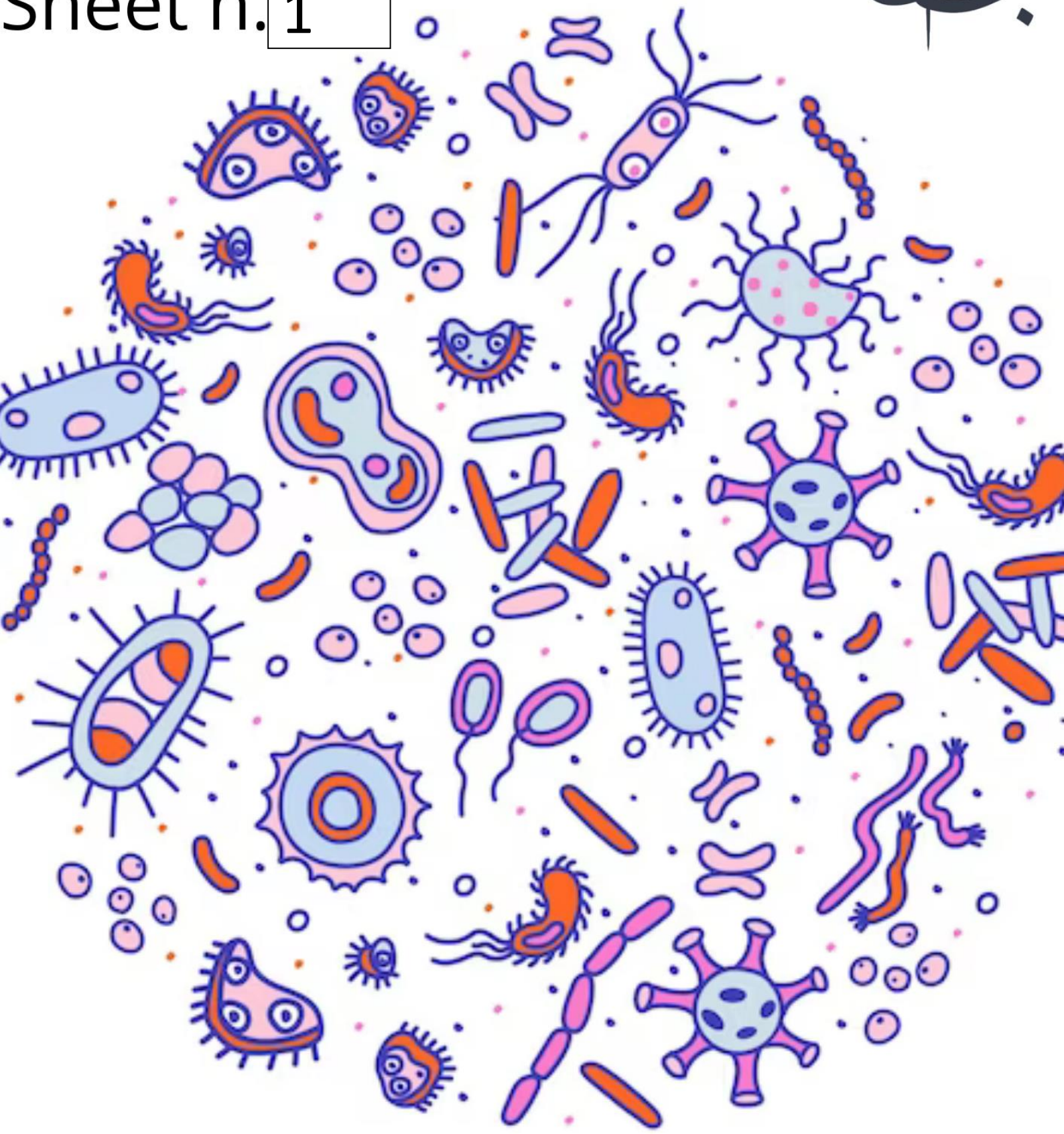
Microbiology

Sheet n. 1



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Green: Slides/Black:Doctor's notes/Purple:Additional information

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

In this sheet, we are going to talk about two genera of spore-forming gram + bacteria: bacillus and clostridium species.

