Salmonella

Prof. Md. Akram Hossain
An estimated 22 million cases of typhoid fever and 200,000 related deaths occur worldwide each year.

In Bangladesh, the overall incidence of typhoid fever is 390 cases per 100,000 population annually. i.e. 6.0 lakh per year.
Introduction

- **Ebreth first in 1880 & Gaffkey subsequently from typhoid fever in 1884 so named as Ebrethella typhi**
- **Salmon & Smith** first isolated the bacillus from faeces of Hog cholera in 1885.
- Normally resides in the colon of vertebrates and pathogenic for humans & many animals
  - poultry, pigs, rodents, cattle, parrots
- **Cause enteric fever (Typhoid & paratyphoid fever), food poisoning & septicemia**
Taxonomy

- There more than 2500 serotypes, of which >1400 can cause human infections
- Broad host range
- Highly adapted to human infections
  - S. typhi, S. paratyphi A, S. paratyphi B, S. paratyphi C
  - S. typhimurium, S. enteritidis, S. newport, S. dublin
  - S. cholerasuis
- 3 methods of nomenclature
  - Ewing – 3 species (S. typhi, cholerasuis & enteritidis with many subspecies)
  - Kauffmann White – O & H antigen, 64 “O” groups (A-Z, 15-64)
  - DNA hybridization analysis
Morphology & cultural characters

Motile with peritrichous flagella except
\textit{S. pullorum} & \textit{S. gallinarum}

GNB 2-4 \(\mu\text{m}\times0.6 \, \mu\text{m}\)

- Lactose non Fermenter i.e. form pale colonies in MA medium. Grows in selective media like SSA, Selenite or tetrathionate broth

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Biochemistry:

- Do not ferment lactose, sucrose, salicin
- Ferments glucose, maltose, mannitol
- Negative for indole and positive for methyl red reaction.
- Most strains produce H2S (except S. paratyphii A & cholerasuis)
  - LSS - - -
  - GMM +++
  - IMViC - + - -
Salmonella Serology

- **Salmonella** can be subdivided by somatic (cell-wall) or O antigens and flagellar or H antigens.
  - “O” >65 types, less immunogenic than H, IgM
  - “H” two types phase 1 & phase 2, more immunogenic & long lasting, IgG.

- **Clinical importance:** Titer of “H” is more than “O”
Virulence factors

1. Invasiveness
2. Endotoxin
   - Possess LPS endotoxin characteristic of Gram-negative bacteria
     - composed of an "O" polysaccharide ("O" antigen)
     - "R" core
     - endotoxic inner "Lipid A"
     - Endotoxins evoke fever and can activate complement, kinin and clotting factors.

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Structure of a Gram-Negative Cell Wall

LPS
O polysaccharide
lipid A

outer membrane

lipoprotein

peptidoglycan

cytoplasmic membrane

phospholipid

porin

protein

protein
Details of endotoxin

Summary of Endotoxin

- LPS
- LPS-LBP
- CD14
- TLR4
- TNFα

Circulation

Macrophages

Toxic shock

Fever, tissue damage, IL6, IL8, IL1, PAF

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Salmonella Illness

Clinical infections caused by salmonella varies with different species or serotypes

1. Gastroenteritis or salmonellosis
   - *S. enteritidis* most commonly isolated species, *S. typhimurium*, *S. dublin*
   - contaminated food or water. Most commonly,
   - often from contaminated poultry (turkeys and chickens).

2. Enteric fever:
   - *S. typhi* (no animal reservoirs), *S. paratyphi* A, B, C (have animal reservoirs)
   - transmitted from person to person and involves
   - reservoirs:
     - Contaminated food or water with human feces
     - asymptomatic human carriers

3. Septicemia
   - By *S. cholerasuis*
Epidemiology

- Salmonella gastroenteritis due to eating contaminated food, often chicken and turkeys, but also pigs and cows.
- Meats and animal products (eggs) not thoroughly cooked are main source of infections.
- 20-40,000 cases/yr in USA.
- Typhoid fever - no animal reservoir, human carriers who contaminate food and water; 400-500 cases/yr in USA.
After binding to cell receptors, bacteria taken in by endocytosis but do not escape endosome.

Pathogenesis of Salmonella enteritidis

- Ingestion of organisms
- Colonization of lower intestine (ileum and cecum)
- Mucosal invasion
  - Cytotoxin
- Acute inflammation + ulceration
  - ? Prostaglandin synthesis
  - ? Enterotoxins
- Activation of adenyl cyclase
  - ↑ Cyclic AMP
- Fluid secretion (large and small bowel)
- Diarrhea
FIGURE 24-6 Gastroenteritis is the most common manifestation of Salmonella infection. After passage through the stomach, the bacteria absorb to the brush border of the epithelial cells lining the terminal small intestine and the colon. The bacteria migrate to the lamina propria layer, where they multiply in the lymphoid follicles, stimulating a leukocytic response. Stimulation of prostaglandin-mediated production of cyclic AMP and active fluid secretion also occurs.
**Enteric fever**

