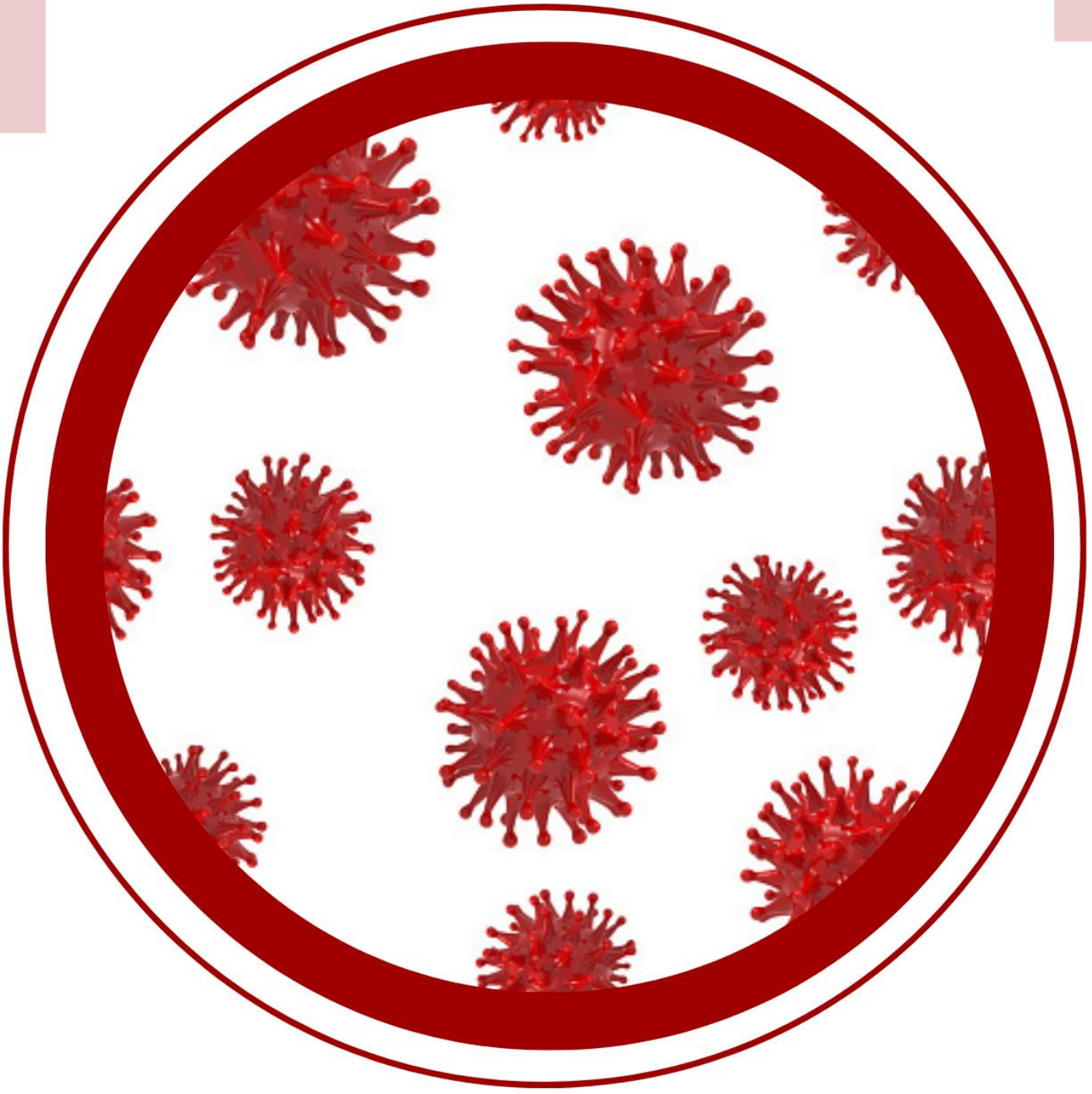
GI
MICROBIOLOGY

Sheet 2



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SPORE-FORMING GRAM-POSITIVE BACILLI: BACILLUS AND CLOSTRIDIUM SPECIES

In this lecture, we will focus on the most common spore forming gram-positive bacteria that cause GI infections. Including BACILLUS and CLOSTRIDIUM species, we will discuss the clinical manifestations, diagnosis, treatment, and prevention of these infections.

