



Microbiology

Lecture No:.....23.....

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Sheet Slide

Enteric Bacteria (cont.)

* Salmonella:

I) Gastrointestinal Salmonella:

We've already talked about the first group of Salmonella, which is associated with gastrointestinal infections or food poisoning.

- This group of Salmonella is widely distributed in birds (especially chicken) so it can easily contaminate chicken meat, eggs or any other chicken products.
- Infections with this organism require at least 100,000 cells to be consumed per 1gram of food in order to produce a clinical case; however some patients may require less number of cells due to an underlying disease for example or in very young infants etc...
 - In short, not only few cells can be enough to induce an infection, so the organism must multiply in large numbers in order to cause a disease.
- Generally in healthy people (who have no underlying disease and not immuno-deficient), the infection is confined (limited) for the intestines and is associated with vomiting, diarrhea which is often watery and sometimes bloody, and it lasts for 24 hours.
- It may also be associated with some elevation in body temperature, abdominal pain, but generally it's self-limiting so there's no need to have antibiotics (it's not treated with antibiotics).
- On the other hand, in certain patients especially infants and very young children who have an underlying disease, or are immuno-deficient, this group of Salmonella which is called gastrointestinal salmonella might be associated with more severe infections, and the organism might reach the blood producing sepsis and in infants it might produce meningitis. Keeping in mind that sepsis and meningitis are fatal, even if we used antimicrobial drugs.

- Last important feature of gastrointestinal salmonellas is that they're considered less invasive in comparison with typhoidal salmonella group.

II) Typhoidal Salmonella:

- It is considered invasive and highly virulent (pathogenic) and it produces as a classical feature of the disease **Typhoidal fever** (which was in the past, especially in our country, the middle east countries and other developing countries, one of the most types of diseases that are associated with mortality "death" due to the severity of the disease and lack of antimicrobial drugs, so it was a very serious disease and caused death of millions of the population following the infection with Salmonella Typhi and paratyphi)

-Here we have three serotypes; if you still remember, we had classified the Salmonella into two groups;

1) Gastrointestinal salmonella which produces food poisoning salmonella, this group has about 2000 serotypes

2) The second group that produces typhoid fever, it has only 3 serotypes

- i. Salmonella Typhi
- ii. Salmonella Paratyphi: A, B and C.

- There is no difference in the clinical feature between these 3 serotypes; the main difference between salmonella typhi and paratyphi in the clinical signs and symptoms → they give the same clinical picture as a result of having the same route of infection and the same virulence factors.

- However, salmonella typhi is more dangerous due to the presence of special layers within its cell wall, called Vi layer. Which is a slime layer like the capsules, associated only with Salmonella Typhi and it contributes for the severity of the disease. Whereas Salmonella Paratyphi A, B and C are not associated with this Vi virulence factor.

* **Dose of infection:** (number of cells needed to induce infection)

In relation to *Salmonella typhi* and *paratyphi*; few cells are enough to induce infection; because these few cells once reach the intestinal tract they'll slowly multiply during the incubation period, which ranges from few days to about 3 weeks depending on the number of ingested cells.

- These cells usually begin slowly to invade the mucosa and sub-mucosa of the intestinal tract, as well as slowly producing inflammatory reactions and carry the organism later by mesenteric lymph nodes of the intestines → to the blood stream, producing blood septicemia.

- They're later disseminated from the blood stream to the gallbladder and liver resulting in abscess formation, infections and it might as well reach any part of our internal body; it might reach meninges (in CNS) producing meningitis, bones (causing bone infection), urinary tract infections (affecting kidneys). So this organism is invasive and highly virulent and produces systemic infections.

- In relation to *salmonella typhi* and *paratyphi*; the organism is highly virulent, once it reaches the intestine (in the majority of cases) it's associated with the clinical features of Typhoid Fever.

