

بسم الله الرحمن الرحيم

TODAY'S LECTURE WILL INCLUDE INSHALLAH THE FOLLOWING
BACTERIA SPECIES : SALMONELLA , SHIGELLA, DIARRHEAGENIC
E.COLI ,AND CAMPYLOBACTER

1.salmonella

One of the two major species of salmonella is called salmonella enterica , it's subdivided into six subspecies and has about 2000 serotypes (serovar)

These serotypes are characterized by 3 surface antigens :

1. H antigen : flagella
2. O antigen (somatic) : cell wall
3. Vi antigen : virulence antigen

Salmonellosis

1. food-borne disease
- 2.salmonellosis is divided into : **enteric fever salmonellosis** (cause typhoid fever) & **gastroenteritis/food poisoning salmonellosis** (no typhoidal fever)
3. The type of salmonella most commonly associated with infections (salmonellosis) in humans is called **Non-typhoidal salmonella**, which produce " gastroenteritis salmonellosis" , this type can only reach blood stream or other parts of body esp. meninges, in infants with certain immune deficiency or malnutrition etc., neonates (aged up to 1 month) are more susceptible, in addition to any patient that suffers from immune compromised condition or deficiency, that's how gastrointestinal salmonella can reach blood causing sepsis.

4. Another rarer type of salmonellosis is caused by **typhoidal salmonella** ,which produces typhoidal fever ,and salmonellosis is called here " enteric fever salmonellosis"

Non –typhoidal salmonella ,infects both humans & animals, if the infection reach the blood stream (as in the previously mentioned cases; neonate, IC patients, etc.) , it should be treated with antimicrobial drugs , but with normal healthy individuals ,there's no need for antibiotics ; only supportive treatments can be used such as rehydration ,because the disease will be self-limited.

Typhoidal salmonella, infects only humans & carried only by humans, it is usually transmitted through direct contact with the fecal material of an infected person.

LAB DIAGNOSIS OF SALMONELLA

1. looking for bacteria in food is impractical: Sometimes it isn't easy to detect the causative agent especially in relation to the type of food, we should also recognize the clinical features but they are common with other bacteria (entero-toxigenic e.coli and enetero-pathogenic e.coli)

2. Isolation from stool is most appropriate

3. serological test or "widal test" is used, but it's not significant in diagnosis, however, if used, only anti-H antibodies can be detected and not somatic.

2. shigella

Strains of shigella species are typical members of the Enterobacteriaceae & are between salmonella and diarrheagenic e.coli (entero-pathogenic e.coli and entero-toxigenic e.coli).

Shigella is divided based on biochemical & serological grounds into 4 species :

1. Sh.dysenteriae
2. Sh . flexneri
3. Sh . boydii
4. Sh .sonnei

the last 3 species usually produce mild watery-bloody diarrhea in relation to the release of cytotoxins from cell wall, they produce irritation in surface of intestinal mucosa of mainly large intestine not small.

The mechanism of action of shigella is to some extent similar to salmonella; however, there are differences that can be recognized based on the type of Diarrhea

SHIGELLA MECHANISM OF ACTION

Shigella invade and multiply in the mucosa of large intestine, resulting in an inflammatory response and cellular disruption. During this inflammation there will be an increase in the secretion of cytokines, which together with the protein secreted from cell wall which is a type of cytotoxin and endotoxin produce the feature of inflammation and cause secretion of the fluid which is recognized as watery diarrhea.

But generally, if you examine the stool you will find large no. of pus cells and WBC's (polymorphic nuclear cells), which is a feature of shigellosis. While in salmonella if there is inflammation you will find less no. of WBC's.

Vomiting is not common in shigellosis, the watery diarrhea is much more common.

In general, shigella is not associated with blood sepsis, and it's less invasive than salmonella, however, it can reach blood in young children with malnutrition or underlying disease.

