



University of Jordan
Faculty of Medicine



Medical Committee
The University of Jordan

Introduction to
Microbiology

Title :

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Types of Gram-Positive Bacilli
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: 16

Slides

Handout

Sheet

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♣ In this sheet :

We will continue with our topic about gram-positive bacilli, we have mentioned in the Previous sheet the first group which is corynebacterium diphtheriae, this sheet will talk about the second group which is **spore forming bacilli**. MRS chap.6 page 48

♣ Spore forming bacilli:

♦ Spore forming bacilli are divided according to growth pattern into:

(This is important in relation to the types of diseases associated with each group of bacilli and to the laboratory tests needed to diagnose infections caused by these bacteria.

1-Aerobic spore forming bacilli: we will take three types: (1-bacillus cereus, 2-bacillus subtilis, 3-bacillus anthracis).

2- Anaerobic spore forming bacilli also called “clostridia”: we will take four types: (1-clostridium tetani, 2-clostridium perfringens, 3-clostridium botulinum, 4-clostridium difficile).

Both Spore forming bacilli whether aerobic or anaerobic are widely distributed in nature and are associated with: soil, dust, vegetations, and dry materials. And they are found in large numbers in the intestines of humans and animals. So, they are found everywhere.

As an example if you simply put your hand on table and then plant a sample on a culture media you will recognize the presence of spore forming bacilli (aerobic or anaerobic), so it's often associated with our body and can easily contaminate our food. So, it's an important causative agent of food poisoning as we will see.

I-Aerobic spore forming bacilli “bacillus”:

If we look at the first group of aerobic spore forming bacilli, the growth can be recognized within 24-48 hour and this rapid growth is like facultative anaerobic either gram – or + .

The first group of importance in clinical medicine is:

1-bacillus cereus:

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Bacillus cereus as spore forming bacilli often associated with rice, especially dry rice, cooking the rice will not necessarily kill all spores ,cooking might kill only vegetative forms which has converted from spore forming to vegetative.

If you eat this cooked rice, in the first 24 hours you will not suffer from any type of illness, but if you keep this rice for another 24 hours at room temp, especially if there is still vegetative forms then you expect to elaborate a potent toxin called “heat stable enterotoxin of bacillus cereus” and this can be a causative agent of food poisoning which is manifested in the form of vomiting and diarrhea **but** not fever, because it’s a type of intoxication not a type of infection. (enterotoxin: type of chemical substance which act as a toxin but inside the intestine of the patient).

It’s more related to Chinese food especially (Fried rice) because frying rice might not necessarily kill all available spore forming bacilli or even vegetative form. Also, in association with our mansaf, if you eat remaining rice in 2nd or 3rd day, it might be associated with heat-stable enterotoxin of bacillus cereus.

bacillus cereus cannot invade tissues and due to this it is rarely associated with wound infections and sepsis etc. , it is more related with intoxication and often there is no need to give an antibiotic because we are dealing with toxins not with infections “not the pathogen itself “ ,and there is no vaccine available.

Bacillus cereus is one of the common causative agents of food poisoning in our country exactly like Staphylococcus aureus both they have similar clinical features and often we can’t distinguish between them.

2-bacillus subtilis :

Opportunistic pathogen, less virulent and it found in our skin and maybe in our intestine. And only under certain conditions, especially if there is immunocompromised condition “immune deficiency “ , the organism can be invasive and might produce sepsis or wound infection (in contrast to B.cereus which is usually not associated with such feature)

(Opportunistic bacteria: bacteria that usually don’t cause disease in a healthy host until there is an opportunity for them like when the host’s immune system fails, they cause infection).

3-bacillus anthracis:

It is a very common spore forming large bacilli found in intestines in large animals like camels, horses and cattle and less found in smaller animals like sheep and dogs etc.

◆There are three patterns of infection by these bacteria:

1-During animal grazing (eating grass) animals may suffer from injuries in their intestines and if these injuries allow a certain number of bacilli to reach their internal organs and blood stream, the end result will be fatal because bacillus anthracis produce severe necrosis in liver and gall bladder and other internal organs leading to death. These organs become so dark and black and that’s what the term “anthracis” refers to (the blackness).

