GI

Microbiology

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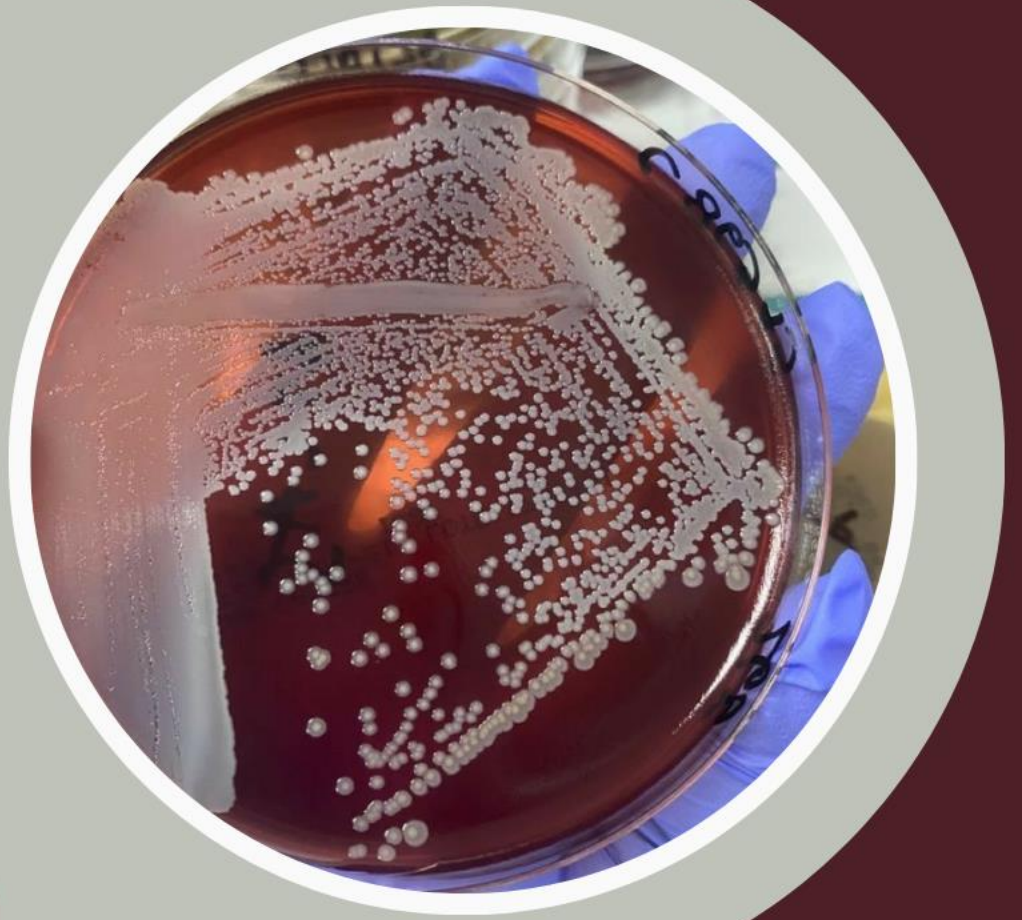

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Enteric Gram-Negative Rods (Enterobacteriaceae)

Today, we are going to talk about shigella and salmonella

By: Nader Alaridah MD, PhD

Shigellosis (Bacillary dysentery)

- The natural habitat of shigellae is limited to the **intestinal tracts of humans** and other primates (Like gorilla), where they produce **bacillary dysentery. (bacillary—> from bacilli and dysentery—>inveasion , WBCs and mucus in affected patients)**
- NOTE: when shigella is mentioned always remember it is invasive causing inflammatory diarrhea.
- Shigellae are slender **gram-negative rods**; coccobacillary forms occur in young cultures but in late culture forms bacilli. Shigellae are facultative anaerobes but grow best aerobically. Convex, circular, transparent colonies with intact edges reach a diameter of about 2 mm in 24 hours.
- All shigellae **ferment glucose**. With the **exception** of **Shigella sonnei,** they do not ferment lactose, they are late lactose fermenters. The inability to ferment lactose distinguishes shigellae on differential media
- **Non-motile**,**non-lactose fermenters**,**do not produse H2S**, and produce a **colorless** colonies in EMB.

