Microbiology

◉ Sheet ○ Slides

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Subject: Gram negative bacteria (continued)

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We have mentioned the major groups of enteric bacteria which mostly can reside in intestine.

groups of enteric bacteria that we have mentioned in the previous lectures :

-E.coli

-salmonella which has two types :

* typhoidal salmonella (enteric salmonellosis).
*gastrointestinal salmonellosis.

now we'll talk about another group which is shigella

shigella

The name shigella in relation to the name of Japanese scientist called Shiga , who has discovered the importance of this organism as a causative agent of diarrhea (outbreak of diarrhea) related to fish contamination.

shigella is related only to the humans (it can't affect animals), exactly like salmonella typhi and paratyphi .

shigella species :

We have four Shigella species associated with shigellosis three of those don’t release enterotoxins or exotoxins (that produce severe diarrhea).

Shigella boydii, sonnei and flexneri >>>>>> associated only with localized inflammation in the intestine causing bloody diarrhea.

In contrast with gastrointestinal salmonellae, Shigella require antibiotic administration as it cause more necrotizing (damage) in the intestinal tract especially the colon .

the 4th type of shigella called shigella dysenteriae , often we use the term Dysentery referring to severe gastrointestinal infection caused by S.dysenteriae.

It is the only type that can release an enterotoxin (a strong one) in human intestine, It is associated with:

1- severe bloody diarrhea
2- severe abdominal pain

3- It might give impression of having meningitis (due to releasing shiga neurotoxin), that means that the enterotoxin of shigella dysenteriae might affect the CNS.

Few cells of dysenteriae might induce the infection so the infection dose is limited to these few cells during multiplication in intestine within incubation period of 48 hours. The first symptoms to appear are diarrhea (not vomiting), high fever and severe abdominal pain.

Infectious dose (ID) is the amount of pathogen (measured in number of microorganisms) required to cause an infection in the host.

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The infection mostly follows contamination of water or fresh dairy products, but it might be associated to direct contact with an infected person. The organism can contaminate food easily then infect people (high standard of hygiene can prevent the personal infection).

Shigellosis is not invasive, It rarely penetrate the intestinal tract reaching blood stream causing sepsis (normal healthy patient infection). It might cause blood sepsis among immunodeficient patients.

vaccination :

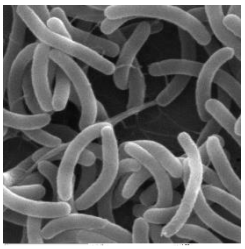
There is a vaccine available but it is used only in wars (for army), because general population should be protected by sanitation measurements (mainly safe water).

lab diagnosis:

Like salmonella, Shigella is not easily detected in MacConkey agar due to the fact that it is lactose non fermenter bacteria. We have to use a selective media to suppress lactose fermenters and allow the growth of lactose non fermenters, so we use S-S agar OR hektoen-enteric agar, then some biochemical tests are done for lab-diagnosing purposes.

Vibrio cholerae :

vibrio means curved rod (comma shape) .In gram stain, when using fresh culture, their unique shape is recognizable (curved rod) .



Notes:

- Vibrios group includes non-pathogenic vibrios & pathogenic vibrio. Pathogenic vibrios are represented mainly by vibrio cholerae (the causative agent of cholera).
- This organism is widely distributed in contaminated water (with human feces).
- vibrio cholerae mainly infect humans and rarely infect other types of living organisms (animals) ,which means that humans are the source of infection in the community . Often, the infection follows fecal-oral route.

special characteristics of vibrio cholerae :

1- it is mainly the only organism which survive at high pH up to (9) pH, this characteristic can be used to detect the presence of it in feces .

2- it is NOT a part of commensal intestinal normal flora ,so often if it is found in intestine , it is associated with the clinical features of the infection .

Note:

- Remember that most human pathogens grow in neutral medium (7 – 7.2 pH).
- vibrio cholerae is cultured in a special selective medium (TCBS agar) that supports the growth of vibrios and suppress others'.

3-Vibrio cholerae - according the somatic antigen (the classical one) and according the composition of the cell wall – recognized as (01) , but there are other somatic antigens which are mutated from the classical one known as 0139 or El-Tor type.

4- Vibrio cholerae produce a very highly potent heat labile toxin in intestines as enterotoxin , this type of toxins can within few hours produce severe damage in the intestinal mucosa , which causes rapid fluid loss from the intestines and blood, example :

within 2-3 hours the infected person might lose 1-3 liter of his/her body fluids . that means severe dehydration , blood acidosis , damage of RBCs , kidney failure , hypotension, failure of all vital organs in the body!>>>> which leads to death (sometimes within few hours) .

