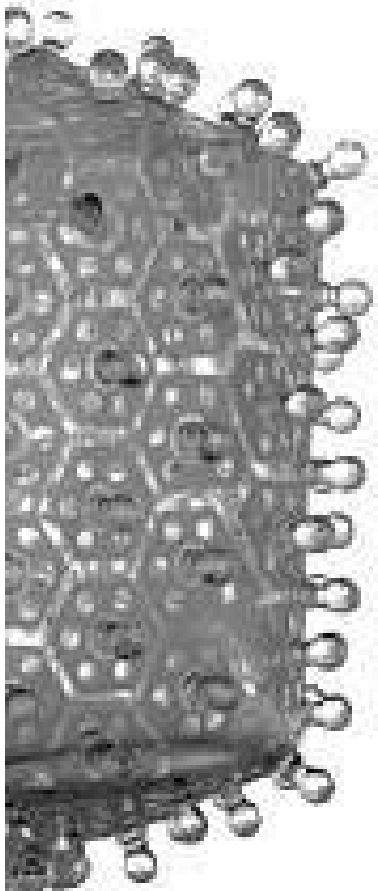


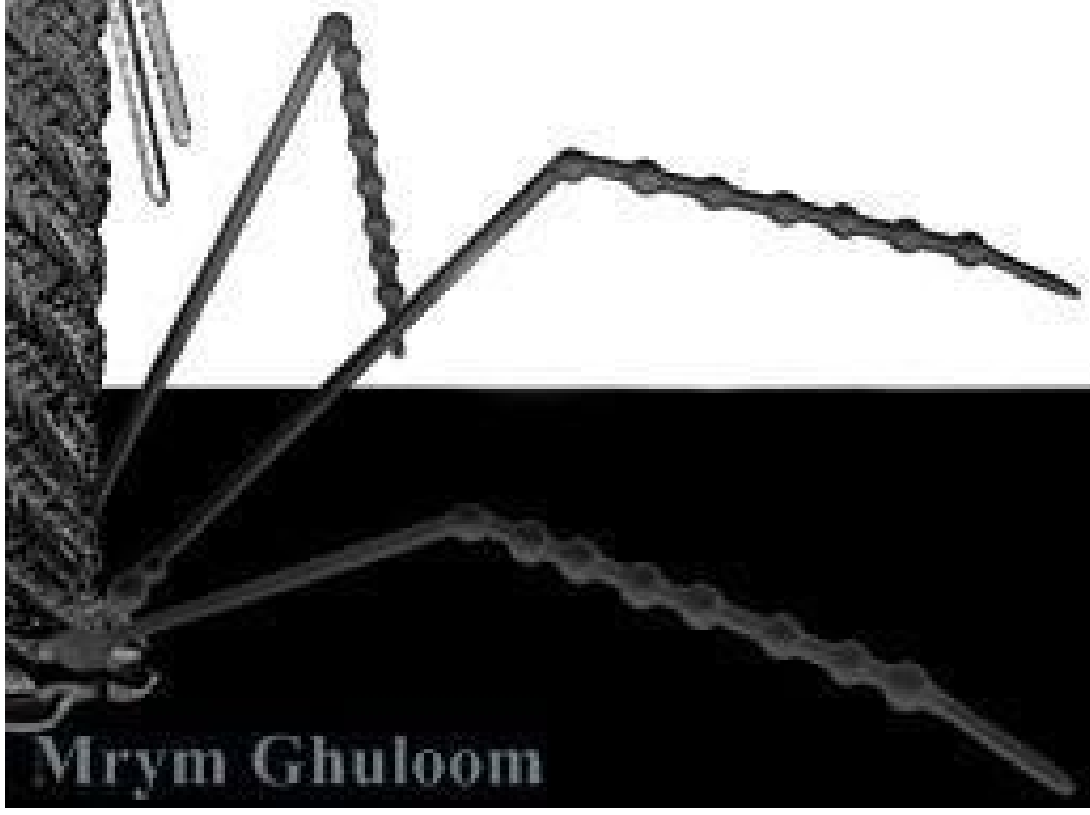
Microbiology

Lecture No: 19

Dr Name: Dr. Asem

Done by: Ali Khresat

Sheet ■ Slide □



Mrym Ghuloom

Anaerobic spore forming bacteria

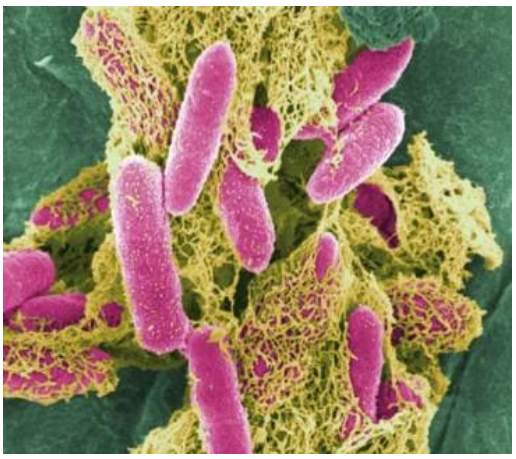
-If you are reading this sheet this means that you have survived the mid-exams week congratulations I hope everyone did well :D

-this lecture contains 2 topics :-

1-Anaerobic Spore forming bacteria

2- Gram Negative Coccobacilli

Clostridium Perfringens



-it's a gram positive rod shaped anaerobic spore forming bacteria

-it has an important clinical feature which is contamination of deep wounds and in fact there are many other species associated with that same clinical feature

- if these deep wounds (in the subcutaneous tissue) in any part of the body _especially the hands ,lens and legs_ were contaminated with this spore forming bacteria , once this bacteria manage to reach the damaged tissue it will **germinate** from the dormant spore to the vegetative form and start replicating, because in the damaged tissue there is an anaerobic condition because of the breakdown of blood supply there and the accumulation of the dead cells and this will enhance the growth of the spore forming bacteria to the vegetative form .

Written by Ali Khresat

-within a very short period after the contamination C.perfringens will release very potent toxins which will cause more severe damage , and increase the absorption of toxins by the cells increasing the damage more and more .

- The toxins that are secreted from C.Perfringines are :

