# Genus Salmonella

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# The Genus *Salmonella*

- **Family: Enterobacteriaceae.**
- **This genus includes more than 2000 serotypes.**
- **All Salmonella are similar in:**
  - **Morphology.**
  - **Cultural characters.**
  - **Sugar fermentation.**
- **They can be differentiated by: antigenic structures.**

# The Genus *Salmonella*

## Medically important Salmonella Species are:

a) Salmonella causing enteric fever:

1. *Salmonella typhi* →→ typhoid fever.
2. *Salmonella paratyphi* A, B,C →→ paratyphoid fever.

**Both diseases are called collectively enteric fever.**

b) ***Salmonella typhimurium*** **and** ***S. enteritidis*****:**
**they cause salmonella food poisoning or enterocolitis.**

c) ***Salmonella cholerae suis*****:**
**it causes salmonella bacteraemia with focal lesions in lungs, bones and meninges.**

# The Genus *Salmonella*

## Natural Habitat:

- All salmonella are obligate parasites.
- Salmonella typhi and paratyphi are restricted to man.
- The other salmonella are parasites of animals (poultry, pigs, rodents, cattle).

## Morphology:

- Gram-negative bacilli.
- Motile.
- Non-capsulate except Salmonella typhi.

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## Cultural Characters

- All Salmonella species can grow on nutrient agar, MacConkey's agar.

- Selective media for growth of Salmonella include:
  - Salmonella-Shigella (SS) medium.
  - Deoxycholate citrate agar (DCA) medium.
  - Both media are used for isolation of Salmonella and Shigella from contaminated samples as stool and urine.
  - On these media → colonies are pale yellow or colorless.
  - Colonies appear after 48 – 72 hours.

- Enrichment medium (Selenite broth).
  - This medium helps multiplication of Salmonella and Shigella; and inhibits multiplication of coliform bacilli.

# Biochemical reactions of medically important Salmonella Species

| | S. typhi | S. Paratyphi A | S. Paratyphi B, C |
|---|---|---|---|
| Glucose | ⊥ | + | + |
| Lactose | - | - | - |
| Maltose | ⊥ | + | + |
| Mannite | ⊥ | + | + |
| sucrose | - | - | - |

# Biochemical reactions of medically important Salmonella Species

| | S. typhi | S. Paratyphi A | S. Paratyphi B, C |
|---|---|---|---|
| Indole | - | - | - |
| M.R. | + | + | + |
| V.P. | - | - | - |
| Citrate | - | - | + |
| Urease | - | - | - |
| $H_2S$ | + | - | + |
| Urease | - | - | - |

# The Genus *Salmonella*

## Serological Classification

### (Kauffmann – white classification)

**1. Salmonella are divided into 30 serogroups by means of somatic (O) antigens:**

- Serogroups first identified were designated by capital letters A to Z.
- Serogroups discovered later are given the number (51 – 67) of the characteristic O antigen.
- Each group Has one specific O antigen, and several nonspecific (O) factors.
- Single organism carries several O antigens

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**2. Each serogroup is subdivided into serotypes by means of flagellar (H) antigens:**

- **Some strains in the same serotype carry an H-antigen specific for the serotype (serotype-specific) ➔ phase I antigen.**
- **Phase I designated with small letters (a, b, c, d, …. Z), recently discovered $z_1$, $z_2$, $z_3$, ……. etc.**
- **Some strains carry H antigen which may possessed by other serotypes in the same Salmonella group (Phase II).**
- **Phase II antigens is group specific and designated by Arabic numbers.**

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- **3. Salmonella typhi and Salmonella paratyphi C have Vi antigen (capsular Ag) ⇨ their antigenic formula includes Vi antigen in addition to O and H antigens.**

  **e.g. antigenic formula of S typhi is 9, 12 (Vi) :d:-**

## Antigenic Formulae of Medically Important Salmonellae

| O Group | Serotype | Somatic & Vi antigens | Flagellar Antigen | |
|---|---|---|---|---|
| | | | Phase I | Phase II |
| A | S. Paratyphi A | 1, 2, 12 | a | - |
| B | S. Paratyphi B | 1, 4, 5, 12 | b | 1, 2 |
| | S. typhimurium | 1, 4, 5, 12 | i | 1, 2 |
| C | S. Paratyphi C | 6, 7 Vi | c | 1, 5 |
| | S. Cholerae suis | 6, 7 | c | 1, 5 |
| D | S. Typhi | 9, 12 Vi | d | - |
| | S. enteritidis | 1, 9, 12 | g, m | - |

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## Virulence of Salmonella is due to:

1. Intracellular growth: its ability to survive and multiply within macrophages.

2. Capsule formation: Vi of Salmonella typhi which has antiphagocytic action.

3. Phase variation of H antigens:
   - It helps the organism to evade the immune system.
   - This varies from phase I (species-specific) ⇄ phase II (group-specific). It is spontaneous, revesible variation.