- These bacteria are ubiquitous in the environment and can survive harsh conditions, including exposure to heat, radiation, and disinfectants. That's because of **their spore forming ability**, that make them a potential source of infection for humans.
- While these two bacterial genera share some similarities, there are several differences between them. Such as their oxygen requirement, morphology, spore location, spore resistance and their pathogenicity, which we'll cover by the end of the lecture.

BACILLUS SPECIES (CEREUS, ANTHRACIS, SUCTILIS, THURINGIENSIS)

- **The genus Bacillus includes large aerobic or facultatively anaerobic, 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, which causes anthrax, B. cereus are the principal pathogens of the genus.**

- Most of the bacillus are motile, meaning they are capable of moving actively by flagella.
- Among the four species, two have been identified as potentially harmful to humans (anthracis, cereus), while the remaining two are not. In fact, the last two have useful applications in different fields. Thuringiensis toxin is used as a natural insecticide and subtilis bacterial chromosome has been extensively used in genetic studies including studies of chromosome translocation.

- Bacillus anthracis causes anthrax in 3 clinical forms
  1. Cutaneous anthrax (most common form)
  2. Inhalational anthrax (woolsorters disease)
  3. GI anthrax (extremely rare)

However, our primary focus is bacillus cereus species since it's usually associated with food poisoning and other GI infections.

BACILLUS CEREUS (ONE OF THE MOST COMMON CAUSES OF FOOD POISONING)

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

- ***B. cereus* is associated mainly with food poisoning** (with 2 clinical presentations; emetic and diarrheal).

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

- The most common extra-intestinal infection caused by B.cereus is ocular infections (endophthalmitis).
- In addition to the mentioned predisposing factors, immunocompromised patients are highly susceptible to these infections.
- **Enterotoxins are usually produced by bacteria outside the host and therefore cause symptoms soon after ingestion of *B. cereus*.**

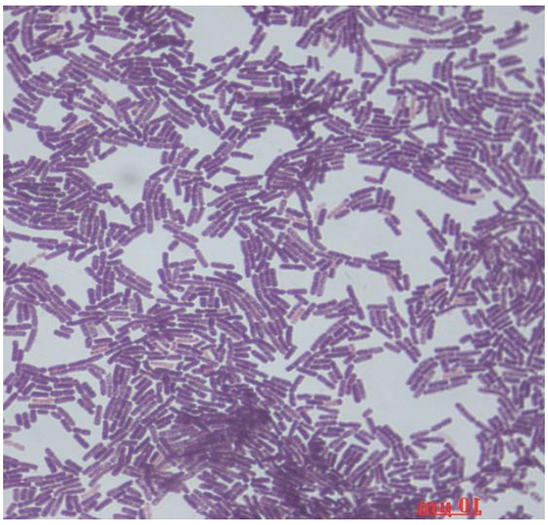
MORPHOLOGY AND IDENTIFICATION

- **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, β-hemolysis, motility, produce licithenase and antimicrobial susceptibility patterns.**

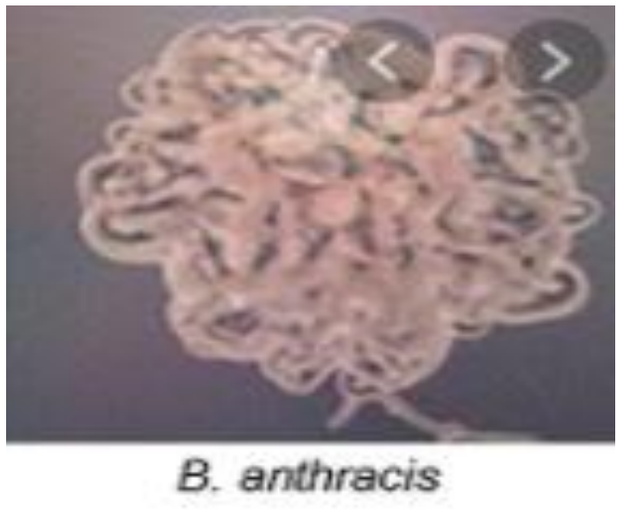
➢ The two species can be differentiated according to:

1. Their colony morphology; B.anthracis tend to have "medusa head" on culture and they have bamboo stick appearance under microscope, while B.cereus have more of feathery appearance on culture, with large square ends undermicroscope.
2. Their hemolytic activity; anthracis is generally non hemolytic, while cereus is hemolytic, so it forms a zone of hemolysis on blood agar plates.

