University of Jordan
Faculty of Medicine

Medical Committee
The University of Jordan

Introduction to

Microbiology

Title :

Enteric Bacteria

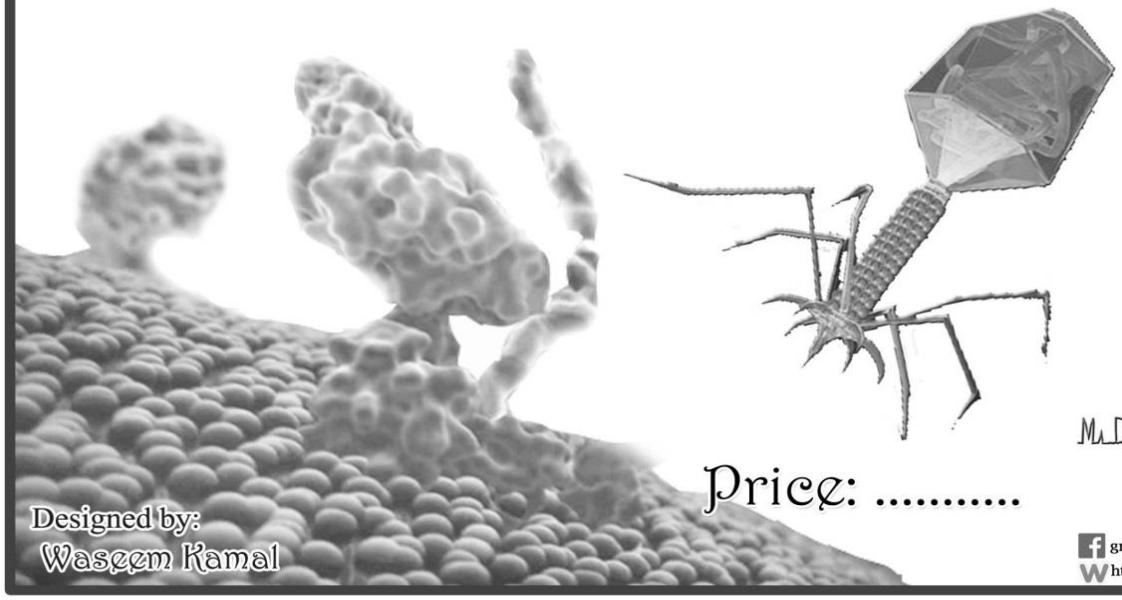
Professor:

Dr. Asem Al-Shehabi

: 20

- ○ Slides
- ○ Handout
- ● Sheet

Done by: Hala A. Makahleh



Price: .....

Designed by:
Waseem Kamal

MD University of Jordan Class of 2018

groups/Doctor2012
http://medstudygroup.weebly.com

ENTERIC BACTERIA

- Continuation of the Enteric bacteria :

1) salmonella

A) We have mentioned the first group of *salmonella* (**salmonella enterica**):

- Salmonella is an obligate pathogen ; food poisoning due to salmonellosis is very common in our country especially in shawrma (chicken) , mayonnaise ,..etc
- GIT : stomach infection cause vomiting ; while Intestinal infection cause watery and to some extend bloody diarrhea ; in some patients the watery diarrhea might accompanied with bloody diarrhea but not so severe as other species .
- Important feature : The presence of fever (presence inflammation in intestine) due to endotoxin which will cause necrosis too ; they release also demonstrated in intestinal cells what we call cytotoxin responsible for pathological changes .
- produce localized immunity " not solid immunity " for small period of time - for some months only - so can be re-infected again .
- they have a large flagella (important to attack the mucosa of intestine) and straight bacilli ; in contrast Vibrio cholera curved bacilli + one single flagellum .
- salmonella is lactose non-fermenter (can't use MaConkey agar) .
- Hekton–Enteric agar appear as black doted colonies due to production of H_2S whereas shigella will appear colorless (no production of H_2S).
- shigella-salmonella agar (S-S agar) will give give both as colorless ; so to have a full identification : one colony is taken + full biochemical test + antiserum (prepared antibodies from immunized rabbits - for example - to attack flagella antigen or somatic antigen) .
- which causes salmonella gastrointestinal infection or food poisoning,
- we have mentioned that this organism is widely distributed among four types of animals birds etc …,
- and contaminate often food and form a case of food poisoning,
- mainly gastrointestinal salmonella can be invasive, can reach blood stream and cause sepsis, in certain category of patients especially Immuno-compromised Patient, very young infants and elderly persons. Otherwise the infection remains localized in the large intestines and only associated with gastrointestinal symptoms.