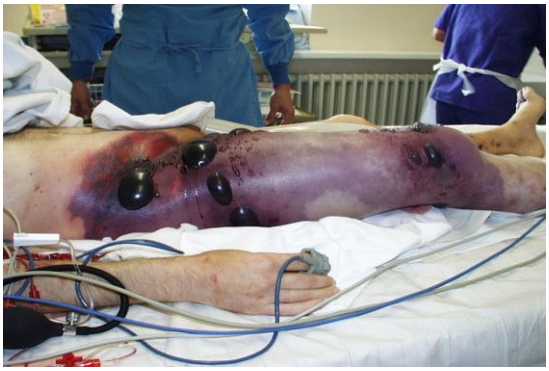
1- Hyaluronidase which breaks down Hyaluronic acid found in the infected connective tissue.

2- Collagenase which breaks down collagen fibers in the infected connective tissue

Both of toxins(Virulence factors) are spreading factors which are involved in the spreading of infection from the infected tissue to the non-infected tissue , and during this period the patient will be experiencing severe pain in the infected area and he might suffer from a mixed infection with other microorganisms other than C.Perfringines that contaminate wounds also .

In addition , this bacteria produces hemolysins which destroy RBCs by attacking their plasma membrane .

Complications associated with C.Perfringens :-



1-Gas Gangrene : it is a condition _which in association to infection and death to the tissue _ there are Gases in the infected tissue. The source of the Gases is from the fermentation process carried by C.Perfringens . Within less than 48 hours the Gangrene will spread from the lower part of the body to the upper part of the body causing death to the patient .

2- Myonecrosis: It is a condition of necrotic damage specific to the muscle tissue . Gas gangrene can cause Myonecrosis at the same time .

3- Cellulitis : inflammation of subcutaneous connective tissue.

✓ **Treatment**:

to treat this medical condition doctors should follow these steps :-

1 – Surgical Debridement : cleaning the wound by removing damaged tissue from the site of injury to improve the healing potential of the remaining healthy tissue .