As we talk about gram + bacilli, there are 4 genera of high-medical importance: the 2 mentioned above (spore-forming) and another 2 non-spore-forming.

The non-spore-forming G+ve bacteria are:

1) Listeria monocytogenes which causes listeriosis.
2) Corynebacteruim which causes diphtheria.

Back to spore-forming G+ve bacteria, they are classified based on their oxygen requirements, bacillus are aerobes while clostridium are anaerobes.

Remember: Spore-formation is a technique used in bacteria to withstand harsh conditions, and when these spores find the right environment & conditions they germinate (convert into vegetative cells).

NOTE: Focus on the distinguishing features (features that help us differ between bacteria).

BACILLUS SPECIES

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

There are 2 important species of highly medical importance regarding the bacillus (pathogenic to humans):

1) Bacillus anthracis that causes anthrax. Clinically speaking, there are 3 types of anthrax:

A. Cutaneous anthrax. Most common

B. Inhalation/Pulmonary anthrax (woolsorter's disease).

C. Gastrointestinal anthrax. Very rare

2) Bacillus cereus: a major cause of food poisoning.

Saprophytic(محللات), prevalent in soil, water, and air, such as Bacillus cereus and Bacillus subtilis.

They are widely spread (ubiquitous) and can deal with harsh environments due to spore-forming.

>>> note that there are more members of high medical importance but not pathogenic, like bacillus subtilis, which are used as role model of bacterial DNA replication.

🔎Saprophytic refers to organisms that obtain their nutrients from decaying organic matter.

Some are insect pathogens, such as B thuringiensis.

So, it's used as Insecticides. Not pathogenic to humans.

B anthracis, which cause anthrax, and B. cereus are the principal pathogens of the genus.

BACILLUS CEREUS

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

>>> it's motile unlike B.anthracis which is non-motile.

B. cereus is associated mainly with food poisoning.

There are two types related to food poisoning, vomiting type and diarrheal type.

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.

>>> rarely associated with localized and systemic infections. Localized infections are mainly in the eye which causes (conjunctivitis and endophthalmitis).

Regarding systemic infections (meningitis, endocarditis and pneumonia)—> immune-compromised patients, IV drug abusers and transplant patients are in higher risk to be infected.

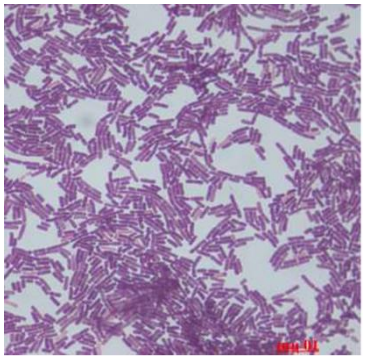
Enterotoxins are usually produced by bacteria outside the host and therefore cause symptoms soon after ingestion of B. cereus.

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.

-This image shows B. cereus under light microscope. ⟶



-Regarding licithenase activity, B. Cereus is positive.

*Licithenase is a phospholipase that disrupts cell membrane.

-colony morphology on blood agar: seems white, large, spreading "<u>feathery</u>". They cause hemolysis (beta hemolytic).

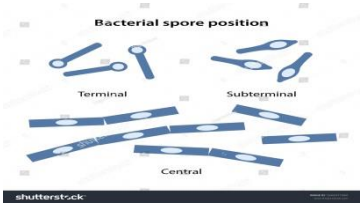
-beta lactamase activity is present, so it's <u>insensitive</u>(resistant) to penicillin and cephalosporins.

-this image shows B. anthracis. ⟶

-B anthracis' morphology under light microscope is called (boxcar-like).

-non-motile.

