Salmonella

Case

A 8 year old boy was admitted to the pediatrics ward with a history of remittent fever which increased gradually in a step-ladder pattern over the previous 10 days. He had taken antipyretics and ciprofloxacin, prescribed by local private practitioner. At presentation to the hospital, he complained of lack of appetite, pain in abdomen and lethargy. On examination, he was found to have fever with anemia and hepatospleenomegaly.

- Investigation:

Blood Count: relative neutropenia

Blood Culture: Salmonella typhi was positive.

Widal test was negative.

IgM antibody for S.Typhi was positive.

Isolate was resistant to ciprofloxacin and so he was treated with ceftrioxone.

He responded after 10 days of therapy.

Salmonella

- 1885 - Diseases Salmon and Smith - discovery of '*Salmonella*'
- *Salmonella* Typhi, the causative agent of typhoid fever was first observed by Eberth (1880) and Gaffky (1884) → Eberth-Gaffky bacillus or *Eberthella* typhi.

Classification

- **Clinical Classification**
- Oldest, user friendly classification widely used

1. **Typhoidal *Salmonella:***

- Includes serotypes *S. Typhi* and *S. Paratyphi*
- Restricted to human hosts → enteric fever (typhoid/paratyphoid fever)

2. **Non-typhoidal *Salmonella or NTS:***

- Colonize intestine of animals
- Also infect humans → gastroenteritis & septicemia

Antigenic Classification (Kauffmann–White Scheme)

Serogroup		Serotype name	O Ag*	Vi Ag	H Ag*	
New	Old				Phase 1	Phase 2
2	A	S.Paratyphi A	1,2,12	-	a	[1,5]
4	B	S.Paratyphi B	1,4,[5],12	-	b	1,2
		S.Typhimurium	1,4, [5],12	-	i	1,2
		S.Agona	1,4,12	-	f,g,s	-
		S.Heidelberg	1,4,[5],12	-	r	1,2
7	C1	S.Paratyphi C	6,7	+	c	1,5
		S.Choleraesuis	6,7	-	c	1,5
		S.Thompson	6,7,14	-	k	1,5

Antigenic Classification (Kauffmann–White Scheme)

Serogroup		Serotype name	O Ag*	Vi Ag	H Ag*	
New	Old				Phase 1	Phase 2
8	C2-C3	S.Muenchen	6,8	-	d	1,2
		S.Newport	6,8,20	-	e,h	1,2
9	D1	S.Typhi	9,12	+	d	-
		S.Enteritidis	1,9,12	-	g,m	[1,7]
		S.Gallinarum	1,9,12	-	-	-
		S.Dublin	1,9,12	+	g,p	-
3,10	E1	S.Anatum	3,10,[15],[34]	-	e,h	1,6

Features of O and H Antigen

O antigen	H antigen
Somatic antigen Part of cell wall lipopolysaccharide (LPS)	Flagellar antigen Made up of proteins –flagellin confers motility to the bacteria.
Heat stable , Alcohol stable	Heat labile, Alcohol labile
Formaldehyde labile	Formaldehyde stable
In Widal test- O antigen of S.Typhi is used	In Widal test- H antigens of S.Typhi, S.Paratyphi A and B are used
O Ag is less immunogenic	H Ag is more immunogenic

Features of O and H Antigen

O antigen	H antigen
O antibody appears early, disappears early – indicates recent infection	H antibody appears late, disappears late- Indicates convalescent stage
When O antigen reacts with O antibody- forms compact, granular, chalky clumps Agglutination takes place slowly. Optimum temperature for agglutination is 55^0C	When H antigen reacts with H antibody- forms large, loose, fluffy clumps. Agglutination takes place rapidly. Optimum temperature for agglutination is 37^0C

Features of O and H Antigen

O antigen	H antigen
Serogrouping is based on the O antigen	Serogroups are differentiated into serotypes based on H antigen
Also called Boivin antigen- extracted from bacterial cell by treatment with trichloracetic acid, first shown by Boivin	Flagellar antigens exist in two alternative phases – Phase I and II. Most of them are biphasic except, (monophasic - S.Typhi)