**FIGURE 24-7** Pathogenesis of enteric fever. After ingestion of bacilli, *S. typhi* passes through the epithelial cells lining the terminal portion of the small intestine and the colon. The bacilli are engulfed by macrophages and then are carried to the cells of the reticuloendothelial system, where multiplication continues in the liver, spleen, and bone marrow. Signs of sepsis are seen after a 10- to 14-day incubation period. The bacilli will spread from the liver through the gall bladder and into the intestines. This stimulates gastrointestinal symptoms.
Pathogenesis of enteric fever

Salmonella typhi, paratyphi A, B, C

Small intestine

Attach, invade epithelial cells & engulfed by MPS where they multiply

Released into blood via thoracic duct– **Primary bacterimia**

7 - 10 days

Cleared from blood by MPS and will lodge in liver, spleen, lung, Lymph node

Parasitized cells undergoes necrosis & bacilli heavily reinvade blood

**Secondary heavy bacterimia**

Localized to gall bladder, liver, spleen etc

From gallbladder to payers patches of intestine-hemorrhage, ulcer

Clinical illness

Occurs at 14th days of infection
Pathogenesis of typhoid fever

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Invasion of M cells by Salmonella leads to destruction of M cell and perforation of intestinal lining. Persistence within phagocytes is basis for systemic spread of organism.
Clinical manifestations

**Gastroenteritis**

6-48h incubation, then nausea, vomiting and diarrhea with fever and chills; lasts 2-7 d

**Enteric Fevers**

10-14 d incubation; gastroenteritis may precede enteric fever;
high sustained fever, malaise, anorexia, headache and myalgias;
complications include intestinal obstruction, severe bleeding, cholecystitis, sepsis, etc.

Approximately 30% of typhoid patients may have rose spots - a faint salmon-colored maculopapular rash on the trunk; organisms can be cultured from punch biopsies of these lesions, and the pathology is characterized as a perivascular mononuclear cell infiltrate.
Evolution of Typical Symptoms and Signs of Typhoid Fever

<table>
<thead>
<tr>
<th>Disease period</th>
<th>Symptoms</th>
<th>Signs</th>
<th>Pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st week</td>
<td>Fever, chills gradually increasing and persisting, headache</td>
<td>Abdominal tenderness</td>
<td>Bacteremia</td>
</tr>
<tr>
<td>2nd week</td>
<td>Rash, abdominal pain, diarrhea or constipation, delirium, prostration</td>
<td>Rose spots, Splenomegaly, Hepatomegaly</td>
<td>Mononuclear cell vasculitis of skin, hyperplasia of ileal Peyer’s patches, typhoid nodules in spleen and liver</td>
</tr>
<tr>
<td>3rd weak</td>
<td>Complication of intestinal bleeding and perforation, shock</td>
<td>Melena, ileus, rigid abdomen, coma</td>
<td>Ulcerations over Peyer’s patches, perforation with peritonitis</td>
</tr>
<tr>
<td>4th weak And later</td>
<td>Resolution of symptoms, relapse, Weight loss</td>
<td>Reappearance of acute disease, cachexia</td>
<td>Cholecystitis, chronic fecal carriage of bacteria</td>
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“rose spots”

a faint, salmon-colored, blanching, maculopapular rash located primarily on the trunk and chest.
Late complications
in the third and fourth weeks

- intestinal perforation and/or gastrointestinal hemorrhage.
- life-threatening, require immediate medical and surgical interventions, with broadened antibiotic coverage for polymicrobial peritonitis and treatment of gastrointestinal hemorrhages, including bowel resection.
The incidence of chronic carriage

- **Chronic carrier:** Typhoid fever
  - Higher among **women** & among persons **with biliary abnormalities** (e.g., gallstones, carcinoma of the gallbladder) and gastrointestinal malignancies.

- The **anatomic abnormalities** associated with these conditions presumably allow prolonged colonization.
Principle: is based on isolation of the Salmonella spp. by blood culture & culture of stool etc also by immunological test i.e. detection of antibody (widal test) and antigen (recently introduced). Detection of DNA by Molecular tests like PCR. Tests of choice varies with duration of illness. Blood culture and antigen detection is reliable in the first week and widal test is helpful in 2nd week onwards. PCR is helpful 1st week onwards.

Steps:

Specimen: Blood, Bone marrow for culture, Ag Detection & PCR in 1st week and for Antibody test in 2nd week.

M/E: is not helpful.

Isolation & identification (Culture): Blood culture can be done in three processes -
  - Traditional - using liquid media or biphasic media
  - Lytic method - after processing in lytic solution directly in solid media
  - Automated - very rapid (within 6 hrs)

Immunological test: Widal test for Ab detection & Ag detection, Dot ELISA for IgM

PCR
Other than a positive culture, no specific laboratory test is diagnostic for enteric fever.

- Leukopenia & neutropenia, the white blood cell count is normal despite high fever.
- Leukocytosis can develop (especially in children) during the first 10 days of the illness, or later if the disease course is complicated by intestinal perforation or secondary infection.
- Liver function tests
- Electrocardiograms.
The diagnostic "gold standard"

- Isolation of organism
- A blood culture positive for *S. typhi* or *S. paratyphi*.
  - As high as 90% during the first week
  - Decrease to 50% by the third week.
- Bone marrow cultures
  - highly (90%) sensitive despite 5 days of antibiotic therapy.