-In contrast, Bacillus anthracis spores are typically located subterminally within the bacterial cell. This means that they are positioned towards the end of the cell, but not right at the tip.



--Regarding licithenase activity, B. anthracis is negative.

- non-hemolytic.

-on blood agar, B. anthracis' appearance is summarized by <u>(medusa head colonies)</u>

--beta lactamase activity is not present, so it's <u>sensitive</u> to penicillin and cephalosporins.

	B. cereus	B. anthracis
Motility	Motile	Non-motile
Spores Location	In the center	Subterminal
Morphology Under Light Microscope	In chains	Box-car like
Hemolytic Activity on Blood agar	B-hemolysis	No hemolytic activity

Colony Morphology on Blood agar	Large white feathery spreading	Medusa Head
Licithenase Production	Present	Absent
B-lactamase Activity	Present	Absent

EPIDEMIOLOGY

-The heat-resistant spores of B. cereus are widespread and contaminate rice and other cereals. the spores germinate if left at room temperature. Mainly in vomiting type

-A heat-stable toxin can also be produced which can survive "flash frying".

>>> usually related to open-buffet, especially to the rice that is kept warm. The vomiting (emetic) form of food poisoning (also called Chinese food poisoning, or open-buffet poisoning) is caused by toxins that are heat-stable and acid-resistant. These toxins can survive (flash fry), which is a cooking technique in which rice is fried on high temperature for a short period of time.

>When we say that a microorganism or its spores are heat-resistant, it means they could survive exposure to high temperatures, to some extent, due to certain resistant structures or mechanisms. However, it doesn't necessarily mean they are completely invulnerable to heat. Heat stability varies among microorganisms and their spores.

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.

>> Don't forget that spores can survive very harsh environments, that's why they are ubiquitous in environment

PATHOGENESIS

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

These toxins listed above are secreted by B. cereus in food poisoning process. There are some notes must be mentioned:

*Distinct phospholipase is licithenase we mentioned previously.

*Emisis inducing toxin has other names (vomiting inducing toxin or cerulide toxin)

FOOD POISONING

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

• The emetic type, which is associated with fried rice & cereals.

Emetic means vomiting, so the chief complaint of the patients is vomiting and nausea, the causative agent is the cerulide toxin (emisis toxin).

• The diarrheal type, which is associated with meat dishes and sauces.

This type is mediated by the three pore-forming enterotoxins: hemolysin BL (HBL), nonhemolytic enterotoxin (NHE), and cytotoxin K. So, the chief complaint from the patient is mainly diarrhea.

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 –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.

***be aware that the toxins in this type are preformed outside the body, so the patient ingests the toxin and has a short incubation period. Usually, it's self-limiting and spontaneously resolving within 24 hours.

>So, it's considered **intoxication**.

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.

In this type, the patient ingests the food containing the spores, and those spores will germinate and become vegetative cells inside the body, then liberate the toxins, so the incubation period needs more time. What makes the watery diarrhea is the increased production of cAMP by the activation of adenylyl cyclase through the toxin, thus leading to hypersecretion of fluid and electrolytes.

>So it's considered **infection** followed by liberation of the toxins.

-Both Forms do not show Fever

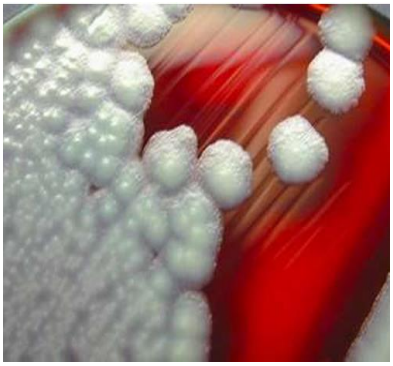
DIAGNOSIS AND TREATMENT

-Clinical grounds.

-Isolation of B. cereus from the suspect food, as well as from the stool or vomitus of the patient.

-Culture and Gram stain of implicated material.

