University of Mustanisiriya
College of Medicine
Third stage

Salmonellae and Shigellae

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The salmonella

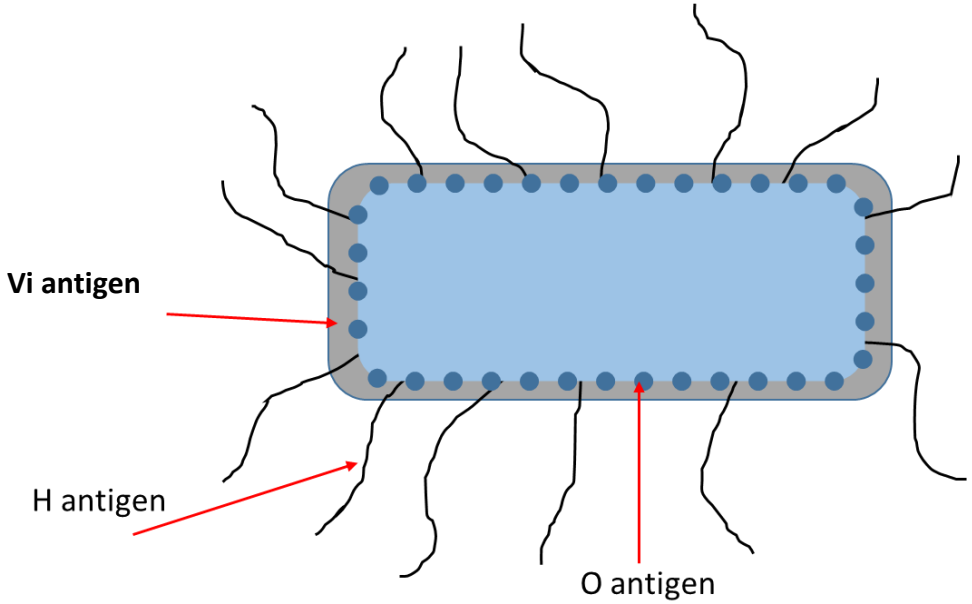
- Pathogenic for humans or animals when acquired by the oral route.
- The bacteria transmitted to human via contaminated food, contaminated water or from person to person
- They cause different infections for human such as :
- Gastroenteritis
- Systemic infection (bacteraemia)
- Enteric fever (typhoid).

The morphology and growth characteristics

- Gram- negative bacilli .
- Most isolates are motile with peritrichous flagella.
- Grow readily on simple media
- They almost never ferment lactose or sucrose.
- They form acid and sometimes gas from glucose and mannose.
- **They usually produce H2S.**
- They survive freezing in water for long periods.
- Resistant to some 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.

Surface antigens and variation

- As a member of the enteric family, Salmonella posses the three antigens :
- Somatic (O) antigen, Fagellar (H) antigen , and capsular K antigen (called Vi in salmonella)
- Fagellar (H) antigen can be found in two forms called phase 1 and phase 2 and the organisms can switch from one phase to another
- This is called phase variation.
- Salmonella can loss H antigens and become non motile
- It can also loss O antigen, and switch from smooth to rough colony
- Vi Antigen can be lost partially or completely



Classification

- Recently, the genus Salmonella is divided into two species.
- ***Salmonella enterica*** and ***Salmonella bongori***
- Based on phenotypic profiles *, S. enterica* were divided into 6 subspecies
- enterica (subspecies I),
- salamae (subspecies II)
- arizonae (subspecies IIIa)
- diarizonae (subspecies IIIb)
- houtenae (subspecies IV)
- indica (subspecies VI)
- The subspecies I strains (written as *S enterica* subspecies enterica) is responsible for the majority of human illness

Kauffmann White classification

- The salmonella are also classified into different O serogroups and serotypes according to the variability in their surface antigens :
  - O somatic antigen , and H flagellar antigen.
  - O groups, or O serogroups as A, B, C1, C2, D, and E
  - Each O group contains a number of serotypes possessing a common O antigenic structure not found in other O group
  - Within each O group the different serotypes are differentiated by variability in the structure of their H antigen(s)
  - The classification then gave species status to each serotype such as naming the species were named according to the disease caused (S. Typhi) or of the animal source (S. Gallinarium),
  - There are more than 2500 serotype has been identified
- Less than 100 serotype are responsible for the majority of infection in humans
- The globally used nomenclature for classification is as follows:
- *S. enterica* subspecies *enterica* serotype Typhimurium
- It can also be shortened to ***S.*** **Typhimurium** with the **genus name in italics** and the **serotype name in roman type.**