Epidemiology

It is a human disease
Mostly occurs in :-
1- asymptomatic subclinical carriers
2- after treatment(chronic intestinal carriers)
3-Paediatric disease <5 yrs
4-Orderly with contact with infected patients and children
5-Homosexual
السجون
**Few hundred bacterial cells are enough to cause an infection
(low infectious dose)which is the lowest between bacterial species

- Man are only reservoir and certain primates are the **only** host.
- Age: **any age but commonly under 5 y/o**. Also, causes in household contacts with children and homosexuals. Causing outbreaks in correctional institution and nurseries.
- It occurs in warm months, temperate climates and rainy seasons in tropical countries.
- **Asymptomatic** infection in endemic areas.
- In industrialized countries , **S.sonnei** is **most common (**in developed countries) with **S.flex** second (common cause in developing countries).
- Transmission: **feco-oral route**, person to person, toilet seat, door handles, contaminated food and water supply and a **vector** causing outbreaks: flies maybe .
- Remember the 4 F's for the transmission: Food, feces, flies, fingers

Etiology

- The genus shigella is subdivided into 4 species (A,B,C and D) according to their biochemical reaction and antigenic composition . **Low number** are required to cause disease : 10-1000.

- Group A **Shigella Dysenteriae** 12 Serotypes**, most imp**. type 1 shiga, most sever disease.

- Group B **Shigella flexneri** 8 serotypes mild disease. (most common in developing countries)

- Group C **Shigella boydii** 18 serotypes.

- Group D **Shigella sonnei** single , intermediately sever disease .

- All of them can produce Shiga toxin, but shigella toxin is encoded by bacteriophage, so if they don't have this bacteriophage; they won't produce this toxin.

Pathogenesis

- Shigella infections are almost always limited to the gastrointestinal tract; bloodstream invasion is quite rare. Shigellae are highly communicable; the infective dose is on the order of less than 10^3 organisms (it usually is 10^5–10^8 for salmonellae and vibrios).

- The essential pathologic process is

  1- **invasion** of the mucosal epithelial cells (eg, M cells) by induced phagocytosis,

  2- **escape** from the phagocytic vacuole,

  3- **multiplication** and spread within the epithelial cell cytoplasm, and

  4-passage to adjacent cells.

- Micro abscesses in the wall of the large intestine and terminal ileum lead to **necrosis** of the mucous membrane, superficial ulceration, bleeding, and formation of "pseudomembrane" on the ulcerated area. This consists of fibrin, leukocytes, cell debris, a necrotic mucous membrane, and bacteria. As the process subsides, granulation tissue fills the ulcers, and **scar tissue forms after granulation.on the other hand, salmonella scar usually doesn't heal .**

Toxins

- **A. Endotoxin**
- It is gram –ve bacteria. Upon autolysis, all shigellae release their toxic lipopolysaccharide. This endotoxin probably contributes to the irritation of the bowel wall. Fever , prolonged fever.
- **B. Shigella Dysenteriae Exotoxin**
- S dysenteriae type 1 (Shiga bacillus) produces a heat-labile exotoxin that is **neurotoxic**, **cytotoxic** and **enterotoxic** .
- Acting as an enterotoxin, it produces diarrhea as does the E coli Shiga-like toxin, perhaps by the same mechanism.
- It is an Enterotoxin that act synergistically with invasion process causing irritation and blood diarrhea.
- In humans, Acting as a "**neurotoxin**," this material may contribute to the extreme severity and fatal nature of **S dysenteriae** infections and to the **central nervous system** reactions observed in them (ie, meningismus, coma).
- The toxic activity is distinct from the invasive property of shigellae in dysentery. The two may act in sequence, the toxin producing an early nonbloody, voluminous diarrhea and the invasion of the large intestine, resulting in later dysentery with blood and pus in stools.

Clinical Findings

- After a **short** incubation period (1–2 days), there is a sudden onset of abdominal pain, fever, and watery diarrhea. The diarrhea has been attributed to an **exotoxin** acting in the small intestine. A day or so later, as the infection involves the ileum and colon, the number of stools increases; they are less liquid but often contain mucus and blood.

- Each bowel movement is accompanied by straining and tenesmus (rectal spasms), with resulting lower abdominal pain.

- In more than half of adult cases, fever and diarrhea subside **spontaneously** in **2–5 days**. However, in children and elderly adults, loss of water and electrolytes may lead to dehydration (fluid therapy is the main approach), acidosis, and even death. The illness caused by S dysenteriae may be particularly severe. They need antibiotic unlike Shiga toxin Ecoli(completely contraindicated)and also they reduced the incidence of haemolytic urimic syndrome .