Due to the short incubation period, the patient doesn't usually go to the doctor asking for help. The diagnosis is based on high suspicion of clinical grounds (Symptoms, Medical history, Physical examination). For epidemiological reasons, we isolate the species from food leftovers, or even from the patient's vomit or stools, but we usually depend more on food leftovers because the time frame of the tests are more than 24 hours and as we said previously it's a transient colonization that resolve under 24 hours which make the stool sample less used.



As we studied before, if we culture it on the blood agar we will see white, large, feathery spread colonies with beta hemolysis. And remember, in gram test it's +.

TREATMENT AND PREVENTION

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

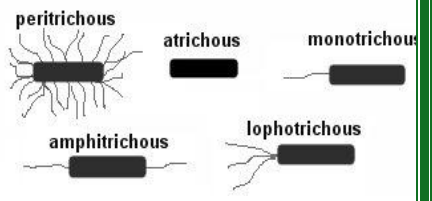
The patient does not need any medication, only supportive care if needed, especially fluid and electrolytes replacement in case of dehydration.

B cereus is resistant to a variety of antimicrobial agents, including penicillins and cephalosporins.

REMEMBER >>>Resistant due to beta lactamase activity. But in case of localized and systemic infections including pneumonia, meningitis and endocarditis, we give the patient vancomycin.

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.



Peritrichous flagella >>>flagella all around the cell which make them motile, **Except**: Clostridium perfringens that causes food poisoning and gas gangrene, it's non-motile.

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

There are 3 aerotolerant species: C.carnis, C.histolyticum and C.tertium.

SPECIES OF MEDICAL IMPORTANCE

In this genus there are more than 200 species, but those are the 4 medical importance:

- **Clostridium tetani -tetanus disease, Rigid paralysis.**

It causes tetanus disease by toxin-mediated mechanism, the toxin (Tetanospasmin) blocks the release of GABA (Inhibitory neurotransmitter), so the patient suffers from sustained muscle contraction >> rigid paralysis.

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

By Botulinum toxin, which inhibits release of acetylcholine (Excitatory neurotransmitter) at the neuromuscular junction, thus sustain muscle relaxation. >>> Symmetrical descending flaccid paralysis, and in infants it's called floppy baby syndrome.

- **Clostridium perfringens- gas gangrene & Food poisoning.**

In case of wounds, compound fractures and postpartum in women (could be the causative agent for toxic shock syndrome in postpartum women). It causes food poisoning by CBE toxin (Clostridium perfringens enterotoxin).

- **Clostridium difficile - pseudomembranous colitis.**

It causes Antibiotic-associated diarrhea (AAD) in its mildest form, and pseudomembranous colitis in its severest form. It's considered an infection control dilemma & the most nosocomial cause of diarrhea (The physician caused the patient to suffer from diarrhea). Its infection control is relatively hard because of its spores.

**An infection control dilemma refers to a situation where there are conflicting priorities or challenges in managing and preventing the spread of infections.

** Nosocomial causes refer to factors or conditions within a healthcare setting that contribute to the occurrence or transmission of infections. (just to understand)

CLOSTRIDIUM BOTULINUM:

DISTINGUISHING FEATURES:

-Anaerobic Endospore-forming gram-positive bacilli.

They are usually subterminal endospores. *C. botulinum* is characterized by a disease called Botulism.

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

(Opposite to Guillain-Barre syndrome which causes ascending paralysis)

-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.

(It colonizes the GIT of vertebrates, opposite to bacillus cereus which colonizes the GIT of invertebrates).

>Finding C. botulinum is problematic when it's a toxin-producing strain.

PATHOGENESIS:

Botulinum toxin:

-One of the most potent toxins on earth, the lethal dose is 1 microgram per kilogram body weight, if someone weighs 70 kg only 70 microgram of this toxin is considered a lethal (cause death) dose.

-It is used in Botox but in very small amounts.

