or produced in the intestine.

Clinical features

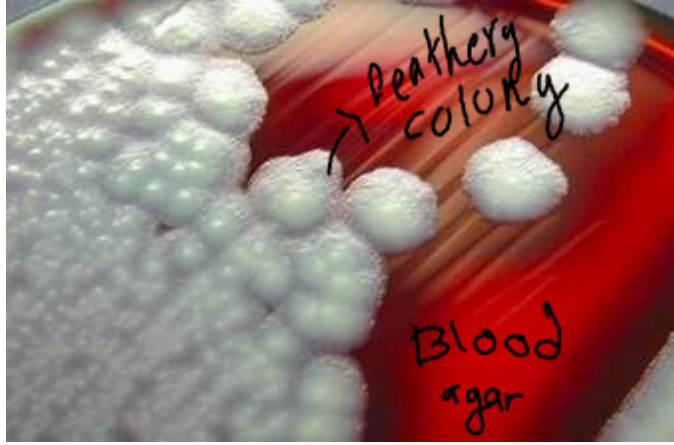
- There are two clinical syndromes produced by the toxins:

1- vomiting type –by a heat stable and acid resistant exotoxin(cerulide): Incubation period 0.5–6 hours(short incubation; **since the toxin is already preformed**),occasionally diarrhea and cramps can occur(rarely)with no fever. The illness is usually self-limiting and over in 24 hours.
The exact mechanism of the cerulide is not elucidated but it is believed that it acts as super antigen

2 - The diarrheal type-Heat labile toxin: Incubation period 6–15 hours (longer period) followed by an **illness similar to that seen with C. perfringens.** The diarrhea and abdominal cramps may be associated with nausea (vomiting is rare)with no fever, but are over in 24 hours ,usually self-limiting.

The difference in the incubation period is due to the state of ingested factor-> preformed, short incubation
Spore-> longer; it'll take time to germinate and release the toxin

Diagnosis and treatment



- Clinical grounds, most commonly to be used for diagnosis.

- Isolation of B. cereus from the suspect food, as well as from the stool or vomitus of the patient, which is used for epidemiological and public health purposes, usually we do not rely on feces or vomitus samples for diagnosis because the bacteria are ubiquitous in the environment, which makes it difficult to determine whether their presence is due to contamination or actual infection. Instead, we typically collect samples from food leftovers. To estimate the bacterial load in these samples, we use a standard index of $<10^5$ CFU/g. However, if feces or vomitus samples are used, a higher threshold—around $<10^6$ CFU/g—is generally considered.

- Culture and Gram stain of implicated material.

Treatment and prevention

In general, the general approach for gastroenteritis usually is fluid and electrolytes replacement, since they are mostly transient and self-limited(except in cases of salmonella ,shigella, and vibrio cholera where treatment tends to be more specified).

- Food-poisoning is self-limiting, therefore antimicrobial therapy is not normally required.

- B cereus is resistant to a variety of antimicrobial agents, including penicillins and cephalosporins.(meaning that there is no need for antibiotics treatment)

CLOSTRIDIUM SPECIES

they can be part of the normal flora of the GIT of humans and animals, and in the human female vagina (specifically C.perfringenes)

- 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 except for C.perfringenes which are non-motile and rarely to form spores in vivo nor on the laboratory media.

- Clostridia are anaerobes; a few species are aerotolerant such as C.tertium and C.histolyticum both of which can cause gas gangrene but very rarely . In general, the clostridia grow well on the blood-enriched media or other media used to grow anaerobes.

Since they are anaerobes they grow very well on thioglycollate(a special media for anaerobes growth) as well as blood culture under anaerobic conditions.

Species of Medical Importance

All of them cause exotoxin mediated diseases

- Clostridium tetani –produces tetanus spasmin which blocks inhibitory NTs like GABA and glycine, Rigid paralysis (tetanus), it has a toxoid vaccine (DTaP) a booster is taken every ten years.

- Clostridium botulinum-botulism by botulinum toxin, flaccid paralysis.

- Clostridium perfringens -gas gangrene & Food poisoning.
  + necrotizing fasciitis

These three are most related to Gi tract diseases:

- Clostridium difficile -pseudomembranous colitis.