Vi Antigen

- Surface **polysaccharide envelope** or capsular antigen covering the O antigen
- Named with belief that Vi antigen is related to **virulence**
- Expressed in **only few serotypes** - *S. Typhi, S.Paratyphi C, S. dublin and some stains of Citrobacter freundii (Ballerup-Bethesda group)*
- Renders the bacilli **inagglutinable with O antiserum** → agglutinable after boiling / heating at 100°C for 1 hour, which removes Vi antigen

Vi Antigen

- **Poorly immunogenic** & antibody titers are low → Not helpful in diagnosis of cases
- Complete **absence of Vi antibody → poor prognosis**
- Disappears early in convalescence. If persists → **carrier state**
- **Phage typing** of *S. Typhi* - using Vi specific bacteriophages
- Vi antigens - used for **vaccination**

TYPHOIDAL SALMONELLA

- *S. Typhi and S. Paratyphi* A, B and C which cause enteric fever
- **Pathogenesis**
- Transmitted - feco oral
- **Infective dose-** Minimum 10^3–10^6 bacilli
- **Risk factors** that promote transmission: Conditions that decrease:

- Stomach acidity (<1 year age, antacid ingestion, or achlorhydria or prior *Helicobacter pylori* infection*)*
- Intestinal integrity (inflammatory bowel disease, prior GIT surgery or suppression of the intestinal flora by Antibiotics)

Pathogenesis

- **Entry through epithelial cells** (M cells) lining the intestine

- Bacteriamediated endocytosis (BME) - mediated by type III secretion system

- **Entry into macrophages:** Salmonellae containing vacuoles cross epithelial layer to reach submucosa → phagocytosed by macrophages
- **Survival inside macrophages:** induces certain alterations on its surface → insusceptible to the lysosomal enzymes

Pathogenesis

- **Primary bacteremia:** From inside macrophages spread via the lymphatics to enter the blood stream (transient primary bacteremia)
- **Spread:** Disseminate throughout reticuloendothelial tissues (liver, spleen, lymph nodes and bone marrow → further multiplication
- **Secondary bacteremia occurs from the seeded organs** → clinical disease

Clinical Manifestations

- Incubation period - 10–14 days
- **Fever (step ladder pattern type of remittent fever):**
- **Other symptoms:** Headache, chills, cough, sweating, myalgia and arthralgia
- **Rashes (called rose spots):** Faint, salmon-colored, blanching, maculopapular rash - trunk and chest (30%)
- **Early intestinal manifestations** - abdominal pain, nausea, vomiting and anorexia
- **Important signs -** hepatosplenomegaly, epistaxis and relative bradycardia

Clinical Manifestations

- **Complications:** Gastrointestinal bleeding and intestinal perforation – in third and fourth weeks of illness
- **Neurologic manifestations –**

- Meningitis, cerebellar ataxia and neuropsychiatric symptoms (“muttering delirium” or “coma vigil”)

- Paranoid psychosis, hysteria, delirium and aggressive behavior

Epidemiology

- **Host:** Humans the only natural hosts
- **Mode of transmission**: Ingestion of contaminated water and food
- **Prevalence:** Worldwide
- **Incidence is:**
- **Highest** (>100 cases per 100,000 population per year) in south central and southeast Asia
- **Medium** (10–100 cases per 100,000) in the rest of Asia, Africa, Latin America
- **Low** (<10 cases per 100,000) in other parts of the world

Epidemiology

- **Locality and age: Enteric fever is:**
- More common in urban than rural areas
- More common among young children and adolescents than in adults
- **Factors that favor transmission:**
- Poor sanitation, contaminated water, food and drinks
- Lack of hand washing and toilet access
- **Typhi vs Paratyphi:** *S. Typhi* infection is more common than *S. Paratyphi A (ratio is 4:1)*

Epidemiology

- **Carriage:** Up to 10% of untreated patients

1. **Fecal carriers**: Multiply in the gall bladder & excreted in feces. More common
2. **Urinary carriers**: Multiply in kidneys & excreted in urine