- Salmonella species can also be divided into “typhoidal” and “non typhoidal” depending on their serotypes
- Typhoidal salmonella refers to the serotypes that responsible for typhoid (enteric) fever which include :
- Salmonella Paratyphi A (serogroup A),
- Salmonella Paratyphi B (serogroup B), S
- Salmonella Choleraesuis (serogroup C1), and
- S. Typhi (serogroup D).
- Non typhoidal refers to other serotypes that do not cause typhoid fever such as Enteritidis and Typhimurium

Pathogenesis and disease

- Infection with typhoidal salmonellae (S. Typhi and S. Paratyphi) believed to be acquired from a human source (as these serotypes is are highly adapted and restricted to human host).
- Non-typhoidal serotypes , which responsible for gastroenteritis, can be acquired from different animal sources as well as from environment.
- Different animal can be carriers for salmonella and act as a the reservoir for human infection; these include poultry, pigs, rodents, cattle, pets and many others.
- Transmission: By oral route, via ingestion of contaminated food or drink.
- The mean infective dose to produce clinical or subclinical infection in humans is 10^5–10^8 salmonellae
- The main diseases that are produced by Salmonellae in humans are:

I. Enteric fever (typhoid)

- Sever systemic infection
- Caused by *Salmonella* typhi (most common), and *Salmonella* Paratyphi
- Transmission : ingestion of contaminated food or drinks
- Incubation period : 10 -15 days
- Clinical meinfistation : fever, malaise, headache, constipation, bradycardia, and myalgia occur after the incubation period
- Fever reach a plateau (39 ^{0}C to 40 ^{0}C)
- The spleen and liver are enlarged
- Rose spots (1-4 mm) blanching pink muscle continuously observed on the chest and abdomen in less than 5% of the cases
- Abdominal symptoms include : diarrhea, constipation , and general abdominal pain

Pathogenesis

- After ingestion of the bacteria with contaminated food and drinks ,
- The bacteria reach the small intestine, enter the intestinal lymphatics and then reach the bloodstream, and subsequently spread to many other organs, including the intestine.
- The organisms multiply in intestinal lymphoid tissue and are excreted in stools.
- The principal lesions are
- hyperplasia
- necrosis of lymphoid tissue (eg, Peyer’s patches);
- hepatitis;
- focal necrosis of the liver;
- inflammation of the gallbladder, periosteum, lungs, and other organs.

II. Bacteremia and other invasive infection

- Bacteraemia and vascular infection occur in around 8 % of patients with non-typhoidal salmonella infection
- Cases of meningitis, septic arithritis and osteomyelitis have reported as a complication of salmonella bacteremia but are rare events
- This is associated commonly with *S choleraesuis* and *salmonella* Dublin but may be caused by any salmonella serotype.
- Bacteremia is more common in infants, elderly and immunocompromised people.
- Mortality rate from salmonella bacterimia in children is less than 10 %
- The mortality rate can be increase with duration of the bacterimia and potential progression to septic shock , and also in patients with concomitant endovascular invasion (ranged from 14 -60 %)

III. Enterocolitis

- *S.* Typhimurium and *S.* Enteritidis are the dominant causes , but enterocolitis can also be resulted from any other serotypes (around 1400 serotype) of serogroup I salmonellae.
- Eight to 48 hours after ingestion of salmonellae, there is nausea, headache, vomiting, and profuse diarrhea, with few leukocytes in the stool
- Low-grade fever and abdominal crumbing are very common
- Diarrhea is self limited typically last for 3 to 7 days
- Inflammatory lesions of the small and large intestine
- Bacteremia is rare (2–4%) except in immunodeficient persons.