#1 causes of nosocomial diarrhea (C. difficile)
CDI infection: mildest form -> antibiotics associated diarrhea
Severest form (common form) -> PMC

Clostridium botulinum

❖ Distinguishing Features:

- Anaerobic Endospore-forming gram-positive bacilli with subterminal spore location.

- Botulism is characterized by symmetrical, **descending**, flaccid paralysis usually beginning with cranial nerves then involving motor and autonomic nerves.

  Unlike Gillian Barré syndrome which starts in an ascending pattern (× aspirin for children with influenza and fever)

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

Pathogenesis

❖Botulinum toxin:

This is the most potent toxin to humans ,which is also used in the cosmetic industry in very minute amount(in nano grams),with a lethal dose for humans by 1 micro gram per Kg body weight (meaning that 70 micro gram dose is a lethal dose for a person weights 70Kg)

- Highly toxic neurotoxin-Coded for by a prophage-

- Seven Serotypes (A-G) including C1 and C2 based on the antigenicity of the botulinum toxin produced, mostly A ,B and E commonly to effect humans, less likely by F, so in cases of botulism one of the treatment options is BIG(botulism immune globulins)which is a trivalent antitoxin for these three major serotypes

The presence of the bacteria itself is not enough since it could be non-toxin producing strain, since that toxin is encoded by a prophage

Mechanism of action

- The most common offenders are spiced, smoked, **vacuum packed, or canned alkaline foods** that are eaten without cooking which contain the botulinum toxin causing the classical form of botulism(gastrointestinal botulism)
- . In such foods, spores of C botulinum germinate; that is, under anaerobic conditions, vegetative forms grow and produce toxin, One of the clues of Clostridium botulinum contamination in canned food is the presence of bulging or swelling in the can, which suggests gas production due to the protolytic enzymes of C.botulinum which produce gases.

- Absorbed by gut and carried by blood to peripheral nerve synapses.

- Blocks release of acetylcholine at the myo-neuronal(NMJ) junction resulting in a reversible flaccid paralysis.

Botulism

❖There are five clinical categories of botulism:

1) Foodborne botulism.

2) Wound botulism.

3) Infant botulism.

4) Adult infectious botulism.

5) Inadvertent, following botulinum IM toxin injection.

Details in the next slide

Notes regarding previous slide

- Infant botulism is most common in Canada and America, while locally food borne and adult botulism are more.
- Food borne and adult botulism have the same MOA, happen when eating spoiled canned food with preformed toxin (intoxication), then these toxins absorbed through gut and start exerting their effects.
- Wound botulism occurs when the wound is contaminated by spores, then spores germinate.
- Infant botulism occurs when infant eat spores, then spores germinate inside gut and liberate toxins, the most common vehicle is **honey.** Spores can't germinate in adults gut as the have mature microbiome.
- Inadvertent botulism occurs when using in inappropriate dose.

Clinical Findings

- Initial symptoms can 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 **in descending manner** . They are followed by inability to swallow, and speech difficulty. In severe cases, extensive respiratory muscle paralysis leads to ventilatory failure.
  - Motor function may be involved but **sensory** function remain **intact.**

- 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.

Diagnosis

- Observing the bacteria only isn't enough, **toxin-producing** C.botulinum must be observed.

- Toxin can often be demonstrated in serum, gastric secretions, or stool from the patient, and toxin may be found in leftover food using ELISAs and PCR.
  - It's a notifiable disease and public health authorities must be notified.
  - Specimens aren't handled in normal labs, instead they are sent to reference labs.
- Mouse bioassay is the test of choice for the confirmation of botulism.

Bacteria are injected intraperitoneally to mouse.
The definitive diagnosis of botulism is confirmed by the death of mouse, this test is called mouse biolethality assay.
If the mouse is antitoxin injected, it will survive.

Treatment

- Clinical advice : Vital signs like heart rate and respiratory rate should be continuously observed to be ready for any medical interventions like mechanical ventilation in case of respiratory muscle arrest.

- Supportive treatment, especially adequate mechanical ventilation, is of prime importance in the management of severe botulism .

- Surgical debridement in wound botulism.

- Antitoxin administration . **Botulism immunoglobulin BIG** A trivalent (A, B, E **the most common serotypes**) anti-toxin must be promptly administered intravenously with supportive care .

- Foodborne botulism is usually **self-limited**.