* **Typhoid Fever:**

- It means severe inflammatory reaction in the intestine as well as in other parts of the body, especially in the blood stream, liver (producing abscesses). Which result in the release of the endotoxins that are associated with very high fever which might reach up to 40-41 °C.

- Due to this high fever, the patient will become confused and suffer from severe malaise, weakness in addition to some gastrointestinal symptoms such as vomiting, diarrhea and bleeding in the intestine due to the perforation in the intestinal tract.

- So the mortality might reach up to 1/3 (30-35%) of the patients who are not treated with antimicrobial drugs, which is very high percentage, especially before the era of antimicrobial drug treatment, but luckily now antimicrobial

drugs can control the infection in most cases especially if the treatment began early.

- Another important feature of this is that during the infection, the body tries to assist the infection, note that typhi and paratyphi organisms are considered as intracellular organisms that multiply usually in monocytes and macrophages, but the body might response by producing specific antibodies (immune response) and this response differs among individuals.

- So typhoid fever is a serious disease transmitted (the causative organism) mainly by contaminated water because usually the organism might be excreted form healthy carriers.

- Normally, following the infection of salmonella typhi or paratyphi, there'll be between 1-3% of infected people who are healthy carriers especially among women, so the organism will reside not in the GIT but in the gallbladder, especially in the cases of having stones in the gallbladder.

- The bile secretion enhances the presence of the organism rather than killing it, in contrast to what it does to other organisms which are usually inhibited by the bile secretion (susceptible to it). So salmonella typhi and paratyphi survive even in the presence of bile solution.

- Therefore, healthy carriers are the major source of infection in the community due to the fact that salmonella typhi and paratyphi are adapted only to humans (not found in animals), and the organism can be excreted in the feces.

- During the acute stage with typhoid fever, the organism can be excreted from feces, and it can be found as well in the blood stream, CSF (cerebrospinal fluid; in cases of meningitis) and the urine (during the acute stage).

- So these sites can be considered for isolation of the organism in order to be used in the lab diagnosis, which helps us to recognize the case of typhoid fever.

- But if the patient is treated partially with antimicrobial drugs, we might not discover the organism by using culture, in that case we have to use a serological test (looking for the presence of specific antibodies against somatic (O antigen) and H antigen) and this test is normally done by using a test known as **Widal Test** {the name refers to the name of the person who had discovered that the use of specific antibodies against salmonella typhi and paratyphi can be used to diagnose cases of typhoid fever}.
- According to the level of antibodies (which is called titer), and in our country [titer of 1:160] of specific antibodies against somatic (O antigen) and H antigen usually indicates the presence of typhoid fever.

*** Treatment:**

- The best treatment is having antibiotics as fast as possible. In the past chloramphenicol was the drug used to treat typhoid fever, which was an excellent drug in eliminating the organisms from internal organs especially the intracellular location of the organisms.
- But unfortunately, chloramphenicol now is less used and it had been replaced with other types of drugs which the doctor isn't willing to mention.

We'll move to another group that is related also to the gastrointestinal tract and is considered as part of enterobacteriaceae (enteric bacteria).

*** Shigella:**

- The name is related to the Japanese scientist Shiga who has discovered this organism during the outbreak of shigellosis infection that is related to the intestinal tract and is associated of developing bloody diarrhea → which means that shigella differs to some extent from salmonella.
- Once shigella reaches the intestinal tract of humans, it produces due to the presence of specific endotoxins in the cell wall and this results in developing bloody diarrhea.

- For example, enterotoxigenic (enteropathogenic) E.coli normally produces watery diarrhea rather than bloody diarrhea, also gastrointestinal salmonella produces watery diarrhea, but shigella normally produces bloody diarrhea. So *Shigella is easily differentiated from other diarrheal diseases.*

***Species of shigella :**

S. Sonnei , Sh.boydii, Sh. Flexneri, Sh.dysenteriae

****The first three are more common****

- The three common serotypes normally have the same clinical features (bloody diarrhea, less vomiting, [less invasive] → they rarely reach the blood stream to produce blood sepsis).