- On recovery, most persons shed dysentery bacilli for only a short period, but a **few remain chronic intestinal carriers** and may have recurrent bouts of the disease. Upon recovery from the infection, most persons develop circulating antibodies to shigellae, but these do not protect against reinfection. Can cause outbreaks.
- Some patients after treatment, shigella stays in their intestines (asymptomatic) and by that , they can transmit it to society especially if they are food handlers. Remember it is a human disease.

Diagnostic Laboratory Tests

- A. Specimens
- Specimens include fresh stool, mucus flecks, and rectal swabs for culture. Large numbers of fecal leukocytes and some red blood cells often are seen microscopically.

- B. Culture
- The materials are streaked on differential media (eg, MacConkey or EMB agar) and on selective media (Hektoen enteric agar or Salmonella –Shigella agar), which suppress other Enterobacteriaceae and gram-positive organisms.

- C. Serology
- Normal persons often have agglutinins against several Shigella species. However, serial determinations of antibody titers may show a rise in specific antibody. Serology is **not used** to diagnose Shigella infections (not enough because there is a lot of cross reacting antibodies as well as fibril glutanase) .
- **The final identification and definitive diagonosis is isolation in the culture.**

Treatment

Remember here we give antibiotics
MOA: they shortening the duration of the symptoms and reducers the excretions of shigella in the feaces of the carrier
And the prevent secondary cases of affected children

- Ciprofloxacin, ampicillin, doxycycline, and trimethoprim–sulfamethoxazole are most commonly inhibitory for Shigella isolates and can suppress acute clinical attacks of dysentery and shorten the duration of symptoms.

- They may fail to eradicate the organisms from the intestinal tract. Causing 1% to be intestinal carriers.

- Multiple drug resistance can be transmitted by plasmids, and resistant infections are widespread. Many cases are self-limited.

- **Opioids** should be avoided in Shigella dysentery. Because opioids decrease shedding period; giving more time to bacteria to invade. SHOULD BE AVOIDED IN CASE OF BLOODY DIARREAH (it mask the pain) as they can increase the risk of sepsis.
- . Also antimotality drugs should be avoided as they can increase the shedding of shigella in the affected patients .
- Keep in your mind that haemolytic urimic syndrome could be caused by shigella infection.

Prevention, and Control

NO VACCINE FOR THIS TYPE OF INFECTION

- IgA antibodies in the gut may be important in limiting reinfection (cornerstone in GI).
- Serum antibodies to somatic Shigella O-antigens are IgM. (Short-lived immunity)
- Shigellae are transmitted by "food, fingers, feces, and flies" (the 4 F's) from person to person (control measures should be directed at the 4 F's). Because humans are the main recognized host of pathogenic shigellae, control efforts must be directed at eliminating the organisms from this reservoir by
- (1) sanitary control of water, food, and milk; sewage disposal and fly control;
- (2) isolation of patients and disinfection of excreta;
- (3) detection of subclinical cases and carriers, particularly food handlers; and
- (4) antibiotic treatment of infected individuals.

The Salmonella-group

Gram –ve, facultative anaerobes causes Salmonellosis – wide spectrum-
Typhi and paratyphi are responsible for human diseases and they are the only types that can cause enteric fever
** they are founded in intestinal tract of chickens , الطيور, الزواحف

- Salmonellae are often pathogenic for humans (chronic carriers, especially in biliary tree in people who have stones, 1-5%) or animals (major reservoir host, especially in poultry-chickens, meat, dairy products, reptiles and birds) when acquired by the oral route.

- They are transmitted from animals and animal products to humans, where they cause **enteric fever** (Typhoid fever, most sever form of Salmonellosis) ,**gastro- enteritis** (enterocolitis, most common form of Salmonellosis) and **systemic infection** (transient bacteremia and focal lesions (salmonella causes infection in GI tract without symtomatolog in this system—> after that they reach blood circulation to make local suburitive infection in distal sites in GI tract , also the may reach meningese in brain(meningitis), heart(endocarditis), lungs (pneumonia) and bone (osteomyelitis)).
- Most isolates are motile with peritrichous flagella. They almost **never** ferment lactose or **sucrose**. They form acid and sometimes **gas** from glucose and mannose. They usually **produce H2S**. (motility, H2S and gas formation are main features distinctive from Shigella)

The Salmonella-group

- They survive freezing in water for long periods. Salmonellae are resistant to certain chemicals (eg, brilliant green, sodium tetrathionate, sodium deoxycholate) that inhibit other enteric bacteria; such compounds are therefore useful for inclusion in media to isolate salmonellae from feces.
- Salmonellae are named by genus (Salmonella), species (**enterica**, infects humans and warm-blooded animals and **bongori**, infects cold-blooded animals), and subspecies (e.g., typhi or enteritidis) .
- Nomenclature of Salmonella

