

HLS MICROBIOLOGY LAB

SALMONELLA

By: **Sima Shihab**

Enterobacteriaceae, G (-) bacilli, Ox (-) Cat (+), has flagellae, present in GIT of animals

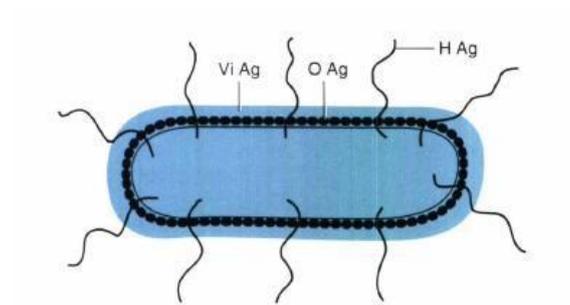


Characteristic Antigens:

H Antigen (flagellar, most commonly)

Vi Antigen (capsular; antiphagocytic; Vi=Virulence)

O Antigen (LPS)



Route of transmission:

FOODBORNE: Ingestion of contaminated food e.g. Chicken, uncooked eggs, dairy products, fish
<main source: animals; oral-faecal route>

Infectious dose: 10^3 S. Typhi & 10^5-8 other types.

(the higher the dose, the more symptomatic the disease is)

Can survive the low pH/high acidity of the stomach, but once it's in the intestines it attaches to the cells and the different types of infections begin (the preferred place is the ileum).

The most common type of infection is: **Gastroenteritis** aka food poisoning. If it crosses the cytoplasm to the lymph nodes & BS & disseminates it can cause a wide range of infections.

{Gastroenteritis is confined to the GIT, but in Typhoid fever there's haematogenous spread of the bacteria}

In **Typhoid fever:** Can cause **colonisation of the gallbladder** after a systematic infection if treatment was not very efficient. This can cause regular shedding of the bacteria in the stools and can serve as a source of infection to others.

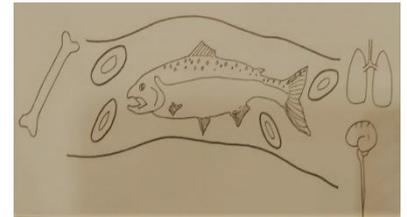
Four forms of Salmonella infection exist:

1) Gastroenteritis:

- Symptoms: Diarrhoea, dizziness, fatigue, vomiting, abdominal cramps (non-bloody stools, with or without mucous)
- Onset of initial presentation of symptoms in patient: within 6-48 hrs of ingestion
- Spontaneously resolves
- Can cause dehydration; treat with fluids and electrolyte replacement.

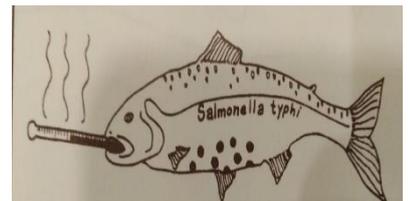
2) Septicaemia:

- Presence & Multiplication of the bacteria in the blood
- Cross to different organs: Liver, spleen, BM... etc
- Symptoms: Fever, malaise, hypotension
- Later stages: Septic shock, DIC, hypotension, renal failure



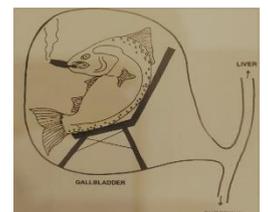
3) Enteric Fever (Typhoid fever):

- Onset of action: 10-14 days (unlike Gastroenteritis which only takes a few hours)
- Takes around 10-14 mins to develop
- How long does it last?
- It has spread to the organs before the symptoms have started
- Common response: fever + pink rash



4) Asymptomatic Colonisation (Carrier state):

- Occurs in 1% of patients after inadequate treatment of Typhoid fever and resolution of its symptoms
- In gallbladder
- Even with gallbladder colonisation, re-endurance of the infection is NOT common.



QUESTION: What is the reason behind Salmonella causing different types of infections in different cases?