2 – treatment with mixed antibiotics, at least 2 or 3 types of antibiotics should be given because usually the patient has mixed infection (caused by C.Perfringens and other Gram positive spore forming bacteria)

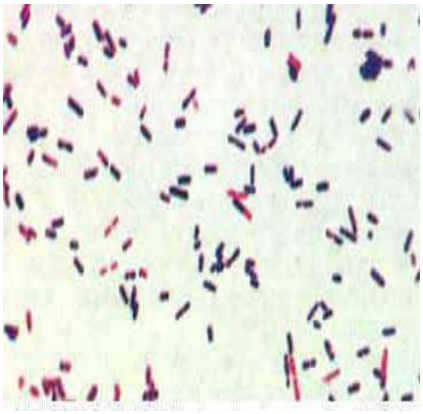
3- giving the patient supportive therapy if possible by keeping him in the hospital for awhile .

- if there was any delay in doing these treatment steps (especially Surgical Debridement), the limb that is infected by Gas Gangrene must be amputated -because the toxins that are secreted from C.Perfringens will spread everywhere in the body and affect all internal organs ,causing kidney failure and liver damage ,that's why the limb must be amputated , to prevent the spread of the toxin .
- there are no toxoids (vaccines and immunovaccines) against *Clostridium perfringens* to protect us from its

complications , that's because it produces a variety of toxins , not like *Clostridium tetani* which we can to some extent immunize the patient against its complications by vaccines .

- Clostridium perfringens is a common cause of food poisoning because it produces Enterotoxins (toxins secreted from the bacteria and targets the intestines causing poisoning) .Most of the cases of food poisoning by C.Pergringens are sub-clinical characterized by watery diarrhea ,abdominal cramps with no fever and can be treated by antibiotics.

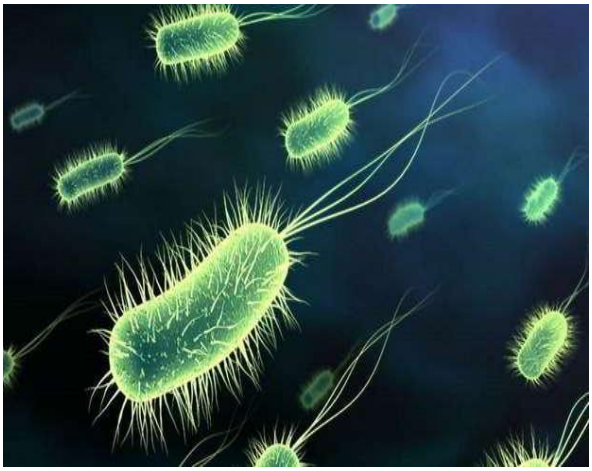
Lab diagnosis and culturing of C.Perfringens :-



-Like Clostridium Tetani, lab diagnosis of C.Perfringens is not easy and it is done by taking a specimen from the infected tissue , culturing it and applying gram stain on it and then the susceptibility test , or you can take an Aspirated fluid (fluid removed from the infected tissue using a needle or syringe) and then apply Gram stain on it , after applying gram stain the culture will be mixed containing Gram positive spores , gram negative spores .. etc, that's why it's hard to diagnose clostridium Perfringens by gram stain alone , so we use **PCR(Polymerase chain reaction)** to diagnose clostridium Perfringens specifically .

But the most important thing that must be done to prevent complications is to rely on the clinical picture for diagnosis , that means all wounds and injuries must be cleaned (Surgical Debridement) and treatment with mixed antibiotics .

Clostridium botulinum



-it is a bacteria that has the ability to produce a neurotoxin called Botulinum which cause a type of intoxication called Botulism .

- we can notice that not the microorganism itself that causes Botulism ,it's the toxin produced by it .

-this toxin is produced outside the body in some types of canned food(like meat, beans and fish) where Clostridium Botulinum can grow in the anaerobic environment provided by the canned food, which enables C.Botulinum to secret its toxin in the food, which will be later ingested by the human causing Botulism .

- Botulinum toxin is the most lethal toxin known in relation to its dose , 1 Microgram of it is enough to kill any person within 24 hours which indicates the high potency of this toxin .

-Botulinum toxin is Heat stable. It can't be inactivated by boiling the food for a short period (10 min or less), is requires at least 20 minutes to be inactivated at a temperature of 100 C^0.

-so the properties of Botulinum toxin is that it's:

1- highly potent

2- heat stable

3- it can attach rapidly to the CNS and inhibit nerve impulses causing respiratory arrest, heart failure and death

4- it inhibits the release of the neurotransmitter Ach.