Genus ---->	species ------>	subspecies
Salmonella	enterica	Typhi or enteritidis

Subspecies o f Medical Importance

- S. enterica subsp. Typhi.
- S. enterica subsp. Paratyphi

→ **Typhoidal salmonella cause typhoid or enteric fever**

- S. enterica subsp. Enteritidis
- S. enterica subsp. Typhimurium

→ **Cause gastroenteritis/ enterocolitis, isolated in USA + worldwide**

- S. enterica subsp. Choleraesuis → **Common cause of bacteremia and focal lesions**
- S. enterica subsp. Dublin → **Cause gastroenteritis, isolated in Europe**

The “Enteric Fevers” (Typhoid Fever)

- We have 2 types of clinical in Salmonella: Typhoidal & Non Typhoidal
- **Typhoidal**: **Four** serotypes of salmonellae that cause enteric fever can be identified in the clinical laboratory by biochemical and serologic tests. These serotypes should be routinely identified because of their clinical significance.

- Salmonella Paratyphi A (serogroup A), Salmonella Paratyphi B (serogroup B), Salmonella Paratyphi C (serogroup C1), and S Typhi (serogroup D).

- **Non-Typhoidal:** Salmonella serotypes Enteritidis and Typhimurium are the two most common serotypes reported in developed world.

Epidemiology

- Typhoid fever is sever systemic disease. (mortality rate is high in pre-antibiotic era, but it has decreased in post-antibiotic era).
- Incidence differ significantly developing vs developed counties 0.2-4 cases to up to 500 in developing countries /10^5 population. (around 0.5 mil deaths yearly)

- Humans are the natural reservoir. The feces of persons who have unsuspected subclinical disease or are carriers are a more important source of contamination than frank clinical cases that are promptly isolated, such as when carriers working as food handlers are “shedding” organisms. It might be found in the gallbladder in stones , urinary bladder and intestines.

- Many animals, including cattle, rodents, and fowl, are naturally infected with a variety of salmonellae and have the bacteria in their tissues (meat), excreta, or eggs.

- Food ,water contaminated with human faeces (**fecal–oral route**), **vertical from infected ,pregnant women to her fetus —>**transmission (trans- placental).

Pathogenesis

- The vast majority of salmonellae, however, are chiefly pathogenic in animals that constitute the reservoir for human infection; these include poultry, pigs, rodents, cattle, pets (from turtles to parrots), and many others
- Stomach acidity and normal intestinal microbiota are important determinants of susceptibility.
- The salmonella invades peyer paches (M cells), remain in endocytic vacuole, where they replicate, ingulfed by macrophages and transported to other intestinal L.N.(lymph nodes) where they multiply in Mononuclear cells to mesenteric L.N. to blood through thoracic duct (**transient bacteraemia**).
- Circulating organism reach reticule-endothelial cells in **liver ,spleen and bone marrow** and circulating endo -toxin **cause prolonged fever**. (Chief complaint)
- Inflame mucosa and lymphatics. Necrosis and sloughing of overlaying epithelium producing **ulcer that may bleed**. Ulcers heal without scarring.
- Cell mediated immunity is important

Clinical manifestations

- **Incubation 7-14 days**. Onset is insidious.
- **1st week:**
- **High** Fever, malaise ,anorexia myalgia headache, abdominal pain ,diarrhoea early and later constipation. **But no GI symptoms in the first week of infection in Typhi, paratyphi**
- Temp. increase in **a stepwise fashion** become unremitting and high (a high platuea).

fever rises one day, falls the subsequent morning, and continues to form peaks in evenings higher than day before.

- **2nd week:**
- High fever, fatigue, cough ,epistaxis. **abdominal symptoms more sever** (alternating bowel habits, watery diarrhoea in the first but it becomes bloody), rose spots and rash on the chest and trunk also coughing .
- **3-4 weeks:**
- If no complications, symptoms & signs **gradually resolve**.
- In the **pre-antibiotic era**, the chief complications of enteric fever were intestinal hemorrhage and perforation, and the **mortality rate was 10–15**%. Some others carry Salmonella as part of their normal tract microbiome, especially in people with cholelithiasis

Enterocolitis **(Salmonella Gastroenteritis)**

- This is the most common manifestation of salmonella infection **in human**.
- **Mainly from animal meat) الدواجن ، الطيور ، الزواحف.**
- In the United States, S Typhimurium and Salmonella Enteritidis are prominent, but enterocolitis can be caused by any of the more than 1400 group I serotypes of salmonellae. **In developing countries (like jordan) we have no information about the responsible serotype of salmonella that cause Salmonella Gastroenteritis.**
- Eight to 48 hours after ingestion of salmonellae**(short incubation period)**, there is nausea, headache, vomiting, and profuse diarrhea, with few leukocytes in the stools. Low-grade fever is common, but the episode usually resolves in 2–3 days. Inflammatory lesions of the small and large intestine are present.