Chronic carriage

- The majority of people who recovered from infection of salmonella still excrete the bacteria in their stools for several days or weeks
- This will be followed by the clearance of the bacteria from the body.
- The term chronic carrier is used for people who continue harbour the salmonellae in their body for prolonged period (a year or more).
- Around 3% of people who convalescents from typhoid fever become permanent carriers for salmonella
- The bacteria can be harboured in the gallbladder, biliary tract, the intestine or urinary tract.
- The development of carriage state is more common in women and in older age groups
- The rate of patients who become carriers increased with age from 1 % in patients under 20 years to up to 10 % in patients over 50 years

Laboratory diagnosis

A. Specimens :
   - Non-typhoidal salmonella : fresh stool
   - Typhoidal salmonella : blood , bone marrow (less practical) , other sterile sites, urine or intestinal secretion

B. Culture : speciemens can be plated on different media including:
   1. Differential media :
      - EMB, MacConkey, or deoxycholate medium permits rapid detection of lactose nonfermenters (salmonella and other genera)
      - Bismuth sulfite medium: rapid detection as many salmonellae form black colonies due to H2S production.
   2. Selective media: such as :
      - Salmonella-shigella agar (S-S agar), Hektoen enteric agar, xylose-lysine decarboxylase (XLD) agar and others
   3. Final detection:
      - Unconfirmed colonies on agar media can then be identified by biochemical tests and agglutination tests with specific sera

***Salmonella* on SS Agar**

Source of picture : https://microbiologyinfo.com/salmonella-shigella-ss-agar-composition-principle-uses-preparation-and-result-interpretation/

C. Serological test

1. Agglutination test : using known sera against unknown culture
   - It is useful for rapid preliminary identification culture
   - commercial kits available to agglutinate and serogroup salmonellae by their O antigens: A, B, C1, C2, D, and E
2. Tube dilution agglutination test (Widal test)
   - Used to detect the antibodies raised against the O and H antigens
   - Serum specimens taken in the 7-10 days of S. Typhi infection (always with high titters at this period)
   - Serial dilution of these sera will be tested against antigens from representative salmonella
   - Higher titers of antibodies against O (<1:320) and H (1;640) is considered as positive
   - 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.

D. Nucleic acid amplification tests

- There are several commercial kits for direct detection of salmonella in fecal samples with acute diarhea

Treatment

1. **Typhoidal salmonella infection**
   a) Uncomplicated enteric fever: treatment with oral azithromycin
   b) Patients with complications: parental treatment with third generation cephalosporins or flouroquinilone for at least 10 days
2. **Non-typhoidal bacterimia:**
   - empirical treatment with third generation cephalosporins (cefitriaxone) and flouroquinilone until doing antibiotic suitable test
3. **Suspected or confirmed endovascular infections**:
   - patients should be treated for 6 weeks intravenously with either Ceftriaxone, or ampicillin or flouroquinilone followed by oral therapy
4. **Non-typhoidal salmonella gastroenteritis**:
   - Antibitic treatment in common cases is not needed as the infection is self limited , but it recommended in neonates , immunosuppression patients, and elderly people(over 50 years) with suspected or confirmed endovasicular diseases
5. In case of **sever diarrhia** : fluid and electrolytes replacement is essential

Prevention and controls

- **Prevention**

- Sanitary measures to prevent contamination of food and water with salmonella that excreted by rodents and other animals
- Thoroughly cooking of poultry , meat, and eggs.
- Prevents carriers from working in food industries and observe strict personal hygiene

- **Vaccination:**

- Two typhoid vaccines are availabele :
    1. Oral live, attenuated vaccine (Ty21a)
    2. Vi capsular polysaccharide vaccine (Vi CPS) for intramuscular use
- Recommended for travellers (who visit endemic area or rural area)

The Shigellae

THE SHIGELLAE

- The bacteria is the causative agents of Shigellosis (Bacillary dysentery)
- The intestinal tracts of humans and other primates is the natural habitats of this bacteria
- The bacteria transmit from person to another fecal-oral route

Morphology and identification

A. Typical Organisms

- gram-negative rods**,**
- facultative anaerobes but grow best aerobically.
- produce Convex, circular, transparent colonies with intact edges.
- **C. Growth Characteristics**
- All shigellae ferment glucose except ***Shigella sonnei,*** they do not ferment lactose.
- Ferment carbohydrates and produce acid, but rarely produce gas.
- They may also be divided into those that ferment mannitol and those that do not ferment mannitol.

• Antigenic Structure

1. **Somatic O antigens** : The shigellae are divided into four ‘major’ O antigenic groups, designated A, B, C, and D.

- Groups A, B, and C also contain minor O antigens, using for subgrouping.

2. **K antigen**: presence in some strains , and it is not important for serologic typing

3. **H antigen**: shigellae is non-motile and does not have H antigen.