- But the disease -in comparison with gastrointestinal salmonellosis- might produce more severe necrotizing inflammatory reactions in the intestine. Therefore it is recommended to treat the patient with antimicrobial drugs to stop the progress of inflammation in the intestine.

- Another important feature for gastrointestinal salmonella that it can survive in harsh conditions (environment, water, moisten soil on the hands of healthy people in relation to food).

- While Shigella (outside the intestinal tract) cannot survive for a long period, within 30 minutes the organism can die specially if found in the stool.

- Stool is normally alkaline, but outside the body certain primitive bacteria begin to produce more acidity so it will become acidic and this acidity will kill shigella rapidly.

- Therefore if the physician suspect in case of bloody diarrhea that it could be shigella he should send the stool without delay or rectal swab for culture in order to recover the organism, otherwise the result will be negative because the organism will die rapidly outside the body if it was especially in the stool.

(Meaning that stool should be cultured without delay to look up for the organism and to give the patient the appropriate antimicrobials)

- We have a special type of shigella called **Sh.dysenteriae** .
- It is the most severe form of bloody diarrhea, due to the elaboration of a special toxin called **shiga toxin**; which is secreted during multiplication of the organism in the large intestine.
- It produces more severe inflammatory reactions similar to clostridium difficile toxins, associated with severe ulceration in the large intestine and patients might die following ulceration and bloody diarrhea if not treated with antimicrobial drugs.
- In addition Sh.dysenteriae is considered to some extent **invasive**, which means It might reach the blood stream of the infected person specially in association with an underlying disease (immunocompromised conditions) , whereas other species of shigella (previous three species) are less invasive (rarely associated with blood sepsis) but S.dysenteriae is more associated with blood sepsis.
- Therefore it is recommended to treat the patient –without delay- by antimicrobial drugs to relieve the patient from severe ulceration as well as to prevent any complications.

***Lab diagnosis:**

- Rectal swab or stool should be send without delay to the lab for culture, culturing of shigella and salmonella requires using of specific type of selective media, because in the stool of any person there are large number of enteric bacteria and other bacteria.
- so it is not easy to recognize few number salmonella or shigella among hundred types of bacterial species, so they should be cultured in special selective media (S-S agar, Hecton-enteric agar) where 99% of intestinal flora will be suppressed and only salmonella or shigella can be recognized .
- Shigella normally cannot produce H₂S during growth, so it produces only transparent colony, whereas salmonella (whether gastrointestinal, typhi or paratyphi) will produce H₂S which will be recognized as black colonies on Hecton-Enteric agar or on Salmonella–Shigella agar (S-S agar).

- So we have two types of selective media and both of them are considered as selective media for isolation of these organisms:

1. Hecton-Enteric agar
2. S-S agar

- But we cannot isolate them on MacConkey agar, because there will be over growth of Escherichia coli, klebsiella and other organisms, so we won't be able to discover the desired Salmonella and Shigella.

- In cases of infections related to the blood; we need to use blood agar or any other type of media for isolation of the organism.

*** Vibrio Cholerae:**

- Now we move to another organism associated with very famous history in developing of the **outbreak of diarrheal disease.**

- The name of cholera has induced panic in the world and in any community, because they know that cholera is a very dangerous disease associated with severe diarrhea and dehydration and the death of the infected person within 24 hours.

- Later, following the knowledge about organism and how to deal with it, cholera became like any other diarrheal disease. It is not so dangerous to be infected with cholera but it is important to know how to deal with the case of cholera.

- There are certain places in the world where they have always endemic cholera (where they have the classical type of organism which is called vibrio cholerae, the causative agent of clinical Asiatic diarrheal disease Cholera).

- Vibrio Cholerae is not part of the enterobacteriaceae, it is a special group of bacteria that belongs to a family called Vibrionaceae which is composed of many species of Vibrio Cholerae and other vibrios which can be found in water

and can be associated with various of clinical infections particularly in association with diarrheal diseases.