Highly toxic neurotoxin-Coded for by a prophage/plasmid.

- *C. botulinum*, *C. Difficile* and *C. Perfringens* can be a part of normal flora, but the presence of the bacteria doesn't mean that it's a toxin producing strain. If this prophage is acquired & encoded in the bacteria then it becomes a toxin-producing strain.

Seven Serotypes (A-G) based on the antigenicity of the botulinum toxin produced (A, B and E, are the serotypes that infect humans).

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.

(If you find any bulging in food cans, this may indicates that there is a spoilage & presence of botulinum toxin, and this case is called foodborne botulism)

Absorbed by gut and carried by blood to peripheral nerve synapses, it blocks the release of acetylcholine at the myo-neuronal junction resulting in a reversible flaccid paralysis (sustained muscle relaxation).

BOTULISM:

There are five clinical categories of botulism: (keep in mind that 1&4 are the same and they are both caused by intoxication)

1) Foodborne botulism (eating canned food with the preformed toxin).

2) Wound botulism (compound fractures, cut wounds and postpartum) —> The spores are implanted on the wound, turn into vegetative cells, and release the toxin (Infection)

3) Infant botulism.

In this case, the contaminated food is honey that contains spores and these spores in infant's gut will transform into vegetative cells then they'll produce botulinum toxin so, it's an infection (not intoxication as in adult botulism). This case usually won't affect adults since they have mature immune system and microbiome (adults are affected by the toxin not spores).

4) Adult infectious botulism. (Eating canned food with

the preformed toxin).

A past exam question: Foodborne and adult infectious botulism (and vomiting type of B. cereus) are: Intoxication

5) Inadvertent (Unintentionally) **following botulinum IM toxin injection.** (the inappropriate usage of Botox for cosmetic and non-cosmetic reasons)

CLINICAL FINDINGS:

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

-Neurological manifestations: **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 ventilatory failure.

(The early manifestations are reversible and will resolve spontaneously after the half-life of toxin ends, but we give the patients with early symptoms antitoxins to prevent advancements and reaching respiratory paralysis in severe cases).

-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:

-Toxin can often be demonstrated in serum (especially in infants since it's rare to find the toxin in their stool), **gastric secretions, or stool from the patient, and toxin** or spores **may be found in leftover food using ELISAs and PCR.**

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

In mouse bioassay we inject the suspected specimen in a mouse intraperitonially, and the mouse will die immediately. However, if we give the mouse an antitoxin it can survive.

*A small note: Botulism is a notifiable disease so, if you suspect a case with botulism, you must notify public health. Also, not all clinical labs can cultivate *C. Botulinum*.

Another past paper question:

The gold standard of diagnosis for botulism is: Mouse lethality assay/ bioassay

TREATMENT:

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

Supportive treatment (We must secure the ABCs: Airway, Breathing, Circulation).

Mechanical ventilation is used in case of respiratory muscle paralysis.

Surgical debridement in wound botulism (in this case there'll be a traumatic implantation of C. Botulinum spores and in the wound, they will germinate and produce toxin).

Antitoxin administration. A trivalent (A, B, E) anti-toxin must be promptly administered intravenously with supportive care.

REMEMBER: Antitoxins don't remove the previously attached toxin but it prevents any further advancement.

Although most infants with botulism recover with supportive care alone, antitoxin therapy is recommended (better be safe than sorry 😊 so we give them the antitoxin).

*Notice that we give patients an antitoxin not an antibiotic, one of the theories explains that giving an antibiotic puts selective stress on C. Botulinum making it release more toxin. But in case of wound botulism because it is a localized infection we can give antibiotics.

PREVENTION AND CONTROL:

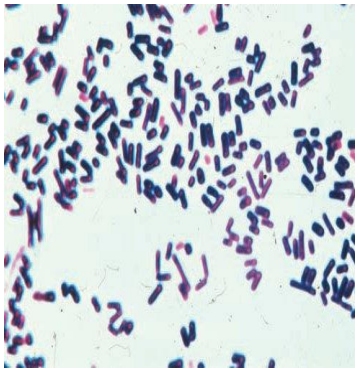
-Canned food must be sufficiently heated to ensure destruction of spores.