These 3 types of shigella are the most common, found in majority of countries but in certain countries you will find shigella dysenteriae, which is of clinical importance:

shigella dysenteriae

1. It's associated with the classical features of dysentery
2. Causes severe mucopurulent bloody diarrhea with severe abdominal pain & fever
3. If not treated, more severe bloody & dehydration with subsequent death (death is related to dysenteriae not the other types)

4. Septicemia is rare
5. This virulence is due to production of heat-labile shiga enterotoxin (neuro toxin) , which affects small intestine , and carried through the blood to CNS causing mild-severe meningitis & comma .
6. High mortality

Diagnosis of shigella infection

1. **Acute case** : Direct stool examination for presence of numerous WBCs & blood cells
2. **direct rectal swab or rapid stool culture of feces on S-S agar , and Hekton-enteric agar**

In Jordan, our labs rarely isolate it, because it is highly susceptible to acidity in the stool and environmental factors, while salmonella can survive for about 2-3 days while shigella, within 30-60 minutes can be killed during keeping the stool specimens at lab temperature, so if you want to find it you have to collect rectal swab and put it directly on the culture media, or ask the lab to culture it without delay, cause any delay will cause the result to be negative.

Treatment

In salmonellosis : not recommended

In shigellosis : antibiotics are recommended, because you have more diarrhea and more inflammation, more inflammatory reaction, more necrosis in large intestine, and more blood loss. Antibiotics that can be used: **ciprofloxacin, doxycyclin ,cotrimoxazole**: rapid treatment will reduce the severity of disease, antibiotics that are used differ from county to another. Before, they used to use amoxicillin and ampicillin successfully, but now cotrimexazole for children and doxycycline for adults and recently more quinolones are used especially ciprofloxacin, in Jordan we have medium percentage of resistance that's why we have to use selective antibiotics.

Rehydration is important , but not enough.

Vaccine is used in wars and endemic areas: The vaccine is exactly like typhoidal oral vaccine but this one is used mainly in military because the military usually can't wait to recognize the outbreak of shigellosis due to contamination of food and water, In a short period they may have thousands of cases, Americans who invaded Iraq were immunized against shigellosis and cholera, but if the country can control water there is no problem.

Prevention

Concentrates on hygienic control of water, milk & fresh food

Q. Clinical close-contact transmission is more common in shigellosis than salmonellosis , why?

Because few numbers of cells (up to 50) is enough to produce shigellosis, while gastroenteritis salmonellosis need at least 100,000 cells in order for the infection to develop
Note that salmonella typhi only need few cells to be transmitted (like shigella).

So, shigellosis is mainly associated with water and fresh food as well as direct contact between persons while in salmonellosis, direct contact is not important because the no. of infected cells won't be enough.

There's no healthy carrier for shigella and gastrointestinal salmonella.

Incubation period for shigellosis is usually between 2 & 3 days and may reach up to 8 days, then the onset of symptoms is usually sudden, and frequently the initial symptom is abdominal colic, followed by diarrhea

Symptoms typically last for 4 days & can continue for 2 weeks or more.

Shigella is only related to humans, the only other species are dogs, while others are immune against this type of infection.

3. Diarrheagenic E.coli

1. E.coli, is one of the predominant species of facultative anaerobes that found in human intestines, and is part of our normal flora
2. Usually, they are harmless to the host, however, a group of pathogenic E.coli can cause diarrheal diseases in humans
3. We have different strains of e.coli; some produce heat labile or heat stable toxins or both (enterotoxigenic e.coli), some produce colistin¹s which are important to control the presence of e.coli in relation to other facultative anaerobes, to have a biological equilibrium. Any change in the biological equilibrium between the different strains of e.coli will cause a diarrheal disease due to one of these types.
4. These pathogenic strains can be transmitted through contaminated food, water or through contact with animals and birds, after ingestion of them, they can reside in intestine and any change in equilibrium in normal flora will allow these pathogenic bacteria to grow and cause the disease.
5. The pathogenic groups are six and include (only the first 4 are required and will be discussed):