Symptoms of Botulism :

*the slide says : Clinical symptoms begin 8-36 hours after toxin ingestion with weakness, dizziness, dryness mouth, Nausea, Neurologic features.. blurred vision, inability to swallow, difficulty in speech, weakness of skeletal muscles and respiratory Paralysis .

Symptoms are not easily recognized. It starts with constriction of the muscles of the jaw , so the person will have difficulty to open his mouth , and he will some have phobia towards water and noise . later on, there will be nausea and neurological symptoms .

Treatment of Botulism :-

-It's difficult to treat Botulism because of the lack of specific antibodies against Botulinum toxin in the 3rd world due to their high price but in countries like Russia and America they have Anti-Botulinum toxin against this type of disease .

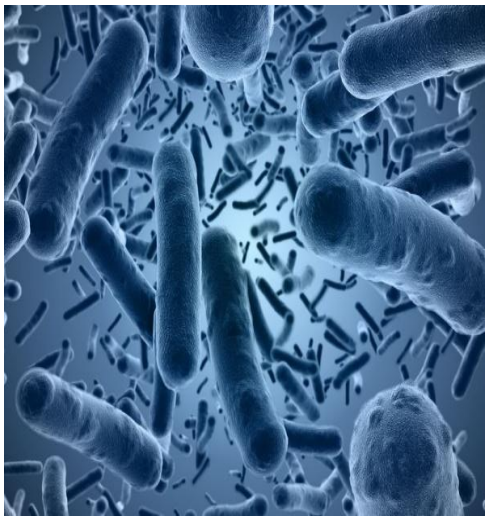
-botulism now is very rare in the world including Jordan , as autoclaves are used to sterilize cans used in manufacturing canned food , so the bacteria and its spores are killed.

- we don't give the patient Antibiotics because we are not dealing with infection that is caused by the microorganism itself , we are dealing with an intoxication from Botulinum toxin produced from the bacteria, not the bacteria itself .

- Note : Clostridium botulinum can be found in the intestine , but usually it is not harmful ,as the conditions in the intestine don't allow it to produce significant amount of the toxin that can cause side effects . it has

been isolated from the feces of normal animals and humans .

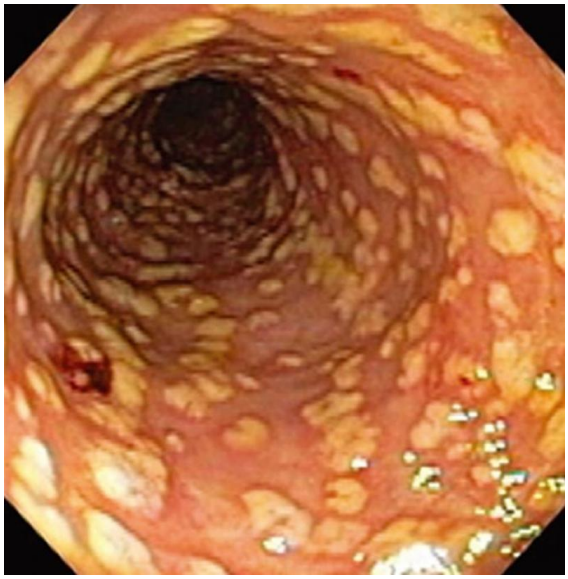
Clostridium difficile



-it's a spore forming bacteria which is found in the intestines as a normal flora of about 20% of the population .

- a study showed that the amount of *Clostridium difficile* in the intestine increases in hospitalized patients to be present in about 30% of the hospitalized patient population , and the patient's chance to be infected by C. difficile increases as the duration of hospitalization increases .

-long Antibiotic treatment specially the ones that are given *orally* like lincomycin , clindamycin (that are used to treat bone infections as they have the ability to penetrate the bone and inhibit gram +ve bacteria) and cephalosporines and amoxicillin causes decrease in the number of facultative anaerobic bacteria in the intestines (like E.coli and other enterobacteriaceae spp) and increase the number of Vegetative spore forming Clostridium Difficile (disruption of normal intestinal flora).



-under normal healthy intestinal flora C.difficile can't produce toxins, but any change in the pH or

disruption of the intestinal flora enhance the growth of Clostridium difficile and the production of toxins which cause a disease known as Pseudomembranous Colitis .

