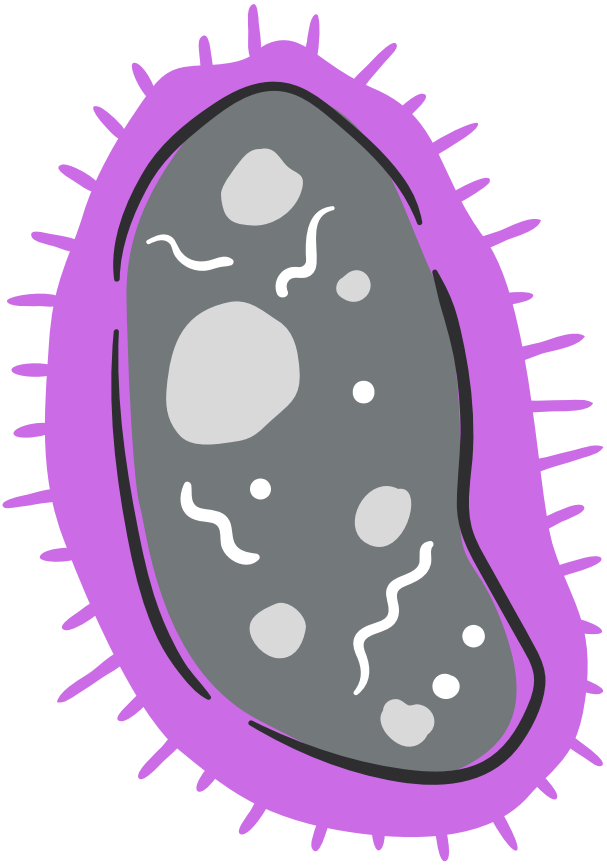
GIS

Sheet no.2

Microbiology



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Introduction:

This lecture's topic will be about spore forming gram positive bacilli; they form endospores, spores are resistant against heat, chemicals and disinfectants. Usually we're referring to two genres classified according to their oxygen requirement into:

1. Aerobes → *Bacillus*
2. Anaerobes → *Clostridium*

Bacillus species :

The genus Bacillus includes large strictly aerobic (some books may refer to them as facultative anaerobes but stick with what the doctor said that they're strictly aerobes), mobile, gram-positive , spore forming rods occurring in chains.

- They decompose organic compounds that's why they're prevalent in soil, water and air(Saprophytic) (in addition to Clostridium).
- Some are insect pathogens, such as B thuringiensis that doesn't cause human disease.

Those with medical importance include:

- Bacillus Anthracis: which causes **anthrax and** can be used as a **biological warfare.**

3 clinical forms of anthrax:

a) Cutaneous.
b) Pulmonary also called **Woolsorters' disease.**
c) Gastrointestinal; very rare.

- Bacillus Cereus: one of the most common causes of food poisoning, and it's the one that we're interested in in the gastrointestinal system.

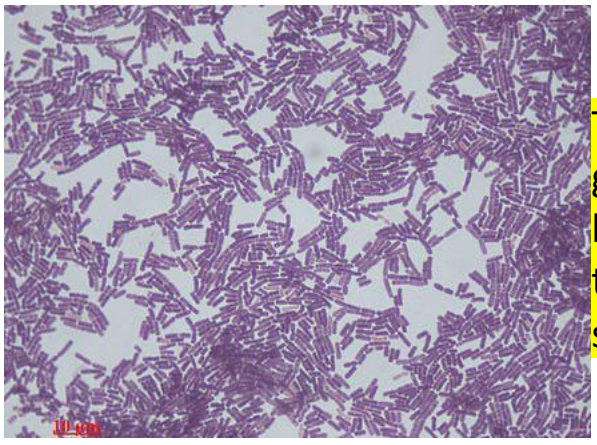
❖ Bacillus Cereus:

Again they're gram positive bacilli , aerobic, motile and widely distributed in the environment.
They are a common cause of bacterial food poisoning which can be shown in 2 forms (both are self-limiting):

a) Food poisoning by ingestion of food contaminated with **already formed toxin (cereulide) "vomiting toxin"** or
b) Food poisoning by ingestion of food contaminated with **spores** that will germinate in the gut then they will liberate the toxin **"diarrheal toxin"**.