3. Their motility, they both have the ability to move by means of peritrichous flagella. However, B.anthracis is described as being slower (or not motile).
4. Their antimicrobial susceptibility patterns, cereus is more resistant.



B.Cereus under microscope, notice their chain-arranged Rods.



B.Anthracis on culture with medusa head appearance (pathogenomic for B.A)

EPIDEMIOLOGY

- **The heat-resistant spores of B. cereus are widespread and contaminate rice and other cereals (food that grows in soil). the spores germinate if left at room temperature.**
- **A heat-labile toxin can also be produced which can survive "flash frying".**

➢ Flash frying is a cooking technique where food is quickly deep-fried in a hot oil for short period of time, the problem is that after cooling of food, B.cereus spores can germinate producing their toxins.

- **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

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

➢ According to its to clinical presentations of food poisoning, B.cereus produces two types of toxins

1. Exotoxins, emesis producing toxins: hemolysins, distinct phospholipases and cereulide.
2. Enterotoxins, diarrhea producing toxins: HBL, NHE, cytotoxin k.

FOOD POISONING

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

- **The emetic type, which is associated with fried rice & cereals.** (Chinese food poisoning)
- **The diarrheal type, which is associated with meat dishes and sauces.**

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

➢ Depending on the site of toxin release, food poisoning caused by cereus is classified into emetic and diarrheal forms. The emetic form is caused by ingestion of a preformed toxin, while the diarrheal form is caused by the ingestion of food contaminated with the bacteria itself or its spores, which will germinate in the gut producing its toxins.

CLINICAL FEATURES

- **There are two clinical syndromes produced by the toxins:**

1- vomiting type –heat stable toxin(cerulide): Incubation period 0.5–6 hours, occasionally diarrhea and cramps can occur. The illness is usually self-limiting and over in 24 hours.

➢ Since it's caused by ingestion of an already preformed toxin, its incubation period is usually shorter. it's presented mainly with nausea and vomiting and rarely with abdominal pain and diarrhea.

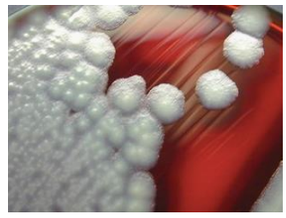
2- The diarrheal type-Heat labile toxin: Incubation period 6–15 hours followed by an illness similar to that seen with C. perfringens. The diarrhea and abdominal cramps may be associated with nausea (vomiting is rare) but are over in 24 hours.

➢ It's also a self-limiting illness, presented with abdominal cramps and diarrhea mainly, nausea and vomiting rarely. (both forms doesn't present with fever)

DIAGNOSIS AND TREATMENT

- **Clinical grounds.**
  - In cases of food poisoning, we don't usually need to identify the causative agents unless we have an outbreak.

- **Isolation of B. cereus from the suspect food** (the sample taken from food is more definitive), **as well as from the stool or vomitus of the patient.** (10^5/g of B.cereus colonies on a culture is considered diagnostic)



- **Culture and Gram stain of implicated material.**

TREATMENT AND PREVENTION

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

  - That include food poisoning caused by every other causative agent. Antimicrobial use is minimal or even contraindicated like in cases of food poisoning caused by enterohemorrhagic E coli.
    - **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.**

  - They are widely distributed in the environment, and they produce the most powerful toxin known to human kind (it's used in biological warefare).

- **Clostridia are strictly anaerobes; a few species are aerotolerant. In general, the clostridia grow well on the blood-enriched media or other media used to grow anaerobes.**

SPECIES OF MEDICAL IMPORTANCE

- **Clostridium tetani -tetanus, Rigid paralysis.** (Has no GI manifestations)

- **Clostridium botulinum-botulism, flaccid paralysis.**

- **Clostridium perfringens-gas gangrene(myonecrosis).** (Also one of the common causes of food poisoning)

- **Clostridium difficile -pseudomembranous colitis (severest form).**

(Antibiotic associated diarrhea in as a mild form)

- They all posses peritrichous flagella except for C.Perfringens that has “gliding movement”, it also has the weakest ability to form spores.
- Rigid paralysis caused by tetani which produces a powerful neurotoxin that blocks the release of inhibitory neurotransmitters, leading to excessive and uncontrolled muscle contraction.
- Flaccid paralysis caused by botulinum which produces a neurotoxin that inhibits the release of acetylcholine, a neurotransmitter essential for muscle contraction.