B) Typhoidal Salmonella

- Now the second group of salmonella which called *typhoidal salmonella* or *systemic salmonellosis* due to the fact that this group despite the fact that the morphological structure is similar to other type of salmonella according to that it is gram negative, facultative anaerobes etc…,
- in relation to *typhodal salmonella* which means type of salmonella which cause systemic infection .
- infection started like the other type of salmonella in intestinal tract acquired by contamination mainly of water and second might be with food ,

- and the second important feature of this salmonella is that only few cells can be enough to infect the intestinal mainly the small and large intestine and by incubation period between one week and three weeks there will be enough number of cells which produce necrosis and later damage to be carried via mesenteric lymph nodes and carried to the intestinal lymph nodes to the blood stream and then cause sepsis and often there will be other organs involved.
- these invasive salmonella can reach any internal organ in particular the liver, produce liver abscess (note : a liver abscess is a pus-filled mass inside the liver.)
- and reach gall bladder and reside in it , due to the fact that this type of salmonella can survive in bile salts and bile fluids and might later associated with the presence of healthy carrier which means that the infected person will carry all his life this salmonella in his gall bladder and under certain conditions this will be execrated in the feces and feces might reach food particles especially infected persons working in restaurants , might produce infections and outbreak of disease of typhoid or for example in dairy product factory or if his feces reach water and contaminate water .
- *typhoidal salmonella* is very serious disease , and the fatality is high especially without the treatment and especially in certain patients who suffering from immune deficiency or infants.
- in our country *typoidal salmonella* killed thousands and thousands of our population in the past before the era of administration of antimicrobial drugs and before the controlling of water especially by chlorination . to give you one example in 1976 there was an outbreak of thyphoidal fever in Al-Salt city associated with contamination of water as resulted of infection of 3000 persons within few days and at least 20 persons died and few hundreds were admitted to hospitals for treatment so you can imagine how this organism can produce outbreak of disease .
- Another feature in relation to this *salmonella* is related only to humans not to the animals there is no source of animal infection , if the salmonella reach the intestines of animals can't produce systemic infection . maybe certain dogs might be infected and might have what we call typhoidal fever , but the majority of animals are immune but might suffer from gastrointestinal salmonella in form of diarrhea and sepsis like our symptoms.

❖ According of the presence of H- antigen and somatic antigen, three important types of Typhoidal Salmonella :

1) First type is the classical one or salmonella typhi the causative agent of typhoid fever .
2) The second type is salmonella paratyphi A , B and C ; mostly B less A and C in our region .but , the clinical feature between three types of paratyphi (A,B,C) they have the same clinical features .difference only in relation to serological immune response in the body in the presence of specific antibodies against typhi and paratyphi etc… therefore if patient is treated with antibiotics , and you know if patient treated by antibiotic you might not discover the causative agent weather from blood stream or from stool because the salmonella during the infection (acute infection) the organism execrated from the intestinal tract to the feces , it might reach blood stream produce sepsis , it might reach CSF and cause meningitis and might execrated by the urine , this mean all of these might be positive but if there is partial treatment there might be negative result and patient might be recognized usually as a fever with unknown origin.

➢ Clinical features :

Salmonella is well known to produce **Hepatospenomegaly** which is enlargement in the liver and spleen which is a very important feature in addition to **high fever** in salmonella typhi and paratyphi temperature might reach 41 and you can't imagine how bad it is in an actual clinical case ; patient cannot speak , cannot move he will collapse with this high fever . In addition to the complications which associated with high fever and **endotoxins** , fever is due to the endotoxins which found in the cell wall of these bacteria .

- Lab diagnosis :

Such a case if we have negative result especially in stool and blood and CSF we might look for the specific antibodies by a **serological test (widal test)** this indicate presence of specific antibodies because during infection (acute infection ,subacute and chronic) the body responded by production of specific antibodies against **somatic** and **H-antigens** , so we can take blood sample look for concentration of these specific antibodies and specific antibodies usually measured by what we call Titer , titer of antibodies means concentration of antibodies in relation to the *salmonella typhi* and *paratyphi* against the somatic (O) antigen and H-antigen . If Titer in both or one of both (O or H) 1-16 then this means that patient is infected by *salmonella typhi* .