- **Duration of shedding:**

- Convalescent carriers 3 weeks to 3 months
- Temporary carriers 3 months to 1 year
- Chronic carriers for more than 1 year

Epidemiology

- **Chronic carriers (1–4% of infected** people) - more common in:
- Women, infants and old age
- Biliary tract abnormalities which leads to increased fecal excretion
- Abnormalities of urinary tract and associated *Schistosoma haematobium infection of bladder*— leads to increased urinary excretion.
- **Food handlers or cooks** who become chronic carriers – dangerous
- Mary Mallon **(‘Typhoid Mary’)** - More than 1300 cases

Laboratory Diagnosis – Sample Collection

Duration of illness	Specimen used and test done
First week	Culture of– • Blood, Bone marrow aspirate, Duodenal aspirate
Second week and Third week	Serum- • For antibody detection by Widal test • For antigen detection Stool and urine culture
Fourth week	Stool and urine culture
Carriers	Stool and urine culture Serum- for detection of antibodies to Vi antigen Sewage culture- indirect way

Laboratory Diagnosis - Culture and identification

- **Blood Culture**
- Sensitivity 90% in first week
- **Clot culture**: Blood is centrifuged → serum for Widal test and clot for culture
- **Culture medium:**

- **Conventional:** Brain heart infusion broth, Castaneda's biphasic medium (BHI agar slope and BHI broth)
- **Automated** blood culture systems - BACTEC orBacT/ALERT

Media for Blood culture

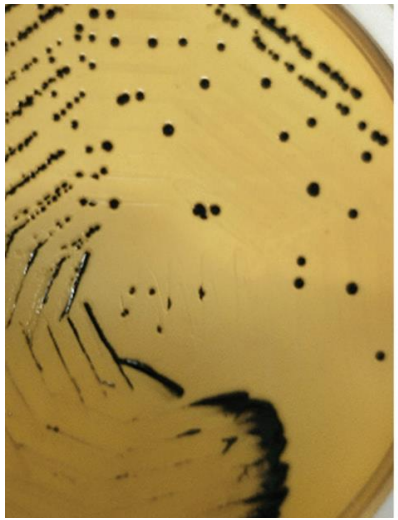
Isolation

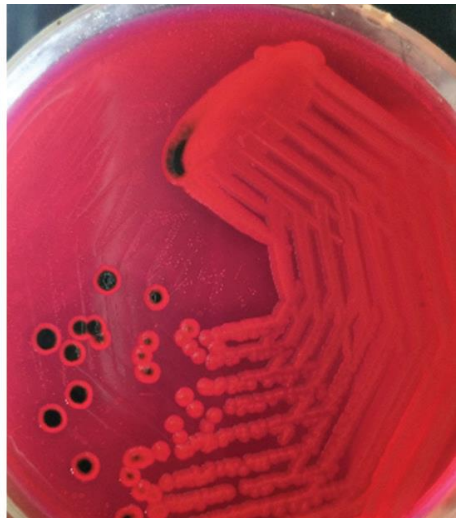
- **Sodium polyanethol sulfonate (SPS):** Anticoagulant, Counteracts bactericidal action of blood
- **Incubation:** at 37°C for up to 1 week.
- **Repeat subcultures:**
- **Monophasic BHI** medium: Periodical subcultures onto blood agar and MacConkey agar
- **Biphasic medium** BHI is preferred over monophasic - subcultures in the same bottle
- **Automated** blood culture systems – monitor growth continuously & positive growth flagged → subcultures done