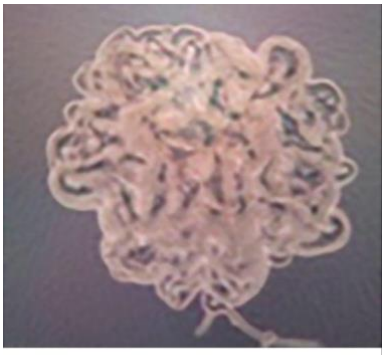
* Immunocompromised individuals and drug abusers are more prone to develop localized or systemic infections by B. Cereus other than food poisoning such as meningitis, endocarditis and osteomyelitis, eye infection, and Pneumonia; presence of prosthetic devices, or IV drug use predispose to these conditions.

- Morphology and identification



This is a gram staining of B. Cereus; gram positive bacilli/rods arranged in long chains with spores, spores are in the middle of the bacilli and have square ends.

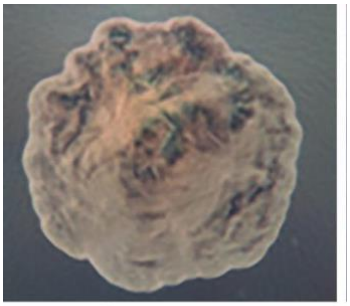
***B. Cereus are motile in contrast to B. Anthracis** (non motile); and this is one way to distinguish between B. Cereus and B. Anthracis. Another way is based on the hemolysis pattern; **B. Cereus produces a double zone of hemolysis while B. Anthracis doesn't**. In addition to that they differ in their colony morphology



B. anthracis

As you see here this is B. Anthracis and we refer to their morphology as "Medusa head"

(Large and dry medusa head colonies)



B. cereus

B. Cereus appear as (feathery, white spreading colonies)

-this pic was added for clarification it wasn't included in the slides-

Also they can be distinguished according to their antimicrobial susceptibility patterns; **B. Anthracis are susceptible to Penicillins and Cephalosporin while B. Cereus are not.**

Bacillus Cereus	Bacillus Anthracis
Gram positive/ spore forming	Gram positive/ spore forming
Aerobic	Aerobic
Motile	Non-Motile
Culture: Large white feathery colonies	Culture: Medusa heads
Double Beta hemolysis	No Beta hemolysis
High Lecithinase activity	Low Lecithinase activity
Resistant to antibiotics (i.e. Penicillins or Cephalosporins)	Sensitive to antibiotics
No vaccine	There is a vaccine for Bacillus Anthracis, and also a capsule consisting mainly of D-Glutamic acid.

Epidemiology

- The heat-resistant spores of B. cereus are widespread and contaminate rice and other cereals. pores 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 .]
- [Spores germinate when they come into contact with organic matter or within an insect or animal host.]

The last 2 points weren’t mentioned by the doctor but were present in the slides, any info stated between square brackets [ ] wasn’t mentioned during the lecture

Pathogenesis

Secreted toxins: hemolysins (since they have double hemolysis pattern) , [distinct phospholipases] , and an emesis-inducing toxin(by the vomiting type), and three pore-forming enterotoxins: hemolysin BL (HBL), nonhemolytic (theses 2 secreted by the diarrheal form) [enterotoxin (NHE)], and
cytotoxin K which can alter the cytoskeleton of the affected cell; actin and actin-myosin cytoskeleton.

Food poisoning and clinical features

Food poisoning caused by B cereus has two distinct forms :

• The emetic(vomiting) type, which is associated with fried rice & cereals (Chinese food) . In this type the toxin (cereulide) is already pre-formed and it’s heat stable it can survive flash frying so as soon as the patient ingest food that is contaminated with the enterotoxin symptoms will start to appear after a short incubation period ranges from 0.5~6 hrs.

Symptoms mainly include vomiting and abdominal cramps occasionally diarrhea may occur but very rarely.