2-people who contact with such animals might also suffer from infection especially in their hands, If they have small injuries in their hand or abrasions, even if it was unrecognizable, bacilli might lodge (تقطن) and produce coetaneous skin lesions (anthrax-chronic lesions) that are associated usually with development of granuloma and chronic Feature and requires surgical treatment by removing these lesions, and the use of antibiotics.

3-This one is more dangerous: inhalation of spores or vegetative form of these bacilli if they reach the lung they might, in a short period less than 48 hours, produce a variety of exotoxins and enzymes which results in development of hemorrhagic pneumonia "التهاب رئوي نزيفي" (damage in the blood vessels of the lung) and septicemia "تسمم الدم" and finally death. This type of spore forming bacilli can be used in biological warfare, but it is not used till now, only in experimental trials in relation to animals, and resulted in 100% killing of these animals who have inhaled it, which means it is a highly fatal disease.

◆Lab diagnosis for aerobic bacilli:

All aerobic bacilli can be diagnosed easily in the lab, you can isolate the organism and you can do few biochemical tests to distinguish between different species.

Generally, in related to food poisoning there is no importance to know exactly it's bacilli cereus or not because the type of ingested food is enough to give an impression which type it is; Staphylococcus aureus or bacillus cereus, bacillus cereus intoxication is usually related to roasted meat or rice especially Chinese rice. But for bacillus anthracis you have to isolate it and identify the causative agent by certain biochemical tests.

II- Anaerobic spore forming bacilli 'clostridia':

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This group of clostridia is associated with variety of clinical diseases of highly importance in humans due to the fact that most of them produce specific types of toxins which are associated with a specific type of clinical disease. Some of these clostridia produce a variety of extracellular products which allow her to be Invasive.

◆There are two type of toxins which are produced by clostridia:

1-Heat-stable toxins 2-Heat-labile toxins

“Heat stable toxins: toxins which require more time to be inactivated by boiling “

◆There are four type of clostridium:

1-clostridium tetani :

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It's a typical type of clostridium which produces toxins during perforation in infected tissue of the body, usually in the subcutaneous tissue following injuries under anaerobic atmosphere, by knife or car accident etc. if the wound was contaminated with soil or dust particles, you have to expect a contamination by clostridium tetani, and clostridium tetani begins to produce toxins known as tetanus toxins or tetanospasmin.

Tetanospasmin: it is a neurotoxin that is associated with contraction of the muscles (spasm\ tetanus) due to the diffusion of the toxin in the infected tissues and later it is absorbed by the lymph system and the blood, then carried to CNS and becomes fixed there which results in muscle contraction (spasm) Represented by difficulty to eat or drink or speak, and later respiratory failure due to a collapse in the muscles of the respiratory tract and finally death within a short period from tetanus.

The only way to cure the patient is to recognize the case, for example in wound infection **1- (surgical debridement):** cleaning the wound and getting rid of the damaged tissue to allow the wound to be exposed to air "remember clostridia are anaerobic", and to allow blood flow then **2- (antibiotics)** we add an antimicrobial drug and **3-**give the patient an antiserum "anti-tetanus toxins".

Extra note: Not required, Just to clarify (antiserum): a blood serum containing antibodies specific only for one antigen. Obtained from an animal that has been exposed to that antigen. Used in the prevention, treatment or diagnosis of infectious diseases in humans. (In case of tetanus it has antibodies for the tetanus toxin to neutralize it).

The best way to be immunized against tetanus is the Triple vaccine "diphtheria-tetanus-bordetella pertussis" it's excellent if it has been administered to the person in first year of life, but later during adulthood or older age, people might require a refreshing dose especially surgeons, people working in heavy industries, and soldiers etc. they should have in addition another tetanus vaccine, an extra one, in order to have a high amount of antibodies for tetanus.

◆ **lab diagnosis:**

In tetanus case it's not helpful to recognize the causative agent, due to two reasons **1-**it is not easy to isolate this organism by normal culturing method. **2-**it will take a long time.