CLOSTRIDIUM BOTULINUM

❖ **Distinguishing Features:**

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

- **Botulism is characterized by symmetrical (bilateral), descending, flaccid paralysis of motor and autonomic nerves usually beginning with cranial nerves.**
- Ascending paralysis (guillain barre syndrome) is caused by shigella and campylobacter and its incidence can increase after GI bacterial infection.

- **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:**

- **Highly toxic neurotoxin-Coded for by a prophage-**

- **Seven Serotypes (A-G) based on the antigenicity of the botulinum toxin produced.**
- The most common serotypes affecting humans are A-B-E and rarely F. In the treatment of botulism, we use a trivalent antitoxin medication which is a combination of three different antitoxins, each specific to one the three serotypes (ABE)

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.**
- The most common vehicle food associated with botulism is canned food in adults, and honey in infants

- **Absorbed by gut and carried by blood to peripheral nerve synapses.**
- Just like B.cereus poisoning, the patient may either ingest contaminated food with already preformed toxin, or with the bacteria itself.

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

BOTULISM

❖ **There are five clinical categories of botulism:**

1) Foodborne botulism.

- Ingestion of contaminated food with preformed toxin (canned food)

2) Wound botulism.

- The highly susceptible group to wound botulism are individuals who use injection drugs, particularly heroin. The bacteria can grow in the heroin, and when the drug is injected into the body, it can introduce the bacteria and its toxin directly into the bloodstream (traumatic implantation).

3) Infant botulism.

- Honey contaminated with the bacteria itself, presents as floppy baby syndrome.

4) Adult infectious botulism.

- Very rare, it has the same mechanism of action as infant botulism (ingestion of bacteria itself)

5) Inadvertent, following botulinum IM toxin injection.

- It can occur in people who receive Botox injections, particularly if the injection is not administered correctly or in right dosage.

CLINICAL FINDINGS

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

(we start with GI manifestations because of the ingestion of the toxin)

- **Dry mouth, and diplopia, dysarthria (4Ds) and blurred vision are usually the earliest neurologic symptoms. They are followed by inability to swallow (dysphagia), and speech difficulty. In severe cases, extensive respiratory muscle paralysis leads to ventilatory failure.**

➢ In cases of severe botulism, the paralysis can affect the muscle responsible for breathing, leading to respiratory failure, hypoxia and ultimately death.

- **The infants in the first months of life develop poor feeding, continues crying, weakness, and signs of paralysis (floppy baby)** (poor muscle control and weakness)**. Infant botulism may be one of the causes of sudden infant death syndrome.**

DIAGNOSIS

- **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.**

➢ When we suspect botulism, it's not enough to simply find the bacteria itself, because its presence alone doesn't necessarily indicate the production of the botulinum toxin. So the definitive diagnosis is by identifying the toxin producing strains in serum, gastric secretions….. using ELISA (Detects the bacterial toxin or antibodies against it) and PCR (Detects the DNA of the bacterial toxin)

- **Mouse bioassay is the test of choice for the confirmation of botulism**

➢ The gold standard diagnosis mechanism of botulism, involves injecting a small amount of the patient's serum into a laboratory mouse and observing the mouse for signs of botulism.

TREATMENT

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

➢ Supportive treatment also includes fluid and electrolyte replacement therapy to maintain hydration and electrolyte balance, especially if the patient is experiencing vomiting and diarrhea.

- **Surgical debridement in wound botulism.**

- **Antitoxin administration. A trivalent (A, B, E) anti-toxin must be promptly administered intravenously with supportive care. (in western they give heptavalent)**

- **Although most infants with botulism recover with supportive care alone, antitoxin therapy is recommended.**

➢ NO ANTIBIOTIC TREATMENT

PREVENTION AND CONTROL

- **Canned food must be sufficiently heated to ensure destruction of spores.**
- **The risk from home-canned foods can be reduced if the food is boiled for more than 20 minutes before consumption.**

➢ Canned food must be avoided if it looks ill and bulging, and must be heated and stored properly. Anyways it's better to use fresh food instead of canned.