Pseudomembranous Colitis : is a sever inflammatory reaction that damages the large intestinal mucosa and it's characterized by bloody diarrhea and its associated later with the death of the patient especially in immune-comprised patients and elderly patients.

Types of toxins produced from C.difficile that cause pseudomembranous colitis :

1-Toxin A which is an enterotoxin that causes fluid accumulation in the intestines .

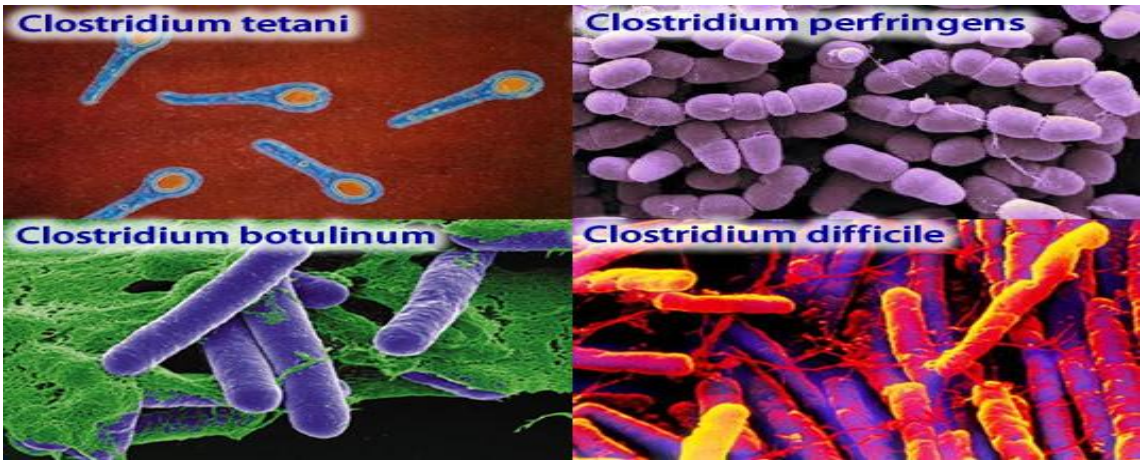
2- Toxin B : which is an extremely lethal Cytopathic toxin which kills the intestinal mucosa cells .

Treatment by stopping the use of antibiotics that C.difficile is resistant against (lincosamine ,cephalosporines) and replacing them with antibiotics that can kill C.difficile like Vancomycin and metronidazole .

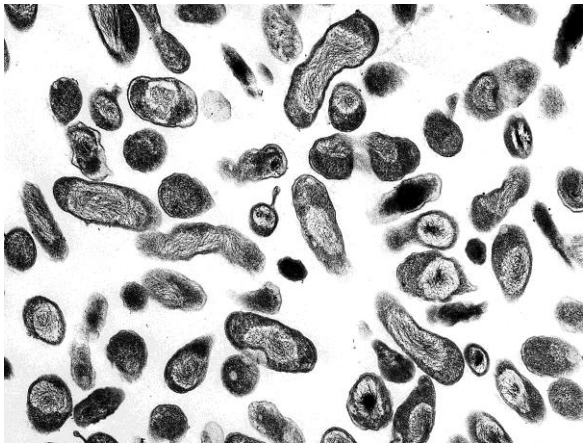
Lab diagnosis

By using a sample of the stool of a patient we can easily recognize the presence of toxins produced from C.difficile by immunological test (ELISA). There is no need to culture the bacteria , the toxins presented in the stool is enough to identify the presence of the disease .

*there is no vaccine available against C.difficile toxins *



Gram-negative coccobacilli



-it has an intermediate shape between cocci and bacilli. It might be recognized in single rounded cocci , diplococci , filamentous cocci or as very short rods ,that's why it might be mistaken with cocci .

-Gram negative coccobacilli includes : Haemophilus group ,Brodetella pertussis ,Chlamydia group and Neisseria.