- **Development of the disease** : Systemic infection is more associated with developing of gastrointestinal symptoms but , in relation to *salmonella typhi* and *paratyphi* it is not necessary to have diarrhea it could be instead of diarrhea a Constipation and abdominal pain and later developing of fever which indicates that the organism has reach the blood stream . the complication of *salmonella typhi* and *paratyphi* that it can reach any part of the body it can produce intestinal perforation for the patient and he will suffer from sever bleeding and might produce sever cirrhosis in the liver and might cause a damage in the kidney and it can affect any part of the body . in the past there were a presence of a salmonella carrier which is the presence of organism in their gall bladder especially among women more than men , because women usually developing more a biliary stones than men , and biliary stones often can be infected by salmonella typhi and reside in gall bladder , and if you eat a heavy meal the gall bladder secrete a biliary solution and this might associated with the release of the organism from the infected part and the organism reach the intestine and after that it is excreted within the stool , but due the previous infection (systemic infections) the body has already certain immunity so it might only have a mild fever without complications, but this person (healthy carrier) might be a source of infection in the community usually by contamination of water or food source or direct contact .
- **Treatment :**The only way to treat the patient before the antibiotics era ,usually the patient will develop immunity and he will recover or the end result which is the death , the morbidity before era of antibiotics differ according to the age and the health condition , but not less than 10% in association with Typhoidal Fever in some communities . In Jordan there is a study it is about Typhoidal fever it is reach 5% , and often with association with perforation in the intestine . there is a specific type of antibiotic they have used it in the past which is chloramphenicol ,now they use other type of drugs ,and there is a human vaccine but it is in fact not necessary , instead it is more necessary to have a high standard of hygiene by using sanitation (by contrling the food and water ,food quality and not to be contaminated by pathogens) which is part of job of the ministry of health which should manage to control the food and source of water to be free of pathogens including the salmonella . in the last 20 years we don't have any cases of salmonellosis especially salmonella Typhi ,there are few important cases from the neighboring countries but in Jordan we are lucky that we have very few cases of salmonella Typhi.

2) *Shigella* group :

The second organism of importance as a causative agent of diarrheal disease called *Shigella* .

- ✓ exactly *shigella* like *salmonella* a name of person (shiga) who is a Japanese scientist who has discovered this organism during a large out break of diarrheal disease in Japan in 1945 where about one million of the population of Japan have been infected with *shigella* and developed bloody diarrhea.
- ✓ *Shigella* exactly like Salmonella is gram negative bacteria , often they also have the same virulence factors which include the endotoxins from the cell wall ,and certain cytotoxins as enterotoxins once they reach the intestine , interact with intestinal mucosa and cause necrosis and often bloody diarrhea, it is more than watery diarrhea , it is bloody due to the inflammatory reaction in the intestines.
- ✓ One important feature of this organism similar to *Haemophilus* and *Neisseria gonorrhea* it is highly susceptible to the dryness and acidic this means that a patient suffering from diarrhea and you want to culture his stool (feces) you have to culture without delay not to give a sample of stool and place it in the lab or home for more than 15 minutes , because the stool have a large number of coliform bacteria and these usually ferment the rest of food in the stool especially the sugar and produce organic acid which can kill Shigella very rapidly where as Salmonella normally resistant to acidic to some extent.
- ✓ so in order to culture shigella from a person suffering from diarrheal disease you have culture it rapidly without delay. Therefore in our country rarely they reported the presence of Shigella in stool although clinically the patients have bloody diarrhea and all the features that indicate the infection with *Shigella*. In our hospital we have recovered 10-15 cases of *Shigella* due to obtain the samples rapidly from the infected patient and send it to the lab. Therefore, it is very important in clinical practice.

- **Species of *Shigella*:**
  - Now we have many species of *Sh.Sonnei, Sh.flexneri, Sh.boydii, (* which is very common) often associated with what we call Purulent-Bloody-Diarrhea, it is means that there is diarrhea associated with the presence of WBCs and RBCs therefore examination of the stool might be indicate that there is an infection with the *Shigella* ,but we have to explore certain parasites that produce the same feature. Therefore the stool might help to certain extent the more important is to culture a specimen in a special culture media like S-S agar (which is *Salmonella-Shigella* agar) ant Hecton-Enteric agar.