-The risk from home-canned foods can be reduced if the food is boiled for more than 20 minutes before consumption (denaturation or neutralization takes place that's how we get rid of these toxins).

-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 and Necrotizing fasciitis**) if** traumatic implantation of spores is **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.**

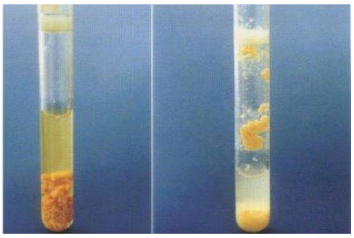


C. Perfringens might cause endometritis or toxic shock syndrome in females in postpartum period.

*It is characterized by gas in the subcutaneous tissue that's why if you palpate the affected tissue you'll find crepitation.

DISTINGUISHING FEATURES:

-Large gram-positive, spore-forming rods (spores rare in tissue) (we can rarely induce spore formation in vitro, they form spores mainly in vivo)**, non-motile** (this is an exception, all clostridia posses a peritrichous flagella except C. prefrengins).



-Anaerobic: "stormy fermentation" in litmus **milk media** we'll find coagulation, bubbles, gases and turbidity (look at the tube on the right).

-Double zone of hemolysis (beta and alpha hemolysis on blood agar).

-Reservoir-soil and human colon (it can be part of normal flora in colon and vaginal canal).

-Transmission---foodborne (food poisoning) **and traumatic implantation** (Gas gangrene**)**.

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.

Gas gangrene and food poisoning are mainly caused by type A, other types barely affect humans except type C.

A more serious but rare illness (necrotizing enteritis or pigbel disease in children and neonates**) 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 (causing crepitation).

Toxins have lethal, necrotizing, and hemolytic properties. The α and theta toxins. Some strains of C. perfringens produce a powerful enterotoxin as well. (foodborne mainly by the effect of CPE (Clostridium Perfringens Enterotoxin) but sometimes alpha and theta toxins have a role in the diarrhea caused by food poisoning).

(Alpha toxin is a lecithinase, so it disrupts cell membranes of enterocytes, lymphocytes and RBCs causing massive hemolysis and more invasive disease.

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 and abdominal cramps**— usually without vomiting or fever** or nausea**—in 7–30 hours. The illness lasts only 1–2 days** (self-resolving).

* C. perfringens can be part of normal flora of the large intestine not the small intestine, once they reach the small intestine they will multiply and release Clostridium perfringens enterotoxin (CPE).

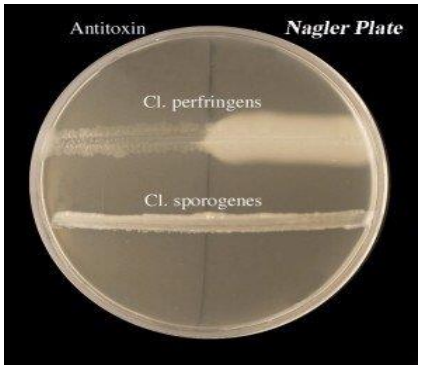
DIAGNOSTIC LABORATORY TESTS:

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

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.

*C. perfringens grow perfectly in thioglycolate, but we are not interested in their growth rather, we want to find the toxin or toxin producing strains so, the definitive diagnosis of C. perfringens is through Nagler test (the agar plate is divided into two halves, one with antitoxin and one without then we streak colonies of clostridium that were obtained from the specimen, if there are toxin producing strains the antitoxin will diffuse toward them (look at the top of the picture).



TREATMENT AND PREVENTION:

-In the case of gas gangrene; **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 helpful. It is said to "detoxify" patients rapidly.

-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.