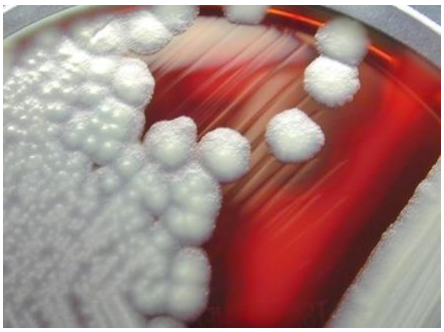
• The diarrheal type, which is associated with meat dishes and sauces. It occurs as a result of consuming meat dishes or sauces that were inadequately cooked, theses foods would be contaminated with B. Cereus bacteria and their spores once they're in the gut they germinate, sporulate and release enterotoxin which is heat labile (in contrast to the emetic one).

The outcome would be manifested as diarrhea and abdominal cramps may be associated with nausea and rarely vomiting, incubation period here is a bit longer ranging from 6~15hrs until the symptoms start to appear.

*Luckily in both types the disease is self limited, recovery within 1~2 days and the emetic type is even quicker.

• Diagnosis

Usually clinical grounds are enough through history and physical examination of the patient so no need for samples, if needed, samples can be obtained from vomitus(vomiting type) or stool(diarrheal type)and usually we don't rely on the patient's stool because B. Cereus are common contaminant of stool so we rely more on leftover food we look for the gram positive bacilli with their spores, bacterial count 10^5 or more is diagnostic in food leftover that the food is contaminated with B. Cereus or we can culture them on blood agar they appear as large white feathery spreading colonies (typical for b. Cereus)



• Treatment and management

First line of management is basically fluid and electrolyte replacement in diarrheal diseases or any disease that causes fluid and electrolyte loss then you do further assessments to decide whether there's a need for antibiotics or not but in this case usually we don't need antibiotics this is a benign food poisoning caused by B. Cereus and self recovery within 24 hrs but keep in mind that if you decided to give antibiotics B. Cereus are resistant against penicillins and cephalosporins so they won't do much.

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** (flagella on single pole).
- Clostridia are **anaerobes** -in contrast to bacillus-; a few species are aerotolerant such as C. Perfringens but In general they're considered anaerobes, the clostridia **grow well on the blood-enriched media** or other media used to grow anaerobes.

- Also they're ubiquitous and prevalent in the environment, again this is a feature of all gram positive bacilli.

4 Species with medical importance include:

- Clostridium tetani: causative agent of tetanus characterized with a neurotoxin called tetanospasmin which causes rigid paralysis. (not so important in this course since it affects the nervous system).

And those which we're interested in include

- Clostridium botulinum : causative agent of botulism characterized by "flaccid paralysis" especially in infants (floppy baby).
- Clostridium perfringens : common cause of gas gangrene (myonecrosis) and food poisoning.
- Clostridium difficile : a causative agent of antibiotic associated diarrhea or in its severest form "pseudomembranous colitis".

❖ Clostridium botulinum

Anaerobic, Endospore-forming gram positive Bacilli, and motile.There are many types of botulism but all of them characterized by symmetrical, descending, and flaccid paralysis starts from the cranial nerves and involve later on motor nerves.

Habitat : Since it is **found in the soil**, it may contaminate vegetables cultivated in or on the soil. They can be part of the normal flora of some individuals as well as the gastro -intestinal tract of other animals like fishes, birds and mammals .

● Pathogenesis

- Botulinum toxin: Highly toxic neurotoxin coded by prophages, it's the one of the most fatal toxins on earth dose of 1 microgram/ 1kg of body weight can be fatal and cause death

- Seven Serotypes (A-G) based on the antigenicity of the botulinum toxin produced; serotypes A,B,E,F are the ones that usually affect humans while serotype G doesn't cause disease.

- It's important to know that it's a toxin mediated disease meaning that the presence of the bacteria alone without the toxin doesn't indicate presence of the disease.

● Mechanism of action

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

* similar to Bacillus cereus Clostridium botulinum can also present as an already preformed toxin in one of these offenders or as -in the case if infant botulism for example- disease can be acquired through ingestion of food contaminated with bacteria and spores that will germinate, sporulate inside the guy then they will liberate toxin.
[Absorbed by gut and carried by blood to peripheral nerve synapses]

- toxin works by blocking release of acetylcholine at the myo-neuronal junction resulting in a **reversible** flaccid paralysis.Always keep in your mind that it's reversible flaccid paralysis in all its forms.

- Botulism

There are five clinical categories of botulism:

1) Foodborne botulism.