Note : Shigella - in contrast to *Salmonella Typhi* and *Paratyphi*- is not invasive rarely can reach blood stream might be in patient who suffering from Immuno-compromised.

- But we have single strain or clone which called *Shigella.dysenteriae* the causative agent of shigellosis (bacillary dysentery) This strain exclusively is more dangerous and more associated with production of enterotoxins and type of toxins known as Neurocytotoxin which means that the toxins that usually elaborate from the cells will be absorbed and affect the central nervous system and the impression of a patient have a form of meningitis, despite the fact that the organism cannot reach the

meninges and cause meningitis ,but the toxins affect the central nervous system and might also produce sepsis, which means that it is more invasive than other species , patients should be treated without delay with antibiotics otherwise he will suffer from complication and death .

- **Lab diagnosis :**

Only isolation of the organism from feces, blood usually cannot be use for identification , also there is no serological test . control sanitationand hygiene is so important exactly like the salmonella , and there no animal source of Shigella it is only restricted to the humans, cannot produce infection in the animals .

3) vibrio cholerae

Now we move to another important organism which is *vibrio cholerae* the causative agent of cholera. This organism is not part of the classic enteric bacteria it is a gram negative bacteria and grow under aerobic condition, but it is a special organism:

1) this organism survive more in alkaline medium than in neutral or acidic medium .
2) this organism not associated with developing of healthy carrier like salmonella Typhi and Paratyphi.
3) and it is not found in animals only in humans and produce only infection in humans.
4) the infection in humans often associated with developing of severe watery diarrhea .

- In 1976 there was outbreak of cholera in our country where few thousands of population were infected within few weeks ,and associated with huge number of positive case .
- **Life or Death** in relation to this organism is few hours , because within few hours where the organism is arrive the intestine or large intestine it produce a potent toxins known as cholera toxins and this toxins induce out flow of fluids from the intestine and the body , so in short period less than few hours infected persons with this organism might lose 2-3 liter of his body fluids the end result is dehydration of the fluids from the body which cause concentration of RBCs and this is lead to destruction and associated with kidney failure, respiratory failure, heart failure etc… and death.
- **Treatment :** The only way to cure the patient and to prevent the dehydration is to give the patient large amount of fluids in form of normal saline and glucose , the patient must receive the same amount of fluids that he lost , we have to maintain equilibrium in order to prevent the development of dehydration, this is the only way to cure the patient and this is can be done within a few hours, not orally fluids it is cannot be given because the stomach will be hypersensitive and the acidity will be decreased and amount of fluid will result in vomiting and more complications . the patient should only receive an intravenous fluids otherwise the patient will suffer from shock and died .
- **Clinical features :**

Therefore the only way usually to recognize the presence of cholera is clinically by the following **symptoms** : severe watery diarrhea (when the patient went to toilet he might not return back) then it will develop in dehydration and produce coma , then we have to cure him by : a) administration of fluids ,b) there is no need for antibiotic (in fact antibiotic cannot to cure the patient because cholera have already been elaborated) . The incubation period for the cholera is short (between the infection and the developing of the clinical symptoms might be only 24 hours) it is usually depend on the number of cells that had been ingested with water or food etc… mainly the infection associated with water and stews not solid food (solid food usually cannot be contaminated with large number of *cholera*) .

Vibroio cholera species :

1) *Vibrio cholera-01*

   (01) ⟶ In relation to the somatic antigen on the cell wall of this group of bacteria . It is a classical type which associated with the production of cholera toxins which cause severe dehydration and watery diarrhea .

2) *Vibrio. cholera El-Tor*

   - ✓ (El-Tor) ⟶ in relation to El-Tor mountain in Sina' where outbreak of the disease has been occurred in the 18th century and killed thousands of Pilgrims during their travel to Macca from an African country .
   - ✓ This type is the prevalent type in the world because it is more resistant to the environmental factors than the classical one.
   - ✓ Certain countries in the world like India and Pakistan they have all the year cases of cholera and this is due to the presence of organism in water especially in association with certain types of sea animals the organism still presence but not in humans , there is no human carriers it can only reside for short period in the intestinal tract and the patient will be free of the organism, the important is the cholera toxins, it is heat resistant toxins similar to the enterotoxins of E.Coli

❖ Note : following the infection of the cholera there will be certain intestinal immunity , but it is not a solid immunity the patient will be immune for about 6 months to 1 year not life long .