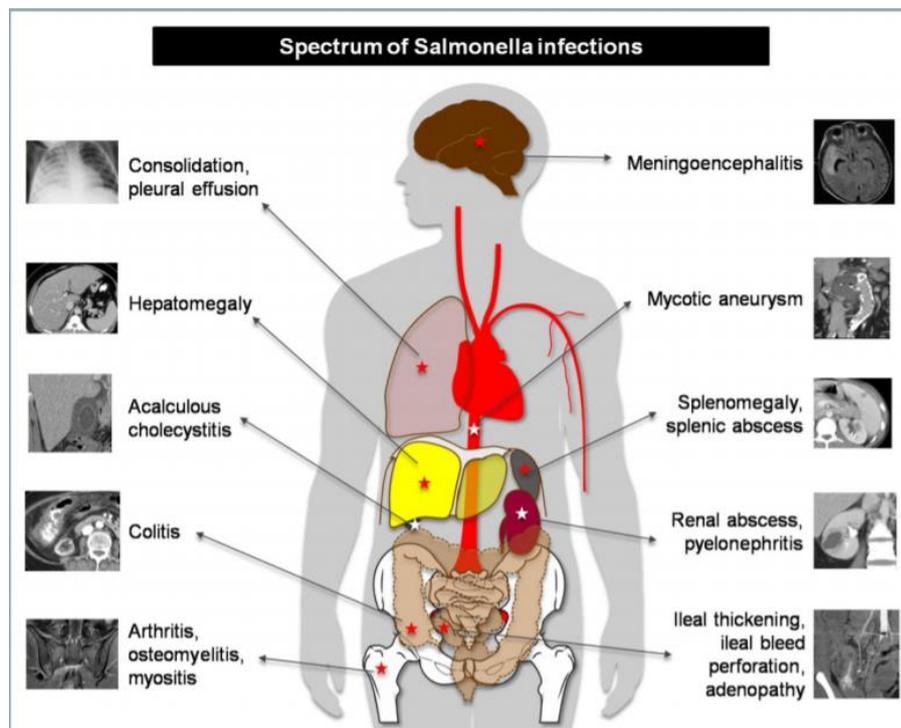
Depending on the SPECIES.

Typhoid: S. Typhi & S. Paratyphi & rarely S. Cholerasuis

Gastroenteritis: S. Gastroenteritidis & S. Typhimurium

Diagnosis of infection (of Typhoid fever):

- 1) **Physical Examination:** the previously mentioned symptoms; abdominal cramps, rashes, raised body temperature
- 2) **Labs & Tests:**
 - a. Blood culture (week 1 POSITIVE; afterwards NEGATIVE due to the formation of antibodies)
 - b. Stool culture (week 1 NEGATIVE; week 2 POSITIVE)
 - c. Blood serology (after 10 days the Antibodies' titre would be significant)
UNCOMMON: urine test for concomitant appearance of antigens in the urine
- 3) **Imaging:** Ultrasound {hepatosplenomegaly}



However, all these diagnostic methods are clinically insignificant as when a patient presents with fever, they are directly started on empirical treatment with a broad-spectrum antibiotic prior to lab results.

WIDAL TEST: a serologic test depending on antigen-antibody reaction causing agglutination.

- Sample is: Serum (containing the antibodies against O or H antigens)
- Application of Widal Test: Salmonella
- Principle & Result Interpretation: Two main types:

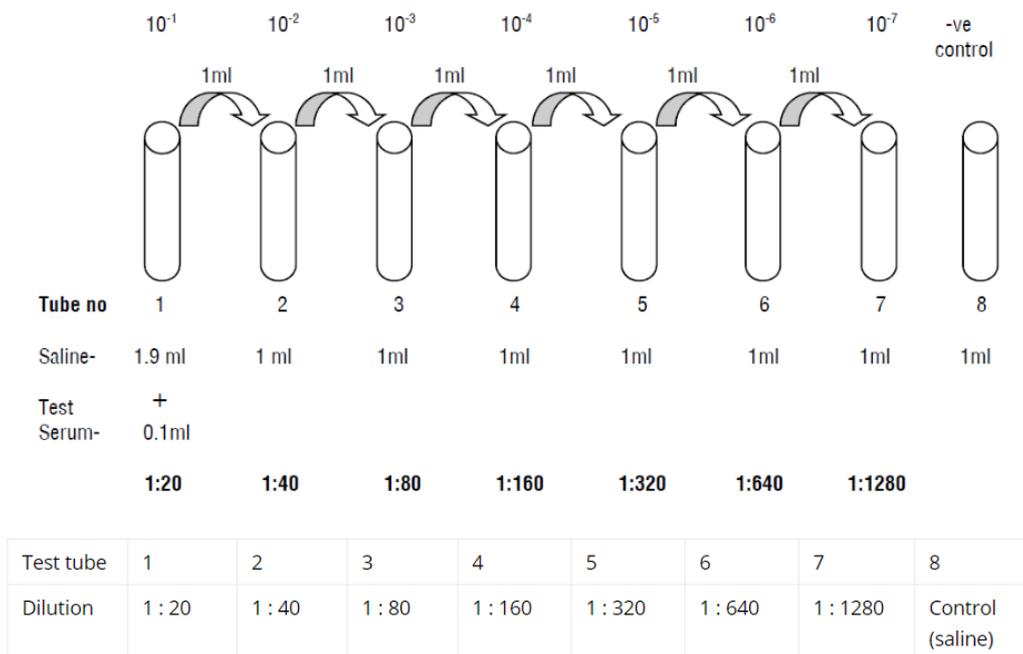
1) **qualitative:** test for antigens H & O of Typhi & Paratyphi
 Agglutination-> positive for presence of Ab in the blood.