So we start the treatment without delay, without the Laboratory. Laboratory might only help us later to confirm that this is a case of tetanus or not, especially in diabetic foot we should start treatment by antimicrobial drugs and surgical debridement in order to cure the damaged tissue in the diabetic foot.

In laboratory if we have isolated organisms we can do spore forming stain or gram stain then we can demonstrate it in specific features like the presence of large

capsules in it like tennis racket, as you can see in slide 7 it easy to recognize it in lab but as we say it needs long time at least one week, so it is not necessary to wait because if we did, we might lose the life of the patient.

2-clostridium perfringens:

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Clostridium perfringens: it's more important than clostridium tetani due to fact that it's not only a toxigenic organism it's also an invasive organism which means that it might be associated with not only wound infection ,but also with sepsis "septicemia", because during the infection of the wound it can reach the lymph system and the blood causing blood sepsis. It also releases toxins exactly like clostridium tetani.

It is special that is doesn't only produce enterotoxins and exotoxins, but also it is important as a producer of a variety of extracellular products which help them diffuse rapidly in the infected tissue, like 1-collagenase 2-hyaluronidase. And these two enzymes usually digest C.T and muscles allowing the organism to spread easily from side to another.

Clinical cause (1): if there is a severe wound infection that has reached the subcutaneous tissue and C.T following a car accident or the usage of any invasive instruments (knives ,etc.); you have to suspect this might be associated with clostridium perfringens especially if there is a first sign of myonecrosis(damage in the muscle tissue ,it is deeper than the epidermis and dermis, a damaged tissue that allows the organism to increase in number in short period and release toxins) and a bad smell, in such case, we do a radical surgical treatment which means to do amputation to the leg or the hand (the infected part),because any delay means the toxin will spread to the upper part of the body like abdomen or heart or liver and finally leading to **death**.

So, the treatment is not only to use antibiotics, this will not help, you have to do a surgical debridement (get rid of damaged tissue) and give antibiotics to 1-cure the patient or 2- prevent the developing of severe gas gangrene

In addition there is no vaccine available (in contrast to tetanus) and no antiserum available to neutralize the action of toxins.

Clinical cause (2): if the strains produce exotoxins that are found outside the body in food, exactly like bacillus cereus and Staph. aureus, this will produce intoxication 'food poisoning', in a short period the patient will suffer from more intense watery diarrhea than vomiting, but if you eat food which contains a type of c. perfringens strains that don't elaborate exotoxins in food, but once it reach intestine in large or few numbers it starts to produce another type of toxins; enterotoxins, and this type of enterotoxin will cause severe watery diarrhea and abdominal cramps or pain without vomiting, while exotoxins in the food produce both vomiting and diarrhea.

Q: are all the types of enterotoxins unable to cause vomiting?

In generally most of them, because enterotoxins are related to development of inflammation within the mucosa of large intestine, they will not be absorbed from large intestine and will not affect vomiting center in brain because the toxins were formed in the lower part of GIT, whereas exotoxins usually will be absorbed from small intestine and it will reach brain center which responds for vomiting.

◆lab diagnosis:

It's Not easy to isolate *C. perfringens*, therefore, most cases especially without septicemia relay on clinical Features of the disease, there are important feature which are gas gangrene or myonecrosis which associate with car accident and wars (bullets).

As you see in slide 9 it is not only related to clostridia but also a mixture of gram + and gram -, and this mean in order to treat such a patient you have to use more than one type of antibiotic to cover all the spectrum of the infection (which may be caused by 4 or 5 types of organisms) and in this case the infection will be severe and you will recognize a very bad smell and a severe tissue damage, and this will indicate that it is a mixed infection.(In the pic. You can see RBC's and WBC's).

3-clostridium botulinum :

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Causes food intoxication which known as "botulism", it is a spore forming anaerobic bacteria that is found in few numbers in human's intestinal tract especially children but without any dangers. These bacteria can contaminate food that was preserved under anaerobic condition (whether in home or industry) such as: canned food, which contains fish, meat or beans. in the past autoclave which was used in industry wasn't perfect like nowadays.