- Culture of intestinal secretions (best obtained by a noninvasive duodenal string test)
  - can be positive despite a negative bone marrow culture.
Widal’s reaction

Widal’s test

- Associated persons:
  Max von Grube
  Georges Fernand Isidore Widal

Description:
Serological agglutination test for typhoid fever.

The test is based on the presence of agglutinating antibodies against paratyphoid bacteria in the patient’s serum. It is mainly used for indirect confirmation of salmonellosis (as against typhus abdominalis, paratyphus, and brucelloses), but also for diagnosing infectional diseases like shigelloses, tularaemia and spotted fever.

Bacterial agglutination was discovered by Max von Gruber and Herbert Edward Durham (1866-1945).

- It was first briefly reported by Durham, and then described by Gruber and Durham. Their discovery was the basis for Widal’s test for typhoid.
If blood, bone marrow, and intestinal secretions are all cultured, the yield of a positive culture is >90%.

- Stool cultures,
  - negative in 60 to 70% of cases during the first week,
  - positive during the third week of infection in untreated patients.
Q. What is enteric fever? Why it is so called? Name tests used for diagnosis in the 1st week of illness.

Ans: Enteric fever is a systemic illness, manifested by fever and other symptoms and caused by Salmonella typhi, S. paratyphi A, S. paratyphi B, & S. paratyphi C. They are so called because their main lesion occurs in the intestine (payers patches).

Blood culture and detection of salmonella antigen from the blood and other specimens are the reliable tests used for diagnosis in the first week of illness.
What is Widal test?
- It is a serological test for detection of antibodies against S. typhi, S. paratyphi A, B

Which antibodies?
- "O" & "H" antibodies
- TO = Somatic antibody of S. typhi,
- TH = Flagellar Antibody S. typhi
- AO = Somatic antibody S. paratyphi A,
- AH = Flagellar antibody of S. paratyphi A
- BO = Somatic antibody S. paratyphi B,
- BH = Flagellar antibody of S. paratyphi B
What is the role in the diagnosis of enteric fever?
- It is helpful
  - where culture facilities is not available
  - When patient comes late (2nd week)
  - When patient takes antibiotics
  - If interpreted cautiously
  - Epidemiological important
History of widal test

- Georges Fernand Isidore Widal, a French physician discovered in 1896
  
  - In 1896 Widal developed a procedure for diagnosing typhoid fever based on the fact that antibodies in the blood of an infected individual cause the bacteria to bind together into clumps (the Widal reaction).

- During World War I, Widal prepared an antityphoid-paratyphoid vaccine that appreciably reduced typhoid contagion among the allied armies.
Titer

<table>
<thead>
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<th>TO</th>
<th>AO</th>
<th>BO</th>
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<td>1:80</td>
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Widal test

Days of illness

1st week

2nd week

3rd week

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What is the interpretation of widal test?

- Following points must be considered for interpretation of widal test:
  - “O” antigen – is somatic, sharing, Polysaccharide, weak, T- independent
  - “H” antigen – flagellar, specific, protein, strong, T- dependant
  - Antibodies develop after 2\textsuperscript{nd} week of illness
  - Local titer (titer in the healthy population) varies from region to region – TO, TH, AO, AH = 1:80, BO, BH = 1:40
  - “Rising titer” rather than single high titer is diagnostic
  - Effect of early administration of antibiotic
  - Administration of vaccine
How will you interpret widal test?

1. Any high titer before 2\textsuperscript{nd} week is to be interpreted cautiously.
2. Very High Single titer either of “O” or “H” (1:320) is \textit{significant}.
3. Rising titer with paired samples (done 5-10 days apart) is \textit{diagnostic}.
4. Anamnestic reaction can occur and is manifested by moderately high titer of only “H”
5. Early use of antibiotics hamper with rise of antibody titer.
6. Even after successful treatment high titer persists for 3-6 weeks.
Treatment

**Gastroenteritis** - Supportive, no antibiotics

**Typhoid fever**
- 1st choice – Fluroquinolone or 3rd generation cephalosporins - for 14 days
- Azithromycin for 7-10 days

**Septicemia or enteric fevers** = Parenteral with double dose
Prevention

- Fluoroquinolones to treat carriers of *S. typhi* (1-3% of infected pts may become carriers, usually bacteria in gallbladder)
- Public health sanitation standards
- Typhoid vaccine - Typhrix
Review questions

- What type of bacteria is Salmonella?
- What is its clinical importance?
- Name the diseases caused by Salmonella spp.
- Name some clinically important salmonella spp.
- What is enteric fever? Why it is so called?
- Pathogenesis of Enteric fever?
- How can you diagnose enteric fever if the patient comes in a) 1st week b) 2nd week?
- What is the gold standard test for diagnosis of Enteric fever?
- What is blood culture? Short notes on Blood culture. What are the other specimens that can be cultured for diagnosis of enteric fever?
- What is widal test? Which antibodies are detected? What is its clinical importance
- Interpretation of widal test?