Treatment :

The most important treatment to cure a patient (change the course of disease) or at least to prevent the fatal outcome of vibrio cholerae is to restore the loss of fluids, so if the patient for example lost 1 liter of body fluids we give him 1 liter of saline with glucose (IV fluid) to restore the loss of fluids within a short period .

Note:

- Treatment with antimicrobial drugs can help only to reduce the prevalence of vibrios in the community but it won't change the course of the disease.
- The heat labile toxin of vibrio cholerae is to some extent similar to the heat labile enterotoxin of enterotoxigenic E.coli but it is more potent (X100 time than that of E.coli).
- Vibrio cholerae is not invasive it can't reach the blood stream to produce sepsis , also it rarely produce localized infections .
- Other types of cholerae (vibrio parahaemolyticus) might produce blood sepsis& infections ,it is considered as food poisoning organism but it is not related to classical one of vibrio cholerae (it doesn’t produce that heat labile cholera toxin) .

lab diagnosis :

We can't use hektoen-enteric agar , S-S agar or MacConkey agar to culture Vibrio cholerae from feces. As mentioned previously we use TCBS agar (Thiosulfate Citrate Bile salts-Sucrose medium) that support the growth of vibrio cholerae and inhibit the growth of other G-ve bacteria. Vibrio cholerae can be easily recognized in yellowish color due to fermentation of sucrose.

Note:

- Gram stain gives an impression that this is →vibrio cholerae← but we can't depend on this only, we have to culture it and do biochemical tests to identify it.

In 1976 , there was a huge outbreak of cholera in Jordan (vibrio cholerae attack) ,and this involved more than 5 thousand person , few of them died and others were cured (by restoring the loss of body fluids) , but we have observed the following :

1- it is not necessary for all infected persons to have the clinical feature of severe watery diarrhea (not bloody diarrhea → because intestines usually will be affected by toxins not by invasion & inflammatory reaction (no necrosis)).
2- In families, one or two members suffered from the disease and the rest had the organism in their intestines without any clinical feature

but why ?!

-The acidity of stomach variation is the key answer, if the person has low stomach acidity (because of taking anti acidity drugs for example) he will be more susceptible to develop cholera. Also, if the person has a full stomach he is more susceptible to develop cholera.

-Cholerae is highly susceptible for the acidity of stomach, so if there is no direct contact with the acidity of stomach the organism might escape to reach colon and produce its toxins there which results in clinical features of infection.

Brucella (Malta Fever/Brucellosis)

Brucella is the name of an English physician who had discovered this organism in Malta island by accident, there was an outbreak of sever fever associated with enlargement of spleen & liver and respiratory difficulty which give an indication that this is typhoidal salmonella infection, but this physician had observed the following:

1- Most of soldiers in the island drunk unpasteurized milk.
2- In the same period, sheep and gouts abortions were recorded.

He connected the infections of humans with these of animals, and later it has been confirmed that Brucella is related more to animals and it might infect humans, specially by the consumption of unpasteurized milk & milk products OR (rarely) by direct contact with infected animals (for example if dust particle related to infected animal reached the conjunctiva, the organism might produce localized infection in the eye, and later it may reach blood stream to produce infection there too).

For Brucella, the route of infection is related to the GI tract (to contamination of food specially milk products), so it reaches the intestines few in number and after the incubation period (one to four weeks → relatively long) clinical features of the disease start to be observed.

The incubation period of Brucella is long as it reaches the intestines in low numbers, so the number of infected cells is relatively small. But these Brucella cells slowly multiply, especially in the lymph node of intestine (mesenteric lymph nodes) , then it reaches the blood stream, then the liver (producing liver necrosis) , then return back in larger number to the blood and the clinical features begin .

Clinical features of brucellosis are not easy to be recognized in the first stage, it is a puzzle for most physicians!.

The most important clinical feature starts in form of intermittent fever not continuous fever (which means that at early morning the patient has a normal body temp. of (37.5 ^{o}C) , (38 ^{o}C) at mid of the day, (40 ^{0}C) at the night and decline again to (37.5 ^{o}C) at the following early morning . This cycle is due to intracellular infection with Brucella.

Brucella usually multiply in the cell (intracellular multiplication), multiplication takes place in cells like macrophages & monocytes. Within 24 hours releasing of Brucella out of the damaged host cells occurs, and this means releasing of endotoxins (because Brucella is a gram negative bacteria that carry endotoxins in its cell wall) , but the Brucella endotoxin is more virulent than endotoxins of many other types of organisms .

lab diagnosis :

Generally , Brucellosis can be easily diagnosed by culturing blood or CSF (cerebral spinal fluid) due to the fact that Brucella is associated to meningitis , in chronic cases it found in the bone marrow and it can be isolated (bone marrow) , but generally blood specimens are the best. There is no need to culture stool because it cannot be discovered from the stool OR urine.