- 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 .
  - Discard bulged cans and those that have improper canning techniques and storage.
- The risk from home-canned foods can be reduced if the food is boiled for more than 20 minutes before consumption.

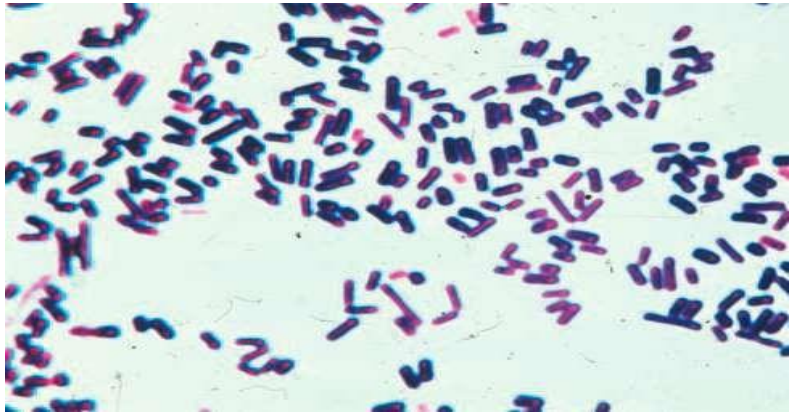
- No honey for the first year infants.

Clostridia that produce invasive infections

- Many different toxin-producing clostridia can produce invasive infection (including myonecrosis and gas gangrene) if introduced into damaged tissue post traffic accidents.
- 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 .especially type A

  - Type C C.perfringens causes bigbel (necrotizing enterocolitis) disease in neonates .
  - C.perfringens is associated with puerperal sepsis postpartum or after abortion causing septic shock. So, it can be found in GIT and vagina.

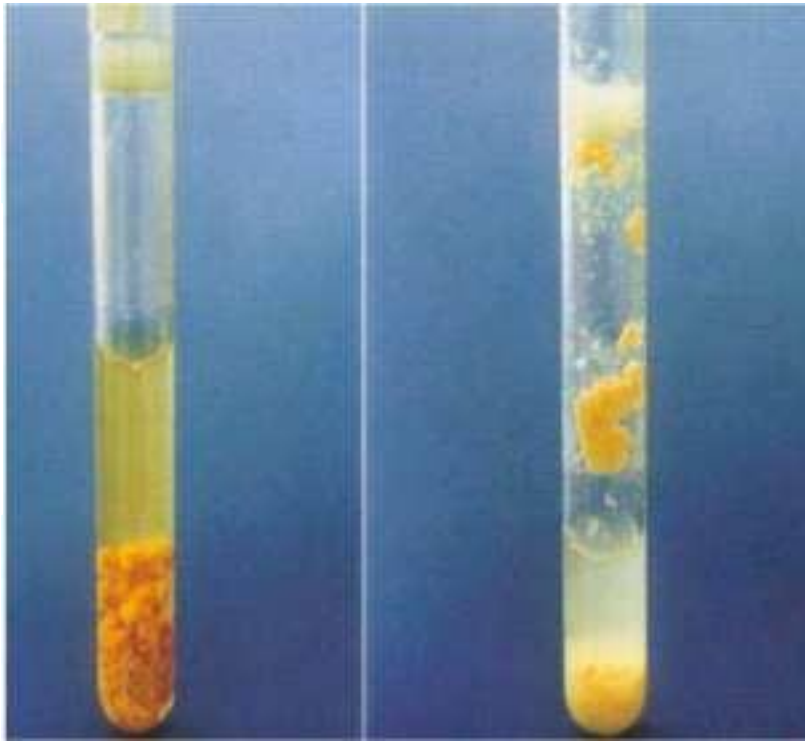


Distinguishing Features

- Large gram-positive, spore-forming rods (spores rare in tissue), **non-motile**

In contrast to other clostridium species

- Anaerobic: "stormy fermentation" in milk media
  - Litmus milk is converted into coagulant and gases in between coagulants.
- Double zone of hemolysis
  - Because more than one type of toxins are elaborated (alpha and beta toxins)
  - Alpha toxin is lecithinase (phospholipase), which is a major component of cell membrane(that's why spreading occurs).
  - Another types of toxins C.difficele produce :iota, epsilon, edema.
- Reservoir-soil and human colon
- Transmission---foodborne and traumatic implantation



Epidemiology

- C. perfringens is widely present in the environment, in the intestine of humans and domestic animals and can contaminate meat during preparation for consumption. Small numbers of microorganisms may survive subsequent cooking particularly in large pieces of meat, and multiply during the cooling down and storage resulting in food poisoning.