- A. Enterotoxigenic E.coli (ETEC)
- B. Enterohemorrhagic E.coli (EHEC)
- C. Enteropathogenic E.coli (EPEC)
- D. Enteroinvasive E.coli (EIEC)
- E. Enteroaggregative E.coli (EAEC)
- F. Diffusely-adherent E.coli (DAEC)

A. Enterotoxigenic E.coli

- common in domestic animals, poultry and humans
- produce heat stable enterotoxin (in animals) or heat labile enterotoxin (in humans), or both
- they usually express **fimbriae** that attached to enterocytes of small intestine
- **heat labile enterotoxin** is similar to cholera toxin; during evolution
- There might be an exchange in genes between these two types, which can be transmitted from one to another; generally e.coli associated with plasmid and heat labile toxin will acquire the second heat stable toxin from the other type of e.coli; that's why you can find **ETEC** that produces both **LT & ST**

¹ Colistin: an antibiotic like substance produced by e.coli helps in inhibiting the growth of some bacteria in the intestine

- the action of HLT and cholera toxin is similar , because in the evolution of microorganisms world **heat-labile toxin** is transmitted from vibrio cholera through conjugation , and by that **HLT** is " plasmid borne " , the only difference is that cholera toxin is produced about 10-100 times more than heat labile toxin of E.coli .
- both toxins (**LT & ST**) , can cause prolonged hypersecretion of water and electrolytes and inhibit absorption of sodium , resulting in mild-severe watery diarrhea which lasts for 24-48 hours (rarely severe or bloody) , abdominal pain , vomiting (not common), and no fever is seen (because there is no inflammation in intestines because these are toxins, but there might be a small elevation.) .
- they are an important cause of diarrhea in infants-very young children in developing countries.
- We are lucky because in our country and most other developing countries we contact these enterotoxigenic e.coli in an early stage of our life, so we might suffer from a mild type of diarrhea due to this organism which might not be recognized. Therefore, usually when travelling from countries with higher standards of hygiene (developed countries) to a country with lower standards of hygiene (developing countries), the tourists will first suffer from diarrhea due to entero-toxigenic e.coli. So, it is a common cause of traveler's diarrhea in developed countries
- transmitted through contaminated water, dairy products and fresh vegetable food
- self limited , no need for antibiotics , supportive therapy like rehydration is recommended.
- There's an available vaccine for chicken mainly.
- Don't rely on any gram stain to demonstrate the presence of salmonella or shigella, because you won't be able to distinguish between them, you should use culture, gram stain can be of help in blood stream or CSF but not in the stool.

B. Enterohemorrhagic E.coli

- It produces two types of toxins similar to shigella toxins; type 1 and 2, these two toxins originated from shigella, in the past they were called **Vero-toxins** in relation to vero cells extracted from kidney of green African monkeys, and used in culture , but now we call them shiga-like toxins 1 and 2
- this name suggests the transmission of toxin gene from shigella to this strain of E.coli , but how since there's no plasmid for transmission in conjugation ???
- Through lysogenic cycle of reproduction , in which a specific bacteriophage nucleic acid integrates into the host bacterial genome , then the bacteria complete division normally .**
- it will be transmitted through contamination of milk & grounded beef meat: it resides mainly in the intestines of large animals, in particular cows, from cows it can be secreted to the environment and might contaminate meat of cattles, also if this grounded meat or the milk of cows wasn't sterilized or pasteurized it might be contaminated with this type of organism
- causes outbreaks of gastroenteritis ; in the last 2 years in north germany , there was an outbreak in which 4000 cases affected , 700 were admitted to the hospital for dialysis (which is a large no. for the hospital to endure) resulting in death of almost 55 people. Each year there is an outbreak in a certain country, it doesn't occur in sporadic cases but in large numbers
- it's associated with more severe gastrointestinal symptoms , severe inflammation & ulceration
- severe enterocolitis (more damage in the mucosa of the colon) and bloody diarrhea develop , and it's called **Hemorrhagic colitis**.