Antigens used (6 total):
 S. Typhi O
 S. Typhi H
 S. Paratyphi A O
 S. Paratyphi A H
 S. Paratyphi B O
 S. Paratyphi B H
 + Negative & Positive Controls
 H is marked Red and O Blue

{if agglutination occurs this does NOT mean there's a significant infection and therefore we have to perform the quantitative test}

2) **quantitative**



- Requirements:

- 8 test tubes containing H Antigen
- 8 test tubes containing O Antigen

In either, the first tube's concentration is 1/20, the second 1/40, and so on...
 [2-fold dilution]

1st tube: 1.9 mL Normal Saline + 0.1 mL of serum containing the Abs.

We take 1 mL of the 1st tube (contains 0.05 mL of serum) and add it to the 2nd tube

*Recall: $D=X/(X+D_i)$

- If agglutination happens at a lower concentration, this is indicative of a more serious infection.
- Result Interpretation: Infection is of **clinical significance** if there's agglutination at **1/80 or higher for Ab O** or **1/160 or higher for Ab H**
***only one titre would be raised at a time; either O or H.**

QUESTION: why did we start this test with 1/20 concentration in the 1st tube and NOT 1/2?

Because very minimal concentrations of the Antibody are needed to indicate a significant infection; therefore if we start at 1/2 we'll need too many tubes and dilution processes which is unnecessary.

QUESTION: Why do we observe the serum being positive to only ONE antibody (Anti O or Anti H) but not the other in the course of one infection?

Our immune system recognises one epitope (surface antigen) in each infection and therefore cannot produce 2 different antibodies for 2 different antigens at the same time.

QUESTION: In most infections we usually see the serum positive for H not O, why is that?

Ag H is more easily recognised due to its relative location (on the surface outside) whereas Ag O is present inside the capsule/less superficially.

QUESTION: Why is the titre considerably higher for the significant infection with Ab-H (1/160) compared to Ab-O (1/80)?

Most normal flora exhibit Antigen H and with transient bacteraemia we have Antibodies to this antigen normally. (I.E. to exclude cross-reactivity with normal flora) therefore lower dilutions are not significant.

Person-to-person transmission is VERY COMMON; e.g. through contaminated drinks and handling of food.

Most commonly used antibiotic for Enteric Fever: **Fluoroquinolones**.

S. Paratyphi symptoms (in enteric fever) are of less severity and shorter duration compared to S. Typhi.

After ingestion of the bacteria, it reaches the intestines but does not cause disease, then spreads to the blood stream (needs 1-2 weeks) before reaching the body organs and causing symptoms.

Serology tests cannot be performed before 10 days have passed.

Blood/stool samples Cultured on: **S-S Agar**, **XDL Agar**, **DCA Agar**, **MacConkey Agar** (not selective), **Bismuth Sulfate Broth** (inhibits other normal G- bacteria in the stool), **CLED Agar** (may be used for all types of bacteria; non-diagnostic).

Biochemical Tests (IMViC)

I = Indole test

M = Methyl Red test

Vi = Voges-Proskauer test

C = Citrate utilisation test

Indole test: (Media: Tryptone Water aka Tryptone Broth)

contains tryptophan. If bacteria has tryptophanase enzyme, a volatile gas (Indole) would appear at the surface of the test tube:



Indole is detected by using **Kovac's Reagent**-> appearance of a **Red Ring** at the surface of the tube.

SALMONELLA IS INDOLE POSITIVE.



Methyl Red Test:

uses Methyl Red reagent. Will be discussed in GIT.