Treatment:

Treatment of brucellosis is not like any other infection, it need treatment with antibiotics for at least 4-6 week in order to prevent complication.

Note:

Low or short treatment might result in clinical features of chronic brucellosis and the patient cannot be cured later with antibiotics , so the patient will suffer all his life of complications specially in relation to spinal cord and connective tissue.

Brucella culturing requires incubation sometimes up to 6 weeks, but why Brucella needs long incubation in culturing??
Because it is an intracellular organism as we mentioned and it might not be easily released from the infected blood cells, so we have to keep the blood specimens for at least 4 weeks to recover the organism.

Vaccination :

There is a vaccine available for Brucella , but this vaccine is recommended only for animals (not humans) .

Prevention measurements:

Prevention measurements against brucellosis should be directed against infected animals. Generally, in Jordan we do Brucella skin test to detect the positive cases specially among sheep, gout and cows (and many other animals even cats and dogs) that can be infected with Brucella. Any positive case should be isolated and send to be slaughtered, and the rest of animals should be vaccinated.

Note:

Eating well-cooked meat of an infected animals in most cases won't affect our bodies. However the liver of infected animal shouldn't be eaten, it should be discarded.

Campylobacter:

This type of organisms is not easily recovered in most clinical laboratories (not like shigella, salmonella & Brucella that are not so difficult to be recovered and identified in clinical laboratories).

Campylobacter requires a special culture media which contain 3 types of antibiotics. In order to recover the organism we have to incubate the culture specimens at high temperature (~42 ^{0}C).

Like Brucella, animals are the origin of Campylobacter. In the last 40 to 50 years, Campylobacter considered a very important causative agent of diarrheal diseases, often diarrhea caused by Campylobacter species is considered mild diarrhea (rarely associated with severe diarrhea), it can be watery with bloody diarrhea but not severe bloody like that in shigellosis.

Campylobacter can contaminate food articles easily (specially milk & milk products) because this organism is found usually in intestines of domestic animals so we have to expect that it will contaminate with milk and milk products and meat may be, later it might reach our oral cavity and intestines, once it reaches the intestine it might cause clinical features of diarrhea mainly in children less in adult, and more in western countries –in comparison with our countries-.

Note:

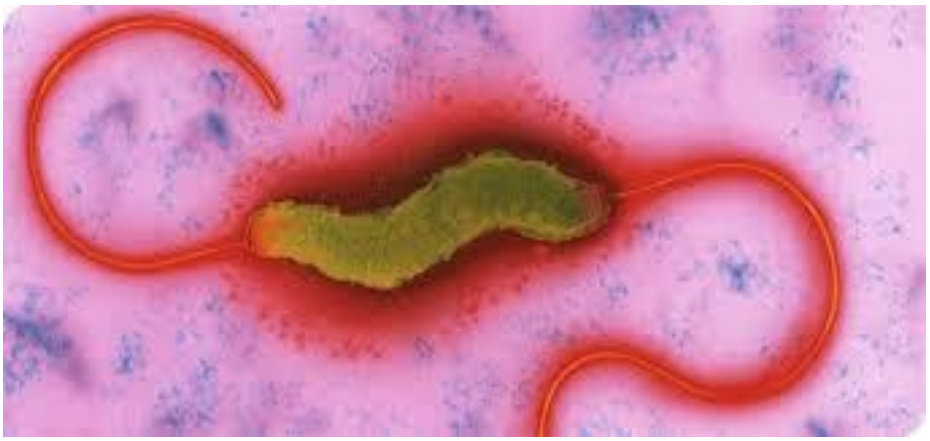
In our country there are only few cases of infection associated with Campylobacteriosis.

Why it is more common in western countries ??
Because they have more pits in their houses specially cats. Cats usually have colonized Campylobacter in their intestines , so it is easy to infect children in the same home , or to contaminate the food in that home .

The problem is that Campylobacter might cause invasive infection (reaching blood stream causing sepsis) only in immunodeficient patients (mostly in children), and this might not be easily detected because the clinical features of sepsis in relation to Campylobacter are not severe , due to the less amount of endotoxins.

Campylobacter has only endotoxins of the cell wall which responsible for inflammation and clinical features of Campylobacter which usually not severe and can be self-limited without using any type of antibiotics.

Campylobacter is a amphitrichous bacteria which has double flagella on both poles , these flagella are responsible for motility and adherence to the small intestines of humans and animals .



culture:

Stool specimens should be cultured in a selective medium that contain 3 type of antibiotics to inhibit other organisms and support the growth of Campylobacter ,later biochemical tests can identify the organism .

vaccination :

there is no vaccine available for humans , however there is one available (but not potent) for animals .

THE END

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^_^ هناك احتمالان لا ثالث لهما : إما أن تنجح ، وإما أن تنجح ^_^

فقط ابذل جهدك وتوكل على الله