2) Wound botulism.

Adult botulism

3) Infant botulism.

-Common offender is honey, so honey is contraindicated in infants (babies with age of less than 12 months)

4) Adult infectious botulism.

5) Inadvertent, following botulinum IM toxin injection. This type started to appear only nowadays with the trend of “botox”, a medical worker may **unintentionally** induce botulism in the patient.

Important notes "يهمني تعرف"

Foodborne botulism caused by an already preformed toxin while in the case of infant botulism, the infant would consume honey that contains bacteria which germinate, sporulate in the gut and release the botulinum toxin.

*You should be able to distinguish between these two forms

Clinical findings

Initial symptoms in foodborne botulism can include **nausea**, **vomiting**, **abdominal cramps** [or diarrhea that begin 18–36 hours after ingestion of the toxic food], notice that there's no fever usually.

- Then cranial nerves symptoms will start to appear like dry mouth ,blurred vision, and diplopia (doubled vision),inability to swallow, and speech difficulty. After that motor symptoms will come to the scene as **symmetrical, descending flaccid paralysis.** In severe cases, extensive respiratory muscle paralysis leads to ventilatory failure.

- Symptoms of **infant botulism** start as crying, poor feeding, general weakness before reaching flaccid paralysis stage "floppy baby" in the last stage (in which the baby appears like a ribbon) and may be one of the causes of sudden infant death syndrome.

Diagnosis

Since it's a toxin mediated disease and Clostridium can be part of the normal flora without causing problems, assays must demonstrate **the presence of the toxin,** as we said previously the presence of the bacteria alone doesn't indicate the presence of the disease.

[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.]

- The gold standard method to diagnose botulism nowadays is through **mouse lethality assays** also called mouse bio-lethality assays by which we take a sample of gastric secretion or stool from the patient - few micrograms-, we inject the mouse with the sample intraperitoneally, if the botulinum toxin exist the mouse will die immediately.

● Treatment

- Supportive treatment, especially adequate mechanical ventilation, is of prime importance in the management of severe botulism that may cause respiratory muscle arrest, in this case we perform ABC procedure stands for (airway, breathing and circulation).

- In the case of wound botulism which is associated mainly with drug abusers where they get implanted spores of clostridium in their wounds resulting in wound botulism, treatment involves surgical debridement of the affected devitalized (necrotic) tissue.

- Antitoxin administration. A trivalent (A, B, E) anti-toxin must be promptly administered intravenously with supportive care, antitoxin is mostly useful in infant botulism while in adults or foodborne botulism usually we don't need antitoxin, usually al we need is supportive management and fluid and electrolyte replacement if needed. If the toxin is already bound, antitoxin won't help but it can be used to prevent further expansion of the paralysis.
And again all these symptoms are reversible.

● Prevention and control

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

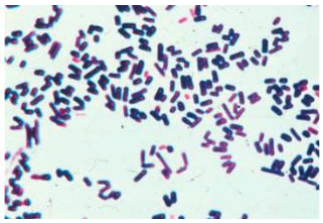
- The risk from home-canned foods can be reduced if the food is boiled or heated with a temperature of more than 60 °C or 65 °C for more than 20 minutes before consumption. And surely autoclave can also kill spores.
- No honey for the first year infants.

❖ Clostridium perfringens

(Clostridia that causes invasive infections)

- It's a common cause of food poisoning through liberation of enterotoxin and can produce invasive infection (including myonecrosis and gas gangrene) if introduced into damaged tissue.

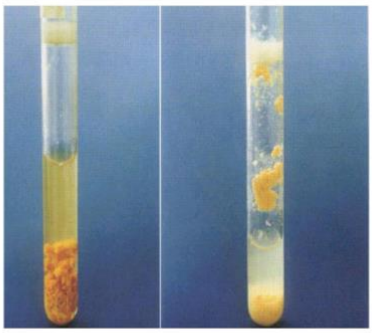
- C. Perfringens is the least spore former among other spore forming species either in laboratory media or in tissue.



● Distinguishing features

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

- They produce turbidity in litmus milk media in which milk is converted into a coagulum with entrapped bubbles of gas, this feature termed as “stormy fermentation” .



stormy fermentation

- They produce a double zone of hemolysis on blood agar.