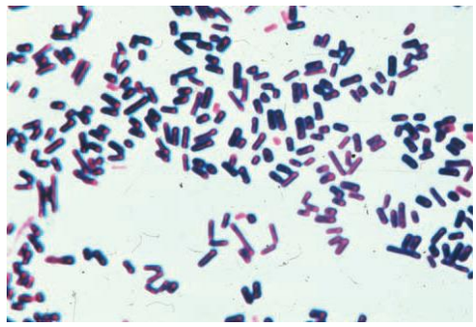
- **No honey for the first-year infants.**

CLOSTRIDIA THAT PRODUCE INVASIVE INFECTIONS (CLOSTRIDIUM PERFRINGENS)

- **Many different toxin-producing clostridia can produce invasive infection (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.**

➢ We have 2 types of C.perfringens depending on the toxin they produce

1. Type A; produces (alpha, theta, epsilon) and causes necrotizing fasciitis, gas gangrene and foodborne illness.
2. Type c; produces beta toxin and causes necrotic enteritis or necrotizing enterocolitis or pig-bell disease.

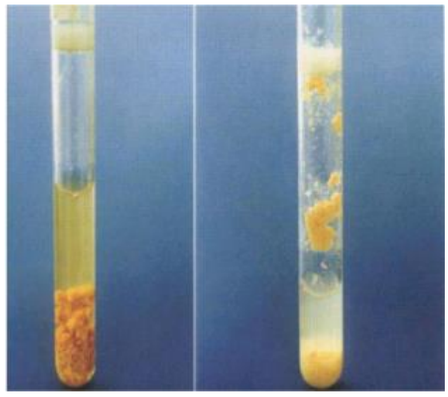


DISTINGUSHING FEATURES

- **Large gram-positive, spore-forming rods (spores rare in tissue and laboratory), non-motile.** (They have gliding movement)

- **Anaerobic: "stormy fermentation" in milk media**
  ➢ When C.perfringens is grown in a tube of milk agar, it can produce a stormy or turbulent fermentation pattern, which is caused by the formation of gas bubbles that rise to the surface of milk and create a frothy appearance.
- **Double zone of hemolysis**
  ➢ Another characteristic feature of C.P that can be observed on a blood agar, that it can produce two zones of hemolysis caused by the two toxins produced (alpha toxin which is a potent toxin that can lyse red blood cells, beta or theta toxin that are less potent)

- **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.**

➢ The main risk factor for C.P food poisoning is the consumption of foods that have been prepared in large quantities and left to stand at room temperature for extended periods, providing an ideal environment for the growth of C.P and its toxin production.

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

➢ Necrotizing enterocolitis is a serious GI disease that primarily affects pediatric population (premature infants and newborns), it's also related to sporadic meat consumption.

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. (causing crepitation, feeling bubbles under the skin)**

- **Toxins have lethal, necrotizing, and hemolytic properties. The α the theta toxins. Some strains of C. perfringens produce a powerful enterotoxin as well.**

- The enterotoxin produced by (CPE) causes GI infection similar to that caused by B.cereus specifically the diarrheal form
  *the emetic form of B.cereus infection resembles staph aureus food poisoning.

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. (GAS GANGRENE)**

- **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.**

- C.P food poisoning is usually associated with consuming meat dishes that cooled down with proper re-heating, and is characterized by abdominal cramps and diarrhea.

DIAGNOSTIC LABORATORY TESTS

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

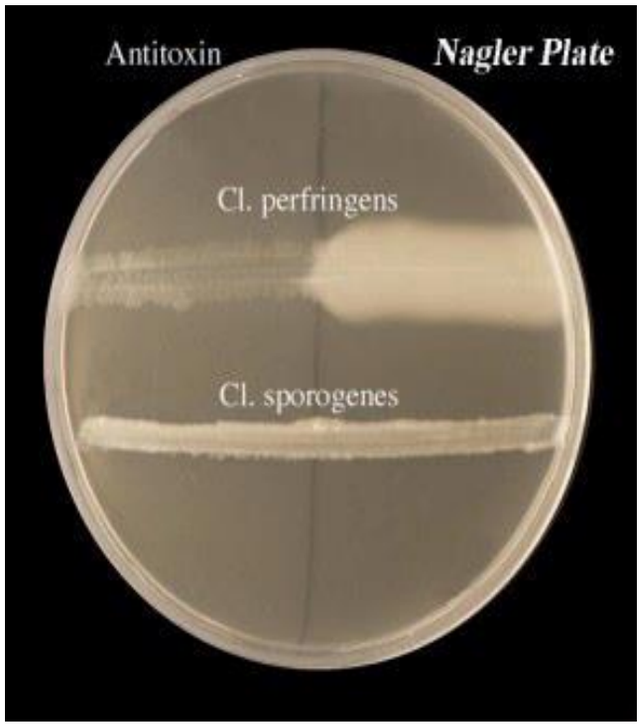
- Again, when suspecting an infection caused by C.P . it's not enough to identify the bacteria itself, but also the toxin producing bacteria in leftover food, pus and tissues.