- Bacteremia is rare**(in immunocompetent)** (2–4%) except in immunodeficient persons.
- **All salmonella species can cause bacteremia, but mainly we see it in typhoid fever and bacteremia with focal lesions.**
- **If invasion process got deeper, it might access the circulation even if they Enteritidis and Typhimurium, not like Typhi and Paratyphi which have capsular antigen "resistant serum killing"**

- **Blood culture results are usually negative**, but **stool culture results are positive** for salmonellae and may remain positive for several weeks after clinical recovery.
- **In Enteric Fever patient, there blood culture is positive (even urine culture is positive) while stool culture is negative.**

Bacteremia with Focal Lesions

- This is **associated commonly with S choleraesuis** but may be caused by any salmonella serotype. After oral infection, there is early invasion of the bloodstream (with possible focal lesions in lungs, bones, meninges, and so on), but intestinal manifestations are often absent.
- **These Focal Lesions usually outside the GIT, most common site is the meninges of the brain (cause meningitis), Lung (cause pneumonia), Heart (cause endocarditis), and Bone (cause osteomyelitis).**
- **In general, the most common bacteria that cause osteomyelitis S.aureus, but in these certain population(children with sickle cell disease) Salmonella is the most common cause of osteomyelitis.**
- **Don't forget the colonization that happen less than 5%, this colonization usually occur in urethra or gallbladder, even it could be found in intestine or urinary system.**
- Blood culture results are positive.

Diagnostic Laboratory Tests

- A. Specimens

- culture : positive in Blood, Bone marrow, Stool & Urine culture results may be positive after the second week.

- In enteric fevers, the stools yield positive results from the second or third week on; in enterocolitis, the stools yield positive results during the first week. A positive culture of duodenal drainage establishes the presence of salmonellae in the biliary tract in carriers.

B. Bacteriologic culturing for Isolation of Salmonellae

1. Enrichment cultures— The specimen (usually stool) also is put into selenite F or tetrathionate broth, both of which inhibit replication of normal intestinal bacteria and permit multiplication of salmonellae.

2. Differential and Selective medium cultures

Then Put bacteria in Differential medium (EMB, MacConkey, or deoxycholate medium) which will give us colorless colonies,
then subculture it in Selective medium (salmonella-shigella (SS) agar, Hektoen enteric agar and xylose-lysine decarboxylase (XLD) agar.)

3. Final identification by serology or serotype— Suspect colonies from solid media are identified by biochemical reaction patterns and slide agglutination tests with specific sera.

C. Serologic Methods

Slide agglutination tests

- 1. Agglutination test— In this test, known sera and unknown culture are mixed on a slide. Clumping, when it occurs, can be observed within a few minutes. This test is particularly useful for rapid preliminary identification of cultures. There are commercial kits available to agglutinate and serogroup salmonellae by their O antigens: A, B, C1 , C2 ,D, and E.
- **After putting the bacteria in selective agar, we will be moving it to serum antibodies against O antigen, H antigen as well as VI antigen from salmonella.**
- **Where agglutination occur, you could know the species, subspecies and serotype.**

Serologic Methods

There is explanation in the next slide

- 2. **Tube** dilution agglutination test (Widal test)—
- Serum agglutinins rise sharply during the second and third weeks of S Typhi infection. The Widal test to detect these antibodies against the O and H antigens has been in use for decades.
- At least two serum specimens, obtained at intervals of 7–10 days, are needed to prove a rise in antibody titer.
- Serial dilutions of unknown sera are tested against antigens from representative salmonellae. False-positive and false-negative results occur. The interpretive criteria when single serum specimens are tested vary, but a titer against the O antigen of greater than **1:320** and against the H antigen of greater than **1:640** is considered positive.
- High titer of antibody to the Vi antigen occurs in some carriers. Alternatives to the Widal test include rapid colorimetric and EIA methods.
- Results of serologic tests for Salmonella infection cannot be relied upon to establish a definitive diagnosis of typhoid fever and are most often used in resource poor areas of the world where blood cultures are not readily available .