- They live as saprophytes in soil and can be part of the microbiome of humans.

- Transmission: foodborne or traumatic implantation in the case of gas gangrene or myonecrosis.

- 2 types of enterotoxin of C. Perfringens
  - Type A ; responsible for food poisoning.
  - Type C ; responsible for a more serious but rare illness (necrotizing enteritis or pigbel disease) in newborns.

● Pathogenesis

- They have the ability to ferment carbohydrates present in tissue, and produce gas thus they give that crepitation sensation when palpating the affected tissue in gas gangrene.
[Toxins have lethal, necrotizing, and hemolytic properties. The α the theta toxins. Some strains of C. perfringens produce a powerful enterotoxin as well]

But what's more important to us in the gastrointestinal module that C. Perfringens is a common cause of food poisoning through elaboration of enterotoxin, usually type A, because type C is associated with necrotizing enteritis.

• 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.]

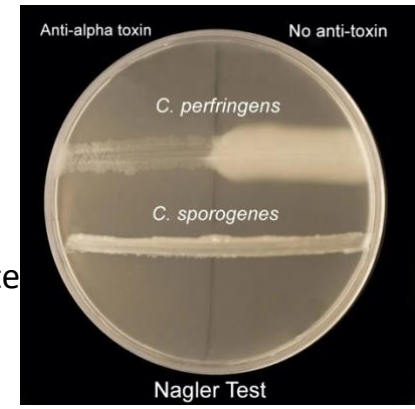
- Foods that are usually associated with C. Perfringens food poisoning include meat dishes, the toxin is formed when the organism sporulates inside the gut. **It's not a preformed toxin!!.**

- Symptoms include diarrhea usually without vomiting or fever, disease is self limited and lasts from 1~2 days.

• Diagnostic laboratory tests

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

[Culture material into thioglycollate 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.
You can use microscopic tests or cultures but we care about the most is whether the bacteria strain can liberate enterotoxin or not which is done by "nagler test"
Basically we use "nagler plate" which contains antitoxin→you culture
C. Perfringens on the nagler plate→ if the antitoxin diffused towards the C. Perfringens that you cultured means that **this strain does liberate the toxin.**

● Treatment and prevention

- In the case of gas gangrene or myonecrosis prompt and extensive surgical debridement of the involved area and excision of all devitalized tissue with administration of antimicrobial drugs, particularly penicillin and hyperbaric oxygen may be helpful.

- Antitoxins are available against the toxins of C perfringens but usually **not given.**

- Food poisoning caused by C. perfringens enterotoxin usually requires only symptomatic care (fluid and electrolyte replacement).

❖ Clostridium Difficile infection (CDI)

● Epidemiology

- They're ubiquitous in the environment as saprophytes and colonize the intestines of 50% of healthy neonates and 4% of healthy adults but don't forget that there are population and individual variations.

- A major cause of healthcare-associated infection(nosocomial); 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 which -as a result- gives more space and nutrients for the overgrowth of C. difficile.
Infection may be endogenous (from your own C. Difficle) or exogenous (getting C. Difficile strain or spores from your neighboring patient while you're admitted in the hospital.

- It's considered a control dilemma, so it's a problem in infection control in hospitals.

Disease

- Antibiotic associated diarrhea : in its mildest simplest form.

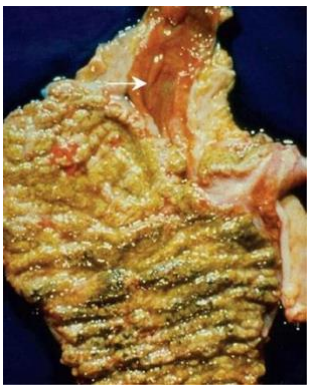
- The classic scenario starts with prescribing broad spectrum antibiotics such as penicillins, cephalosporins or clindamycin.

-After 2 days from the initiation of antibiotics administration the patient will have diarrhea (antibiotic associated diarrhea). It was found that C. Difficile is the responsible microorganism.

- If you look more in the history of the patient you will find out that even before taking penicillins or cephalosporins they had taken other broad spectrum antibiotics like tetracycline weeks or months ago, but at the time of the initiation of diarrhea the patient is usually taking clindamycin or cephalosporins.