- **Culture material into thioglycolate 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.**

- Naglar test is a laboratory test to identify the presence of C.P and its lecithinase activity. A small amount of this bacteria culture is mixed with egg yolk. If it's positive, it will break down the lecithin in the egg yolk causing a zone of clarity as seen in the picture.

- Notice the two zones, the pure one is where the antitoxin is. While the turbid one is where the antitoxin is absent.



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. (MYONECROSIS)**

- **Administration of antimicrobial drugs, particularly penicillin is begun at the same time** (cephalosporins can be used also)**. Hyperbaric oxygen may be of helpful. It is said to "detoxify" patients rapidly. (MYONECROSIS)**

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

- **Food poisoning caused by C perfringens enterotoxin usually requires only symptomatic care.** (self-limiting)

CLOSTRIDIUM DIFFICLE 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 infection; patients taking antibiotics, e.g. cephalosporins, clindamycin, fluoroquinolones, are at increased risk of developing C. difficile antibiotic associated diarrhea.**

➢ The risk of CDI is highest in healthcare settings, particularly in hospitals where patients are often on antibiotics and have weakened immune systems, the bacteria can be transmitted through contaminated surfaces, equipment, and hands of health care workers.

- **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).**

➢ Community-acquired C.difficile infections are less common but they do occur. They can be transmitted from person to person through the fecal-oral route, typically through contact with contaminated surfaces or objects.

PATHOGENESIS

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

1. **Toxin A induces cytokine production with hypersecretion of fluid.**
2. **Toxin B induces depolymerization of actin with loss of cytoskeleton.**

- **Adhesin factor and hyaluronidase production are also associated virulence factors.**

➢ Cytotoxins disrupt the cytoskeleton of host cells, including intestinal epithelial cells.

- **Hypervirulent, hypertoxin producing strains now recognized (e.g. ribotype 027, 078).**

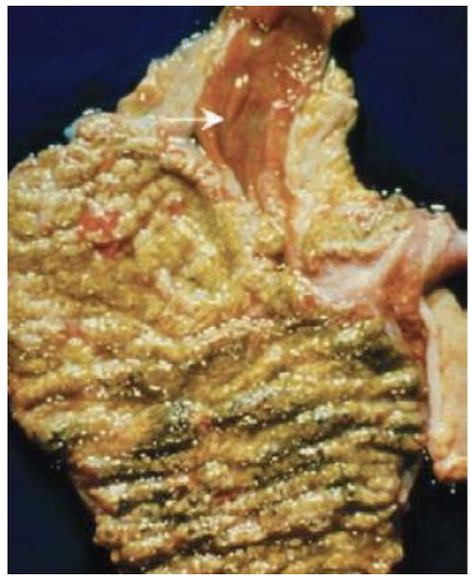
➢ Those strains are often associated with outbreaks in healthcare settings and have been associated with higher rates of mortality and morbidity.

DISEASE

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

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

- The figure shows an autopsy of a patient with pseudomembranous colitis involving cecum.
  As you can notice, the cecum appear dilated with thickened and inflamed wall, with yellowish plaques that form on the surface of mucosa composed of necrotic cells, inflammatory cells and bacterial cells.



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.**
- The diarrhea is defined as change in stool consistency or frequency.
  - **(2) toxin A or B (or both of them) detected in the stool (using: ELISA, latex agglutination, and polymerase chain reaction (PCR)) or culture of C. difficile on selective agar.**
  - **(3) pseudomembranous 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.** (Broad-spectrum antibiotics)

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

➢ IV and oral administration of metronidazole is typically used as the first line treatment, if it's not effective, oral administration of vancomycin is suggested.

➢ PAST PAPER QUESTION: oral vancomycin is only prescribed to treatment of C.D infection, otherwise it's given 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 bed pans (treatment kills spores).**

➢ Autoclave is a device that uses high pressure and temperature to kill microorganisms and bacteria, and is a common method of sterilization in healthcare settings.

GOOD LUCK ;)