This test was used a lot when the doctor was a student😀
Was relied upon for the diagnosis of salmonellosis, but you have to know that shigella and salmonella definitive diagnosis is isolation and identification of the bacteria🦠 (so culture is the definitive diagnosis), serology can't be relied upon any more, but in poor resources countries, it could be used as a suggested or presumptive diagnosis for shigellosis.

In this method, i look for antibodies against the antigens that we talked about (O,H,VI), but in the serum of the patient.

I can't make this in the first week because antibodies need time to appear in the serum of the patient.

Then I put these antibodies with antigens I have in laboratory  (O,H,VI) and you have to take more than one sample to see the rising in antibody titer, sometimes you rely on single take, and if the antibodies against O antigens and somatic antigens more than 320 dilution, it is usually positive. For H antigen if it is more than 640 it is positive.

Can't be relied upon because if the patient is infected with other bacteria gastroenteritis, they can give false positive, and typhoid has vaccine so it could give false positive.

Recently, enzyme immunoassay and rapid colorimetric nucleic acid assays are available for the diagnosis of salmonellosis.

Immunity

- Infections with S Typhi or Salmonella Paratyphi usually confer a certain degree of immunity.
- **Even with vaccines they don't give immunity more than two years.**
- **Shigella infection confers a certain degree of immunity, typically short-lived and usually specific to the crossboundary homologous serotype. So, you would have protection against the same serotype, and this protection doesn't last more than few years.**
- Reinfection may occur but is often milder than the first infection. Circulating antibodies to O and Vi are related to resistance to infection and disease. However, relapses may occur in 2–3 weeks after recovery despite antibodies.
- Secretory IgA antibodies may prevent attachment of salmonellae to intestinal epithelium. **IgA is an important limiting factor for establishing infection in gastrointestinal as well as respiratory tract.**
- Persons with S/S hemoglobin (sickle cell disease) are exceedingly susceptible to Salmonella infections, **particularly osteomyelitis and bacteremia in healthy individual**. Persons with A/S hemoglobin (sickle cell trait) may be more susceptible than normal individuals (those with A/A hemoglobin).

Treatment

Antibiotics are NOT RECOMMENDED in patients with Enterocolitis!!

- Although enteric fevers and bacteremias with focal lesions require antimicrobial treatment(Mandatory), the vast majority of cases of enterocolitis do not.
- Antimicrobial treatment of Salmonella enteritis in neonates is important. In enterocolitis, clinical symptoms and excretion of the salmonellae may be prolonged by antimicrobial therapy. In severe diarrhea, replacement of fluids and electrolytes is essential.
- Antimicrobial therapy of invasive Salmonella infections is with fluoroquinolones, ampicillin, trimethoprim–sulfamethoxazole, or a third-generation cephalosporin.
- Multiple drug resistance transmitted genetically by plasmids among enteric bacteria is a problem in Salmonella infections.
- **It is preferred to be guided by antimicrobial Susceptibility testing because of antimicrobial resistance patterns throw transmissible plasmids.**
- Susceptibility testing is an important adjunct to selecting a proper antibiotic. In most carriers, the organisms persist in the gallbladder (particularly if gallstones are present) and in the biliary tract. Some chronic carriers have been cured by ampicillin alone (it is enough to eradicate the carriage of salmonella Typhi and paratyphi), in some patients it isn't enough so —> in most cases cholecystectomy must be combined with drug treatment.

Prevention and Control

- Three percent of survivors of typhoid become healthy permanent carriers, harboring the organisms in the gallbladder; biliary tract; or, rarely, the intestine or urinary tract.
- Sanitary measures must be taken to prevent contamination of food and water by rodents or other animals that excrete salmonellae.
- Infected poultry, meats, and eggs must be thoroughly cooked.
- Carriers must not be allowed to work as food handlers or **food preparer** and should observe strict hygienic precautions.
- **Two typhoid vaccines** are currently available : an oral live attenuated vaccine**(Ty21a vaccine against Typhi and paratyphi fever ONLY and it isn't widely used due to its low efficacy (<2 yrs), NOT protect against GI)**, and a Vi capsular polysaccharide vaccine**(called ViPS, subunit vaccine)** for intramuscular use.
- Vaccination is **recommended for travelers to endemic regions**, especially if the traveler visits rural areas or small villages where food choices are limited, **efficacy of 50–80%.**not recommended for widely used

The End

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