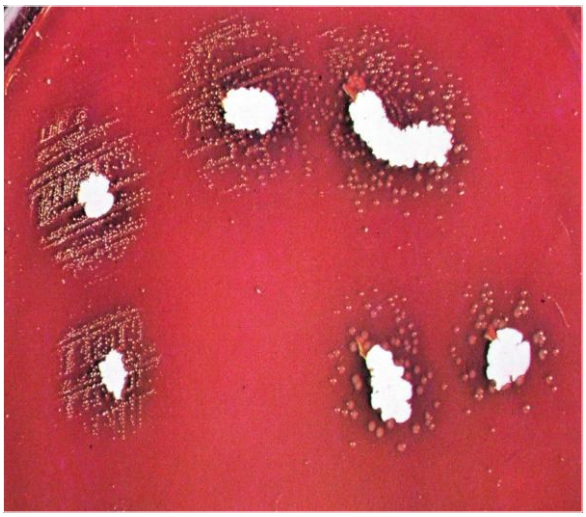
Haemophilus group

-gram negative coccobacilli which is a part of the respiratory tract normal flora.

-It's considered as microaerophilic as it grows under 5-10 % carbon dioxide, it's not a true facultative anaerobe.

- it's not easy to grow it on all culture media because these microorganisms requires a certain cofactors called **V-X-factor** and it grows around hemolytic Staphylococci species .

- Normally we use blood or chocolate agar to culture Haemophilus , at the beginning we grow Hemolytic staphylococci which will destroy the RBC'S releasing 2 types of factors, the first one is called Hemin factor (X factor) and the other is called Nicotinamid adenine dinucleotide (V factor - NAD) .



Both of these factors are required for Haemophilus growth, that's why we culture Haemophilus around Hemolytic staphylococci .

***Note :**

- we can either add these 2 factors on a blood agar to grow haemophilus without growing hemolytic staph or we can grow haemophilus around hemolytic staphylococci spp .

*When haemophilus is grown around staph , its growth is called as satellite phenomena .

-one of the most important clinical feature about Haemophilus is that it dies rapidly outside the body. This means when you collect a specimen from the respiratory tract or the cerebral spinal fluid (in case of meningitis in children which is caused by ***H.influenzae***)you must examine the species in the lab very quickly otherwise in less than 30 min it will die , due to the

activation of autolysins that will destroy the cell wall of this bacteria).

-Haemophilus bacteria is an opportunistic pathogen which means it increase it's pathogenicity in immune-comprised patients .

Haemophlius influenzae type B

-it's an encapsulated Haemophlius influenza .There are also many types of Haemophilius influenzae (type A,C,D) but they are not capsulated .

-it's highly pathogenic because of the presence of 2 virulence factors ***: Capsule and endotoxin (lipopolysaccharide)*** which is responsible for invasive infection

-it's more pathogenic in young children (from the age of 6 months – 5 years) than in adults because children haven't really developed a proper immunity system to protect them against the endotoxins of Haemophilus influenza ,therefore any inflammatory reaction in the upper respiratory tract causes the transfer of that species to the blood causing septicemia ,or to the CSF causing Meningitis .

-

Vaccination : we are lucky that there is a vaccine against Haemophilus called Hib-Vaccine that we give it to infants at 2,3,4 months of age .

Complications of Haemophilus influenza

-it's associated with tonsillitis in children and adults ,remember than Streptococci Pyogen is responsible for about 90 % of the cases of bacterial tonsillitis ,here haemophilus influenza is associated with few percentage .

-it has a very dangerous interaction with Streptococci pneumonia , when both of these bacteria are placed in the nasal cavity, the H.influenza survives because S.pneumonia will start attacking H.influenza which triggers an immune response against S.pneumonia eventually causing the death of S.pneumonia and the survival of Haemophilus influenza .

-Children might suffer from localized infections caused from Haemophilus like otitis media ,sinusitis , Conjunctivitis .

Treatment with antibiotics

It's difficult because Haemophilus develops resistance very rapidly, it can't be treated with B-lactam antibiotics because it has B-lactamase enzyme , but remember streptococci infections can be treated by penicillin .

Lab diagnosis

In a clinical case of meningitis caused by Haemophilus in children we have to do the following :

1-collect the specimen from the CSF and from the blood at the same time

2- culture the specimen in blood or chocolate agar

3- use V-X-factor to grow haemophilus and then antimicrobial susceptibility test .

*we have to consider during testing that many other microorganisms like Neisseria (which is gram –ve) and streptococcus pneumonia (gram +ve cocci) cause meningitis in children as well *

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