- A more serious but rare illness (necrotizing enteritis or pigbel disease) is caused by ingesting food contaminated with Type C strains.

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 α the theta toxins. Some strains of C. perfringens produce a powerful enterotoxin as well. CPE causes diarrhea.

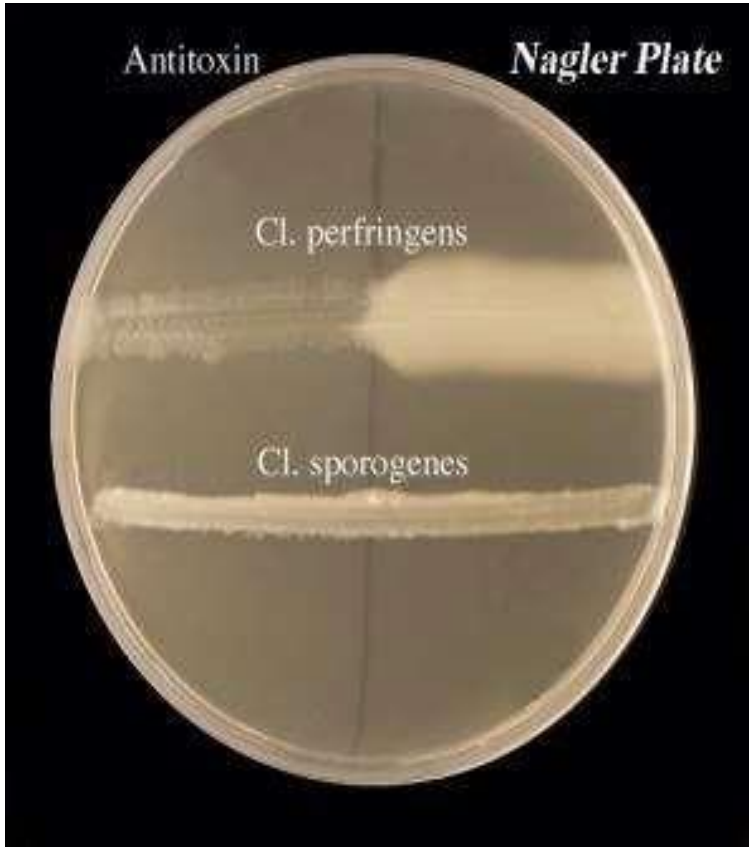
Clinical Findings

- From a contaminated wound (eg, a compound fracture, postpartum uterus), the infection spreads in 1–3 days to produce crepitation because of the presence of gas in the subcutaneous tissue and muscle, foul-smelling discharge as carbohydrates are fermented , 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 if bacteria have the ability to elaborate CPE, with the onset of diarrhea—usually without vomiting or fever—in 7–30 hours. The illness lasts only 1–2 days. It resembles the MOA of diarrheal type of B.cereus.

Diagnostic Laboratory Tests

- Gram-stained smears of specimens from wounds, pus, and tissue.
- Culture material into thioglycolate **universal agar to grow anaerobes** medium 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.
- Final identification rests on toxin production and neutralization by specific antitoxin.e.g. Nagler test.



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Naglar test

- It's the definitive diagnosis of **toxin producing** clostridium perfringens

➢ The agar is divided into two halves, antitoxin (lecithenase) is applied in one half, then we grow a sample C.perfringens on the agar ,if the antitoxin spread to the bacteria, zone of opalescence appears. This indicates that that the C.perfringens is **toxin-producing** strain, and the toxin is **lecithinase**.

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.

- Administration of antimicrobial drugs, particularly penicillin, is begun at the same time. Hyperbaric oxygen may be of helpful. It is said to “detoxify” patients rapidly.

➢ In contrast to botulism, **antibiotics are contraindicated** there as they may elaborate more toxins, patients are given BIG instead, and this trivalent antitoxin will **prevent further advancement** .

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

- Food poisoning caused by C perfringens enterotoxin usually requires only symptomatic care, and fluid ecetrolyte replacement.