- Pseudomembranous colitis (PMC) : severest form

-"Fulminant colitis" colon wall full with microabscess forming pseudomembrane on the mucosa.



Pathogenesis

Two types of toxins released:

- Toxin A ; **enterotoxin** induces cytokine production with **hypersecretion of fluid and diarrhea.**
- Toxin B ; **cytotoxin** induces depolymerization of actin with loss of cytoskeleton.

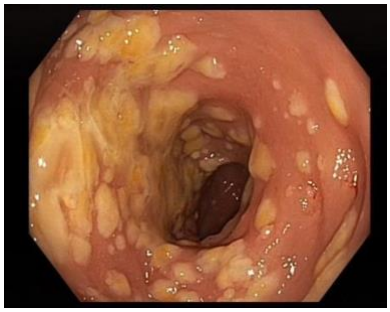
[Adhesin factor and hyaluronidase production are also associated virulence factors.]

- Hypervirulent, hyper toxin producing strains now recognised (e.g. **ribotype 027, 078**), this strain is causing many problems in hospitals around the world it's highly transmissible and resistant, affects mainly elderly people while they're admitted in the hospital and get persistent diarrhea that doesn't stop.

● 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 (we care about consistency and frequency of diarrhea), plus
2. Detection of toxin A or B in the stool (e.g. **ELISA**, **latex agglutination**, and **polymerase chain reaction (PCR)**)or culture of C. difficile on selective agar, since they might be found as a part of normal flora what we care about is whether this strain is liberating the toxin or not.
3. pseudomembranes seen in the colon by endoscopy.



This is how pseudomembranous colitis looks like through endoscopy(sigmoidoscopy).

this photo is extra just for more clarification

- 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.
- Oral administration of vancomycin or metronidazole(flagyl) is recommended for CDI treatment, note that this is the only case in medicine where we use **oral vancomycin** .
- In the nursing home setting, patients who are symptomatic should be isolated.
-Autoclave bedpans (treatment kills spores).

Questions of this lecture from **JAWETZ**
Medical Microbiology book

1- A housewife who lives on a small farm is brought to the emergency department complaining of double vision and difficulty talking. Within the past 2 hours, she noted a dry mouth and generalized weakness. Last night she served home-canned green beans as part of the meal. She tasted the beans before they were boiled. None of the other family members are ill. On examination, there is symmetrical descending paralysis of the cranial nerves, upper extremities, and trunk. The correct diagnosis is which one of the following?

(A) Tetanus

(B) Strychnine poisoning

(C) Botulism

(D) Morphine overdose

(E) Ricin intoxication

Answer: C

2- A food commonly associated with Bacillus cereus food posioning is

(A) Fried rice

(B) Baked potato

(C) Hot freshly steamed rice

(D) Green beans

(E) Honey

Answer: A

3- A 67-year-old man had surgery for a ruptured sigmoid colon diverticulum with an abscess. A repair was done, and the abscess was drained. He was treated with intravenous gentamicin and ampicillin. Ten days later and 4 days after being discharged from the hospital, the patient developed malaise, fever, and cramping abdominal pain. He had multiple episodes of diarrhea. His stool was positive for occult blood and the presence of polymorphonuclear cells. On sigmoidoscopy, the mucosa was erythematous and appeared to be inflamed, and there were many raised white to yellowish plaques 4–8 mm in diameter. Which of the following is the likely cause of the patient's problem

(A) Staphylococcus aureus enterotoxin

(B) Bacillus cereus toxin

(C) Clostridium difficile toxins

(D) Clostridium perfringens toxin

(E) Enterohemorrhagic Escherichia coli

Answer: C

4- Which of the following food items is most frequently associated with infant botulism?

(A) Corn syrup

(B) Canned infant formula

(C) Liquid multivitamins

(D) Honey

(E) Jarred baby food

Answer: D

5- All of the following statements regarding Clostridium perfringens are correct EXCEPT:

(A) It produces an enterotoxin.

(B) It produces a double zone of β -hemolysis when grown on blood agar.

(C) Some strains are aerotolerant.

(D) It is the most common cause of antibiotic-associated diarrhea.

(E) It can cause intravascular hemolysis.

Answer: D