Isolation

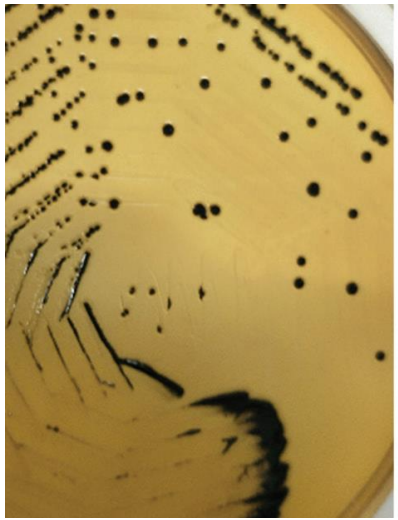
- **Enrichment broth** - Selenite F broth, tetrathionate broth and gram-negative broth are used
- **Selective media such as:**
- **Low selective media** - MacConkey agar – Colourless colonies

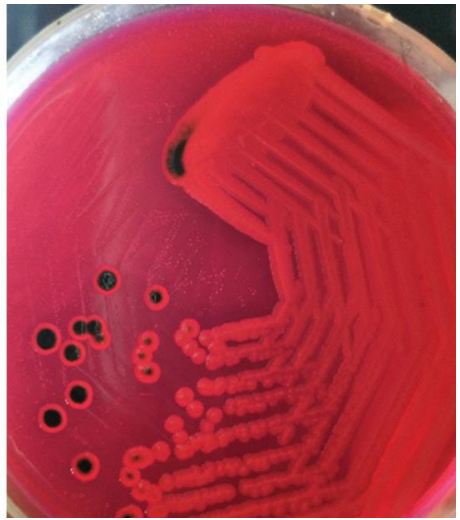
- **Highly selective media:**
- **DCA (deoxycholate citrate agar):** pale colonies with black center





- **XLD agar (xylose lysine deoxycholate):** red colonies with black center





Isolation

- **SS agar (*shigella Salmonella agar):*** colorless with black centers
- **Hektoen enteric agar:** Bluegreen colonies with black centers
- **Wilson Blair’s brilliant green Bismuth sulfite medium** –jet black colored colonies with a metallic sheen due to production of H2S

Other Specimens

- **Bone marrow culture** - first week of illness (55–90% sensitive) when blood culture is negative, especially when patient is on antibiotics
- **Duodenal aspirate culture** - first week of illness if both blood and bone marrow cultures turn negative
- **Combination of blood, bone marrow, and intestinal secretions** culture is the best method in the first week, which shows a sensitivity of more than 90%

Identification

- **Culture Smear and Motility Testing**
- Gram-negative, non-sporing and non–capsulated bacilli
- Motile with peritrichous flagella
- **Biochemical Identification**
- Catalase positive and oxidase negative
- Indole test—negative
- Citrate test—positive (except for *S. Typhi and S. Paratyphi A,* which are citrate negative)
- Urease test—negative

Biochemical Identification

- **Biochemical Identification of Salmonellae**
- TSI shows: Alkaline/acid, Gas present (except for *S. Typhi,* which is anaerogenic*),*
- Abundant H_2S present except for:
- *S. Paratyphi A and S. Choleraesuis:* H2S not produced
- *S. Typhi:* Speck of H_2S
- MR positive and VP negative

Biochemical Identification

- Sugar fermentation test: Gluocse, mannitol, arabinose, Maltose, dulcitol and sorbitol are fermented
- **Decarboxylation test**

- *S. Typhi*—only lysine is decarboxylated
- *S. Paratyphi A*— only ornithine is decarboxylated
- *S. Paratyphi B*— positive for all, i.e. lysine, arginine and ornithine

Slide Agglutination Test

- *S. Typhi:* Agglutinates with O9 antisera
- *S. Paratyphi A:* Agglutinates with O2 antisera
- *S. Paratyphi B:* Agglutinates with O4 antisera
- **Masking effect of Vi antigen:**

- Tested with Vi antisera
- Boiled for 60 minutes at 100^{o}C → testing with O antisera

- **Antimicrobial Susceptibility Testing**

Demonstration of Serum Antibodies

- **Widal Test**
- **Principle:** Tube agglutination test where H and O antibodies against *S. Typhi and S. Paratyphi A and B* are detected
- **Antigens used:** Four antigens are used

1. O antigens of *S. Typhi (TO)*
2. H antigens of *S. Typhi (TH)*
3. H antigens of *S. Paratyphi A (AH)*
4. H antigens of *S. Paratyphi B (BH)*