Clostridium difficile infection (CDI)

Epidemiology

How is the most common nosocomial infection:

1. Endogenous= × toxin producing strain but can acquire it through prophage
2. Exogenous: unsterile beddings and inanimate objects collect the spores of toxin producing strains with antibiotic administration, the patient develops a CDI

- Ubiquitous in the environment and colonizes the intestine of 50% of healthy neonates and 4%(10% in some resources) of healthy adults.

So, C.difficle is part of normal flora, colonization doesn't mean disease.

- A major cause of healthcare-associated infection; patients taking antibiotics, e.g. cephalosporins, clindamycin are at increased risk of developing C. difficile antibiotic associated diarrhea.

- This is due to suppression of the normal bowel flora and subsequent overgrowth of C. difficile. Infection may be endogenous or exogenous (through ingestion of environmental spores).

- Mildest form of CDI is called Antibiotic associated diarrhea.
- Severest form is pseudomembranous colitis manfisted as plaques and micro-absecesses inside colon, they often coalesce to form pseudo membrane. PMC is treated by resection then anastomosis.

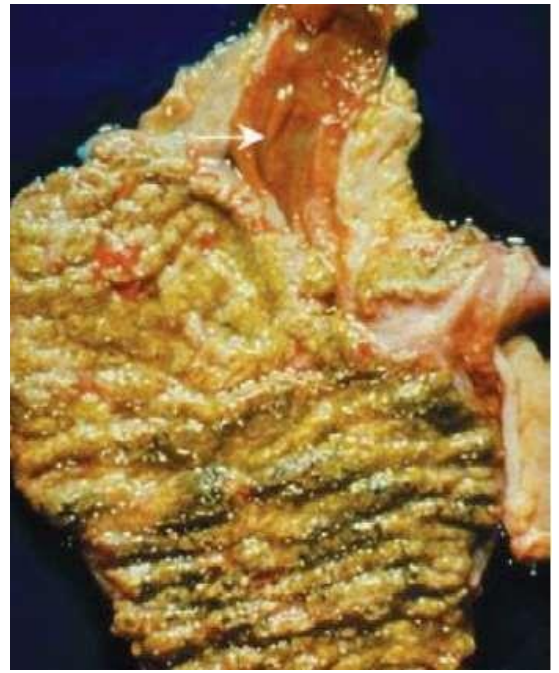
Pathogenesis

- Produces two major toxins: Toxin A (enterotoxin) and Toxin B (cytotoxin).
- Toxin A induces cytokine production with hypersecretion of fluid.
  - Toxin A is chemotactic for neutrophils
- Toxin B induces depolymerisation of actin with loss of cytoskeleton. Adhesin factor and hyaluronidase production are also associated virulence factors.
  - Toxin B excert its effects on small G protein coupled receptors, causing defects in actin cytoskeleton of enterocytes leads to their death. Consequently, plaques and micro abscess are formed -> PMC.
- Hypervirulent, hypertoxin producing strains now recognised (e.g. ribotype **027, 078**). Causes more severe diseases, with high recurrence and relapse rate

Disease

- Antibiotic associated diarrhoea,
- Mild to moderate.

- Pseudomembranous colitis (PMC), fulminant colitis.
- Severe forms



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 plus,

- (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
  - Toxin B is cytotoxic, should be incubated with human cell to observe cytotoxicity.

- (3) pseudomembranes 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 antibiotics therapy.

If antibiotics are given it is recommended to prescribe narrow spectrum antibiotics.

- Oral administration of vancomycin or metronidazole is recommended for CDI treatment.

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

Go to next slide for some notes ...

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

- Autoclave bed pans (treatment kills spores).

As spores are resistant to common disinfectants.

- PMC is the only condition where vancomycin is administered orally.

- The physician should avoid vancomycin as first-line therapy in mild cases and instead start treatment with metronidazole. However, if metronidazole fails or the case is moderate to severe, oral vancomycin is recommended. A single course may not be sufficient, and in some cases, after completing the first course, the patient may have a brief drug-free interval before beginning another course to enhance efficacy and prevent recurrence.

- FDA approved medication is fidaxamicim

- Another treatment (but it's not approved) is fecal microbial transplantation by enema . حقن شرجية

The End

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			

Additional Resources:

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