This food gets contaminated with spore forming *C. botulinum* during harvesting, preparation. under anaerobic condition the organisms starts to convert to the vegetative form and release toxins in a minimum amount (nanograms) but this amount is enough to kill a person, 1nanogram of these toxins is sufficient to kill an adult within a short period of time (less than 48 hours), which means that: **1-** it is a highly potent toxin and **2-** a highly stable toxin.

*We know this canned food is contaminated with *C. botulinum* by these signs:

1-change in color 'blackness on the surface'

2-you might smell the presence of certain organic compounds like acetic acid.

3- Bulging due to the presence of gases during the fermentation process.

Warming is not enough to inactivate toxins because they are Heat-stable toxins, you must get rid of food or you will need at least 20-30 minute of warming to inactivate toxins, these toxins can't be inactivated at lower temperatures.

Once this toxin has been fixed in the CNS, within a short period it will produce neurological Features, seizures, double vision, inability to swallow, difficulty in speech, weakness of skeletal muscles, water phobia and finally respiratory failure, difficulty in breathing, collapsing of lungs and death (respiratory paralysis which is responsible for the death of this person).

◆**lab diagnosis:**

Clinical features of case are important to suspect presence of botulism, laboratory test will not help us to diagnose it, maybe later to detect the causative agent but it requires at least one week.

◆In addition, there are no available specific toxoid antisera available in our country and most countries, they are only found in two centers in the world, and since antibiotics won't help, the patient can only be supported by respiratory tract intubation but generally he will die. mortality 100% in association with botulism.

4-clostridium difficile:

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This type is very important; Nobel Prize was given to an Australian (from Sydney) scientist in 1991 in relation to the detection of the toxin which is produced and the clinical Features which are associated with C. difficile.

C. difficile can be found in the intestines of humans ,in our country we have done three studies and we discovered that 20% of population carry this bacteria in their intestine without presence of any clinical features or diseases ,but hospitalized patients carry and colonize more C. difficile than healthy people outside the hospital, the percentage might reach up to 50% of the hospitalized patients (colonize the c. difficile), due to the wide use of antimicrobial drugs in treatment of diseases, and it is widely distributed in hospital environment because it is resistant to many types of antiseptic reagents.

It is a part of the intestinal flora but if there is a change in the intestinal flora due to the administration of certain types of antibiotics and for a prolonged period, like (amoxicillin, clindamycin, lincomycin and cephalosporins because they can be taken orally for a long period, there are also other types but those are the most associated with the clinical feature of the disease in relation to c. difficile) and this will lead to changes in intestinal flora "biological equilibrium in the intestines will be shifted from facultative anaerobic bacteria to some anaerobic spore forming bacteria especially c. difficile and maybe c. perfringens if present.

◆when *C.difficile* increase in number (also due to the change in the physiology of the large intestine) it starts to produce two type of toxins (A and B) that produce necrosis in the large intestine which is manifested first in the form of bloody diarrhea; the stool usually becomes faint red with blood (few RBC's) but can be easily recognized, the second feature starts after few days which is: more severe necrosis in mucosa of the large intestines which is known as **pseudomembranous colitis**“ the damage of the large intestine may reach 1 meter or more” and the patient will lose huge amount of his blood and he will collapse due to complications associated with pseudomembranous colitis.

Pseudomembranous colitis mild to severe form is recognized in about 5% of all hospitalize patients for more than two week ‘very common in association with antimicrobial drugs’. And the only way to stop this severity is to stop using offending drugs (الدواء المستعمل) and replace it with another drugs like (metronidazole or vancomycin)-these two names are not required from us now- so we should make a change in the drugs we are using, and this might help.

◆**lab diagnosis:**

We can isolate the organism by culture which requires at least few days but we now have a simple test: we have an antitoxin test available in laboratory so upon receiving the stool from the suspected case we can by slide agglutination test, test if toxins A and B are available or one of them, and this is enough to indicate the presence of the disease, especially if there is blood in the stool.

Sometimes we have to exclude other causative agents which might produce the same feature (bloody diarrhea) like a type of parasites and some types of bacteria like (shigella, salmonella, enterotoxigenic e. coli). but at least it can identify the toxin of *c.difficile*

The end



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