- later in the infection, the toxin can reach the blood and go to the kidneys , damage occurs in blood and glomerular cells which leads to the presence of **blood and protein** in the urine can be detected, which is called hemolytic uremic syndrome. if not admitted to dialysis to neutralize action of toxin he will suffer from renal failure and death.

-This feature is more severe in young children, old people and people with severe underlying disease.

In cases of infection with EHEC , the treatment with antibiotics is not recommended , Because it will lead to increase in toxins and developing of hemolytic uremic syndrome, so you should give supportive treatment and hydration without using antibiotics.

So, in general we said that the prevention is better than treatment with antibiotics, and there is a vaccine for this organism.

C. Entero-pathogenic E.coli

- it's related only to infants less than one year , since their intestines have no experience with the cell-wall of E.coli, it causes watery diarrhea without blood
- it results in irritation which might result in invasion, sepsis, meningitis etc. as well as diarrhea according to the composition of cell wall.
- causes outbreaks in infants, and related generally to malnourished infants
- 100 serotypes are found and are difficult to be examined in stool.
- might continue for a long period and progresses into chronic diarrhea, and that is why it is responsible for higher mortality in infants than any other types of diarrheal diseases.
- treatment: you have to enhance the nutritional values of his meals, and by hydration, but not by giving antimicrobial drugs.

D. Entero-invasive E.coli

- like shigella ; produces cytotoxin, irritation and necrosis of mucosa in large and small intestines, and associated bloody diarrhea, abdominal pain & fever.
- affects all ages , but more pathogenic in children
- it is not present everywhere; it is found in certain countries more than others
- no vaccine is available , In adults it develops specific antibodies.

LAB DIAGNOSIS OF DIARRHEAGENIC E.COLI

1. stool culture with macConkey agar is not enough, and can't be demonstrated with gram stain , because we have many other strains.
2. And to look for presence of special toxins by classical methods (using intestines of mice) also is impractical.
3. Identification by PCR is more accurate , and within few hours the results obtained, only important for entero-hemorrhagic e.coli and less in others.
4. There are some information that say that enterohemorrhagic e.coli in contrast to others cant ferment

sorbitol sugars, so you might use selective vitro to recognize sorbitol negative e.coli, and then by using PCR.

4. Campylobacter

-If you compare it to the other causative agents of diarrhea in our country, it is not common here but in western countries like USA where they have many pets (cats dogs etc.), it is the most common causative agent of diarrhea. The Reason is that it is found in the intestines of many pets, sheeps, goats and cows, so by accidents it can infect humans.

- acquired via contamination by feces from animals and birds, particularly contamination of fresh food, less water, milk and direct contact.

- Re-infection is common, since campylobacter doesn't produce permanent immunity, also the same for GI salmonella & shigella, Salmonella typhi produces permanent immunity while the others for only short period.

- It can't be cultured easily by routine culture methods; you have to use a special culture media. It is in spiral form. Treatment is not necessary, all others can't be treated by macrolides but this one is susceptible.

-two major strains are identified

1. Campylobacter .jejuni :

A. Produce typical features between shigella & salmonella; it can produce watery diarrhea or watery-bloody pussy diarrhea, it depends on the no. of infected cells and the health condition of patients, but generally the infection is simple and you don't have to give antibiotics.

B. in this infection we recognize **more vomiting than diarrhea**

C. In healthy individuals it's self limited , and no need for antibiotics

D. pathogenesis is due to cytotoxin known as enterotoxin, but the amount is not enough to produce severe inflammation of the intestines.

E. generally it's not invasive , only in certain cases associated with underlying diseases especially with immune-compromised conditions.

F. It also might produce complications in form of joint arthritis which is not easily diagnosed. You have to demonstrate presence of specific antibodies in order to prove that this arthritis is related to campylobacter, such cases were recognized following repeated infections, repeated infection in campylobacter may cause release of antigens in blood which might reach connective tissue and joints and produce inflammation there.

2. Campylobacter. Fetus :

less common in humans , causes sepsis & abortion in animals

Sorry for any mistake , I've tried my best

Your colleague : Alaa Shqeirat