Lab Diagnosis:

We have to use a specific culture it is a special media called TCBS used for the recovery of the organism (in our body the organism enter from the oral cavity and later reach the intestine and start to colonize and began produce toxins ,which control the release of fluids from the intestine) .

Return to the slide 15 :

➢ Left picture :

- this the TCBS medium the color of colonies appear yellow in relation to fermentation of sucrose in this medium and this can help us in identification.
- it is also contain inhibitory substance which inhibit the growth of other intestinal flora and only support the growth of this type of bacteria.
- In addition the pH of this medium is between 8.5-9 which means an alkali medium (keep in mind that all other cultural mediums we use in the lab have numeral pH ABOUT 7) then this alkali medium will inhibit the growth of all other types of organisms and support the growth o cholera since it can survive in an alkaline media

4) Another type of enteric bacteria is *Brucella*

▪ It is called *Brucella* in relation to (bruce) an English physician who discovered this organism in the island of Malta by accident where there was an outbreak of fever with unknown origin among the British soldiers .

- he has investigated why they have develop this fever in association with other clinical features especially an intensive sweating .
- he has recognized that all of the soldiers that developing a clinical features of brucellosis (at that time the name of the organism was unknown) have something in common that they have drunk unpasteurized milk originated from goats . and so he has investigated that goats carry the infectious agent , in their milk ,blood and urine .
- In addition *Brucella* in animals produce abortion and he note that all the infected goats have already aborted .
- Therefore this organism considered a zoonosis causing infections in animals .
- *Brucella* in fact more important in humans than in animals
- It is originate in the animals induce abortion this means loss of number of animals .
- *Brucella* might infect any type of animals (sheep ,goats ,cuttles , camels , horses ,dogs)
- Therefore we have a specific clones of *Brucella* for each specific animals .
- The most common type of *Brucella* which induce infection for humans and originated from animals are two types :
    1) *Brucella melitensis*
       Melitensis in relation to the Mediterranean sea affect the goats and sheep.

    2) *Brucella abortus*
       Abortus in relation to abortion

These two common species which recognized in our country and which can be associated to Brucella.

- *Brucella* is still present in our country always we have a new cases because people may drink unpasteurized milk ,white cheese and dairy products and there are certain number of animals especially goats and sheep coming from Syria which are not immunized and infected with the Brucella.
- Human *brucella* which known as Malta fever is a very serious disease due to the fact that it might started with low fever without the clinical symptoms and the patient might not give attention to it, but later this asymptomatic feature might converted into chronic feature , it will affect the CNS produce damage and so he will suffer all his life from complications of *Brucella*.
- *Brucella* is gram negative coccobacilli similar to *Salmonella Typhi*, but it is smaller in size and has more specific type of endotoxins which responsible for the pathogenesis of the organism.
- In our country in the 1990s each day we were have a case of the *Brucella* in our hospitals, but now the numbers reduces but still are important .

- The only way to discover the organism is by the infected persons and the more important is to prevent the transmission of the organism from animals to humans and this can only done by vaccination of the animals against *Brucella* and this is job of the ministry of agriculture to control Brucellosis of animals .
- The other feature which is very important in relation to *brucella* infection
    - *Brucella* could be acquired by the respiratory tract by inhalation .
    - Or by Conjunctiva as a dust particle .
    - And might be through a skin injury if you contact with infected animal .
- So the infection might be of different sources not like Salmonella so exactly .

- The only way to treat the patient with specific type of antibiotics and for a period of time 6-8 weeks and the patient should complete its treatment course although he is feeling better and this might develop the chronic case .
- The clinical feature of Brucellosis is easily to be recognized :
  - ✓ High fever
  - ✓ Hepatosplenomegaly
  - ✓ Severe Headache
  - ✓ Sweating in huge amount
  - ✓ Affect the CNS there will be a back pain

- Lab diagnosis : the organism could be discovered in the blood , CSF if there is meningitis , bone marrow in case of chronic infection But not in urine or stool .in addition we can recognize the organism by serological mean by detection of specific antibodies against the specific antigens of Brucella melitensis or abortus , usually in our country in relation to titer of Brucella in thousands 20 up to 90 thousand . in contrast to Salmonella its titer 160-320 etc…
- The prevention of the disease mainly by the presence of Brucellosis in animals and to control the milk products .