Widal Test

- **Procedure of Widal test:**
- Patient’s serum is serially diluted in normal saline in test tubes from 1 in 10 to 1 in 640 dilutions
- Four such sets are made
- To each set of diluted sera, respective four antigen suspensions (TO, TH, AH, BH) are added
- Control tubes containing the antigens and normal saline should be kept to check for autoagglutination
- Test tubes are incubated in water bath at 37°C overnight

Widal Test

- **Results:**
- **O agglutination** - compact granular chalky clumps (disk-like pattern), with clear supernatant Fluid
- **H agglutination** - loose fluffy cotton-woolly clumps, with clear supernatant fluid
- **No agglutination** - button formation & supernatant fluid remains hazy

Widal Test - Interpretation

Widal test result	Suggestive of -
Rise of TO and TH antibody	Enteric fever due to S.Typhi
Rise of TO and AH antibody	Enteric fever due to S.Paratyphi A
Rise of TO and BH antibody	Enteric fever due to S.Paratyphi B
Rise of only TO antibody	Recent infection -Due to any serotype -S.Typhi or S.Paratyphi A or B
Rise of only TH antibody	? Convalescent stage/ Anamnestic response
Rise of all three TH, AH, BH antibodies-	Post TAB vaccination

Widal Test

- **Titer:** The highest dilution of sera, at which agglutination occurs
- **Significant titer: In India**
- H agglutinin titer more than 200
- O agglutinin titer more than 100
- Low titers should be ignored and considered as baseline titers in endemic areas

Widal Test

- **False-positive:**
- **Anamnestic response**: Transient rise of titer due to unrelated infections (malaria, dengue) in persons who have had prior enteric fever
- Antigen suspensions are not free from fimbriae
- Persons with inapparent infection
- Persons with prior immunization (with TAB vaccine)
- **Fourfold rise in antibody titer** demonstrated by testing paired sera at 1 week interval is more meaningful

Widal Test

- **False-negative:**
- Early stage (1st week of illness) & Late stage (after fourth week)
- Carriers
- Patients on antibiotics
- Prozone phenomena (antibody excess)— this can be obviated by serial dilution of sera
- O agglutinins appear early and disappear early and indicate recent infection. H agglutinins appear late and disappear late

Widal test

- O antibodies are serotype nonspecific. H antibodies are specific
- **Other Antibody Detection Tests**
- **Typhidot test:** OMP (outer membrane protein) antigen is used, detects both IgM and IgG antibodies
- **IDLTubex test:** O9 antigen is used, detects only IgM antibodies against *S. Typhi*
- **IgM dip stick test and ELISA** detect anti-LPS IgM antibodies
- **Dot blot assay:** Flagellar antigen is used, detects only IgG antibodies

Other Tests

- **Demonstration of Salmonella Antigen**

- Present blood & urine - ELISA

- **Molecular Methods**

- PCR-based methods - detect and differentiate typhoidal salmonellae - flagellin gene, Iro B and fliC gene

- **Other Nonspecific Methods**

- **WBC count**: Neutropenia (15–25%), Leukocytosis - children

- **Liver function tests** moderately deranged

- **Muscle enzyme** levels moderately elevated

Detection of Carriers

- **Culture:** Stool and bile culture (detects fecal carriers) and urine culture (detects urinary carriers)
- **Detection of Vi antibodies:**

- Tube agglutination test using *S. Typhi* suspension carrying Vi antigen (Bhatnagar strains)
- Titer of 1:10 is also considered as significant

- **Isolation of salmonellae from sewage:** To trace the carriers in the communities

- Sewer–swab technique & Filtration

Drug Resistance

- More drug resistant than typhoidal salmonellae
- **MDR strains** - Resistant to ≥5 drugs - ampicillin, chloramphenicol, streptomycin, sulfonamides & tetracyclines
- Ceftriaxone resistance – AmpC beta lactamase
- Ciprofloxacin resistance - point mutation in DNA gyrase genes

THANK YOU...!