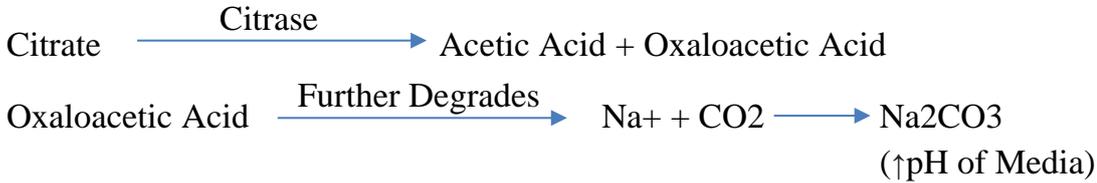
Voges-Proskauer test:

Requires KOH reagent. Will be discussed in GIT.

Citrate Test:

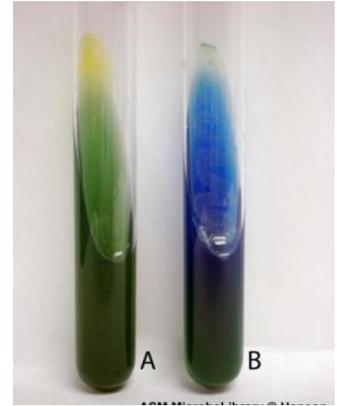
citrate test indicates the bacteria's use of Citrate as the only source of Carbon. Not a broth, only a slant surface. 24-hour incubation.

Citrate Media: green in colour.



Increase in pH of the media is visualised by the blue colour of the indicator **Bromothymol Blue**. <change of colour from green to blue>

THIS TEST IS POSITIVE IN SALMONELLA



A= negative

B= Positive

Another Important test to differentiate between Salmonella & Shigella Gastroenteritis: **S-S Agar**.

Salmonella is H₂S POSITIVE.

Salmonella colonies on **Bismuth Agar** appear black in colour due to the production of H₂S.

Krigler Iron Agar (KIA Agar):

Contains: Glucose, Lactose, and some types contain Sucrose.

Tube composed of two components:
Slant Surface/Slope & Butt.

Bacteria is inoculated and streaked on the surface (aerobic condition), and stabbed with a special type of wire loop to the butt (anaerobic condition). Left for incubation.

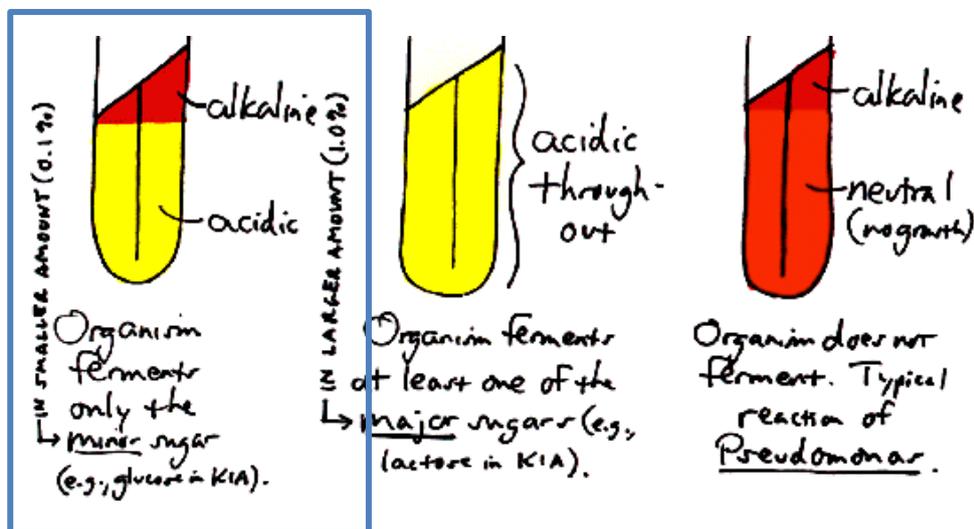
SALMONELLA IS A LACTOSE NON-FERMENTER. (negative)
BUT A GLUCOSE UTILISER. (positive)

Reaction to ONE SUGAR OF THE TWO gives an acid that lowers the pH, visualised in a **yellow** colour due to the indicator phenol red, concentrated in the **butt**, while the surface remains alkaline (before the reaction it's orange; alkaline = orange).

{if it were to utilise both sugars, a bigger amount of acid would have been released and the yellow would have spanned the entirety of the slant & butt}

RESULT: RED/YELLOW or ALKALINE/ACID

*result is written as slant/butt



However, the bacteria releases H₂S, and it accumulates and spreads throughout the whole tube and results in a BLACK colour.

(could be used to distinguish it from Shigella also bc with Shigella it wouldn't turn black)

{these tests are used for Enterobacteriaceae ONLY}