- The somatic O antigens is overlapping with other enteric bacteria such as *E. coli*
- Therefore identification of shigellae should be made by a combination of antigenic and biochemical properties

Classification

- Depending on the combination of biochemical and serological characteristics, Shigellae are classified into four species or groups (A, B, C, D)
- They are also divided into mannitol fermenting species and mannitol nonfermenting species
- There are more than 40 serotypes according the their serologic specificity depends on the polysaccharide

Shigella species	Group	Manitol fermentation
S. dysenteriae	A	-
S. flexneri	B	+
S. boydii	C	+
S. sonnei	D	+

Pathogenesis and Pathology

- The infections is limited to the gastrointestinal tract
- systemic invasion (bloodstream) is very rare.
- The infective dose is around (10-100) organisms (which is low in comparison to salmonellae and vibrios, it usually is 10^5–10^8).
- The essential pathologic process involve :
  - invasion of the mucosal epithelial cells (eg, M cells) by induced phagocytosis
  - escape from the phagocytic vacuole
  - multiplication and spread within the epithelial cell cytoplasm, and passage to adjacent cells.
  - Microabscesses in the wall of the large intestine and terminal ileum lead to necrosis of the mucous membrane, superficial ulceration, bleeding, and formation of a “pseudomembrane” on the ulcerated area.

Toxins

A. Endotoxin

As any Gram negative bacteria , all shigellae release their toxic lipopolysaccharide after their hydrolysis .

Their endotoxin may responsible for irritation of the bowel wall.

B. Exotoxin

- *S dysenteriae* type 1 (Shiga bacillus) produces a heat-labile exotoxin
- It acts as enterotoxin and neurotoxin
- The toxin is antigenic inducing production of antitoxin
- The enterotoxiginic activity: it produces diarrhea as does the E coli Shiga-like toxin, perhaps by the same mechanism. In humans, the exotoxin also inhibits sugar and amino acid absorption in the small intestine.
- Acting as a “neurotoxin
  - it damages endothelial cells of small blood vessels of the central nervous system
  - Leading to neurological complications like polyneuritis, coma and meningism.
- In both activity : It does not involve in the invasiveness of the shigella in dysentery

Clinical Findings of bacillus dysentery

- After (1–2 days) of incubation, there will be a sudden onset of abdominal pain, fever, and watery diarrhea.
- The diarrhea is a consequence of the exotoxin activity in the intestine
- After 1-2 days , the stools increases (but with less liquid) and often contains mucus and blood (due to invasion of intestinal mucosa).
- This is will be accompanied with rectal spasms, with resulting lower abdominal pain
- In adults , the infection is mainly self-limited , and the fever and the diarrhea can disappear spontaneously within 2-5 days in more than 50 % of the cases .
- In elderly and children, dehydration and acidosis can be developed and it can even lead to death.
- The recovered people may carry shigella for short period
- Some people become chronic intestinal carriers (rare) for the dysentery bacilli and may re-infected many times .

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

- Specimens can be streaked on:
  - **differential media** (eg**, MacConkey or EMB agar**)
  - **selective media** (**Hektoen enteric agar or Salmonella–Shigella agar**)
- The growing colourless (non-lactose fermenting) colonies can be inculated into TSI agar
  - **TSI agar: shigella can be diagnostic as it is** H2S –ve , produce acid but not gas in the butt and an alkaline slant in TSI agar

C. Motility test can be done : the bacteria are nonmotile

D. slide agglutination by specific Shigella antisera (not depended in diagnose Shigella infections)

Treatment

- **Antibiotics :**
  - **Ciprofloxacin**, **Ampicillin**, **Doxycycline** and **trimethoprim sulfamethoxazole** are the choice of treatment of Shigella
- They may not eradicate the bacteria completely from the intestine
- Multi-drug resistance features carried on plasmids can be widespread leading to resistant infection .
- Many cases are self-limited.

Epidemiology,

- Shigellae are transmitted by fecal-oral rout , food and flies
- Most cases of Shigella infection occur in children younger than 10 years of age.
- Shigellosis, caused primarily by ***S sonnei***, has become an important problem in daycare centers in the United States.
- ***S dysenteriae*** can spread widely.

Prevention and Control

- Mass **chemoprophylaxis** has been tried for limited time, but resistant strains of shigellae tend to emerge rapidly.
- Some controlling measures can be taken to eliminate the bacteria from infecting human such as :

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**
4) **antibiotic treatment of infected individuals.**

References

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THANK YOU