- So we don't only have *Vibrio Cholerae*, we also have other *Vibrios* known as **non-O1-Vibrio Cholerae**; such as *Vibrio Parahaemolyticus* and others.
- Returning to *Vibrio Cholerae*; why is it important?
 - ✓ This organism once it reaches the intestinal tract of humans (because it's adapted only for humans, like *Salmonella typhi* and *paratyphi*, they cannot be associated with infections in animals) causes inflammation. So humans are the only source for infection in any community (not animals).
- *Vibrio Cholerae* survives in water for long periods; whether it's not clean (rubbish water) or clean water. For that reason, water is the main source of infection in any community when it's contaminated with the feces containing cholera from any person with the infection, whether he is asymptomatic or symptomatic.
- A few percentages of infected people (between 10-50% of patients) can colonize *Vibrio Cholerae* without developing the clinical features of Cholera.
 - Which means that you might have **healthy carriers** for a short period and the organism can be excreted from them in feces, then it might reach a susceptible person (especially by water or direct contact) developing Cholera.
- This organism once it reaches the intestines, it begins to attach to the mucosa of the large intestines, and produces a very potent toxin called **Cholera Toxin**, and this toxin is exactly like the toxins that are produced by the Enterotoxigenic *E.coli* (they both have the same mechanism). But the difference is in the amount of toxin released; *Vibrio Cholerae* releases **10-100 times** more toxins than Enterotoxigenic *E.coli*. The difference is not in the type of toxins but in relation to the amount of toxins.

- This toxin within a short period (less than few hours) begins to affect the mucosa of the intestines, causing the release of a huge amount of electrolytes and water from the mucosal cells. This will result in a very very **SEVERE watery diarrhea**. (A healthy person can “within few hours” become unable to move “even from the toilet to his room” due to outpouring of fluids).

➤ So within a short period, the patient might lose between 1-3 Liters of his body fluids (about 50% of his total body fluid), which means that it'll affect:

- RBCs; higher concentration which will damage them
- Circulation of the blood
- Respiratory tract
- Kidney function; causing kidney failure due to the loss of a huge amount of electrolytes “especially saline; NaCl and K” and water.

- So the patient will die following complex damage in vital organs of the body, especially those that are related to the blood circulation [causing hypotension].

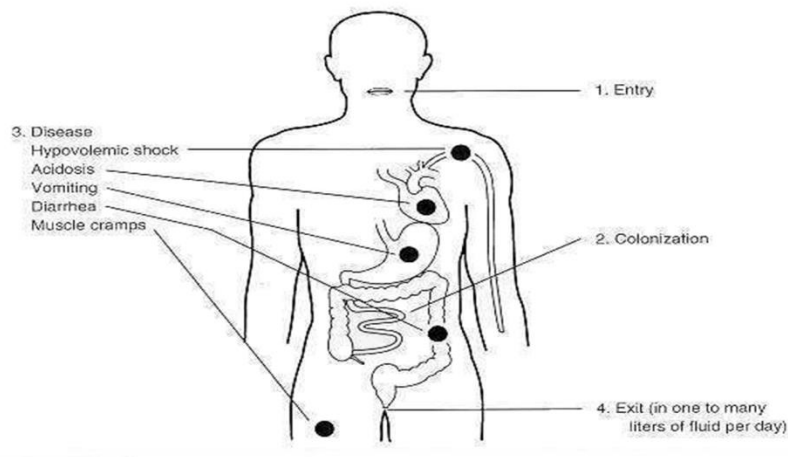
- *Vibrio Cholerae* causes no inflammation in the intestines, it's **not invasive** (cannot reach the blood stream to cause sepsis). Its toxins affect only the outpouring of fluids from the intestines.

- Therefore, the only way to cure such patients is by restoring the loss of fluid, and this can be done in many ways depending on the patient's condition;

- 1) Severe dehydration; cannot be cured by oral rehydration (oral administration of fluids), because the patient will respond by more vomiting due to the change of the acidity in his stomach. So we use IV fluid in this case; which provides a large amount of fluids within a short period of time [about 1 liter per 1 hour] to make sure that the patient has restored the lost amount of fluids and electrolytes.
- 2) Mild diarrhea; the first stage of treatment will be the administration of an oral rehydration solution.

- As you see, cholera is a clinical diarrheal disease that's associated with watery diarrhea, which might end up to a stage that there won't be any types of feces excreted except water.

It might develop some complications, usually to the kidneys due to increasing the concentration and acidity of the blood.



This picture shows the features of Cholera, especially how it affects the large intestines and other parts of the body

* Lab Diagnosis:

- To diagnose cases of cholera, culture is needed. There is a special culture medium called TCBS medium.

- Vibrios are **curved bacilli**, and they can be demonstrated by the gram stain (without much scientific experience). They are also associated with **long flagellum**, which is recognized by its intensive motility, and can be detected by a special motility test.



- Not all Vibrio Choleraes are toxin-producers; you might sometimes isolate the stool (even with watery diarrhea) and find Cholerae without its toxins. Such cases are NOT associated with very dangerous or serious diarrhea.

- Serious diarrhea is related to the toxigenic form of Cholera; which can be cultured on special culture medium called TCBS medium (Thiosulfate -Citrate Bile salts-Sucrose medium), where we recognize **yellow colonies** on the surface of the organism, and this can be done within about 16 hours.
- Later, we do biochemical test to further identify the organism by specific anti-sera against the classical form of Cholera (we have several forms; classical form, El-Tor form..etc).
- Finally, we report it without delay to the treatment physician.
- If there is presence of any cases of cholera in the community, we have to expect at least 50-100 asymptomatic cases or symptomatic with mild-form-of-cholera cases, therefore without delay the health authority of the community must investigate the source of infection and they have to do some preventive measurements to know how patients got infected, because as we said; most infections are caused by drinking of contaminated water or after contact with a positive carrier. In such cases, we can control the Cholera outbreak in any community.
- In 1976, there was a huge outbreak of cholera in many cities of Jordan, about 10,000 of the population got infected and unfortunately few of them died following the infection, whereas the rest got cured by using the suitable treatment, especially by restoring of fluids. Concerning the antibiotic; they are NOT necessarily used.
- So it's important to know that antibiotics cannot cure cases of cholera, they can only:
 - 1) reduce the excretion of Vibrios in the stool of the infected person
 - 2) prevent the dissemination of the organism in the community
 - 3) cut the source of direct contact with the organism
- So the only way to cure cholera is by restoring the loss of fluids, because the antibiotics' action will be too late, because the toxins are already in action and the only way to cure the patient and keep him alive is by giving him the

necessary dehydration solution. Whereas antibiotics can be given only to reduce the excretion of the organism in the stool, as well as to prevent the dissemination of the organism in the community.

- There is an available vaccine, but it's not recommended to be given for the population. It's more important to have hygienic control measurements, to look for the quality of water and for the presence of sources of infections...etc
- The same also applies for *Salmonella typhi* and *paratyphi* and for other gastrointestinal infecting microorganisms.
- Sanitation and hygiene control is more important than giving vaccines in order to prevent occurrence of cases and to begin with the treatment.
- Vaccination is important for certain types of diseases, but for Cholera and *Salmonella typhi* and *paratyphi* (typhoid fever); vaccination is not recommended, because the patient is feeling secure he is immunized so he won't care about have a standard of hygiene in the community. Whereas viral infections, can be prevented by vaccines, but these types of organisms (*vibrio cholerae*) should be prevented by hygiene measurements rather than vaccination; except of soldiers and armies where they can be used.

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Best of Luck All :)