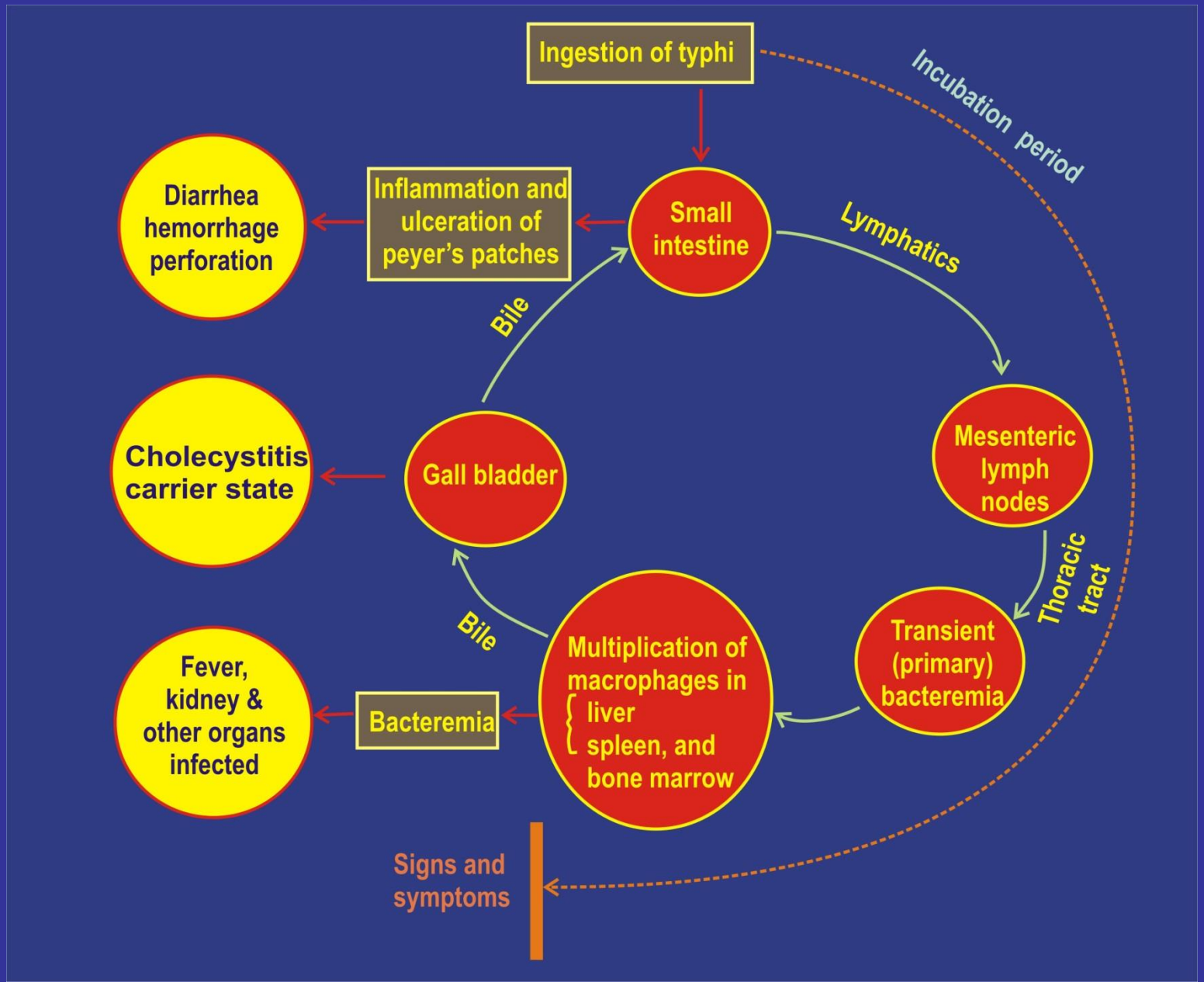
# The Genus *Salmonella*

## Pathogenesis of Typhoid Fever

- **Source of infection:**
  - Case
  - Carrier (fecal &urinary)

- **Mode of transmission:**
  - Ingestion of contaminated food or water by excreta of case or carrier.
- **Incubation period: 14 days.**
- **The organism enters the small intestine, adhere to epithelial cells which engulf them.**
- **Organism multiplies in epithelial cells, pass to submucosa where they are phagocytosed by neutrophils and macrophages.**
- **They are carried in the lymphatic to mesenteric lymph nodes where they multiply within macrophages.**

# Pathogenesis of typhoid fever



# The Genus *Salmonella*

- **Primary bacteraemia (Transient):**
  - Organisms released from Macrophages, drained via the thoracic duct into the blood stream.
  - They are disseminated but do not multiply significantly.

- **Secondary bacteraemia (Septicemia):**
  - Organisms from the blood removed by macrophages of reticuloendothelial system of liver, spleen and bone marrow.
  - They multiply intracellularly until the macrophages are killed.
  - Organisms are released in large numbers into the blood, this marks the onset of serious clinical illness (fever, epistaxis, headache, abdominal tenderness and constipation).
  - The organism can be isolated from the blood at this stage.

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- From the liver, the organism is carried to the gall bladder .
- They multiply freely in the bile, and the gall bladder sometimes becomes inflamed (cholecystitis).
- The flow of bile into the small intestine leads to re-infection of the intestinal tract.
- The organisms now localized in distal ileum and causing inflammation, necrosis and ulceration (typhoid ulcer).
- Thus during the second week of illness There is sustained fever and diarrhea with typical "pea-soup stool" and the organisms are present in the stool.

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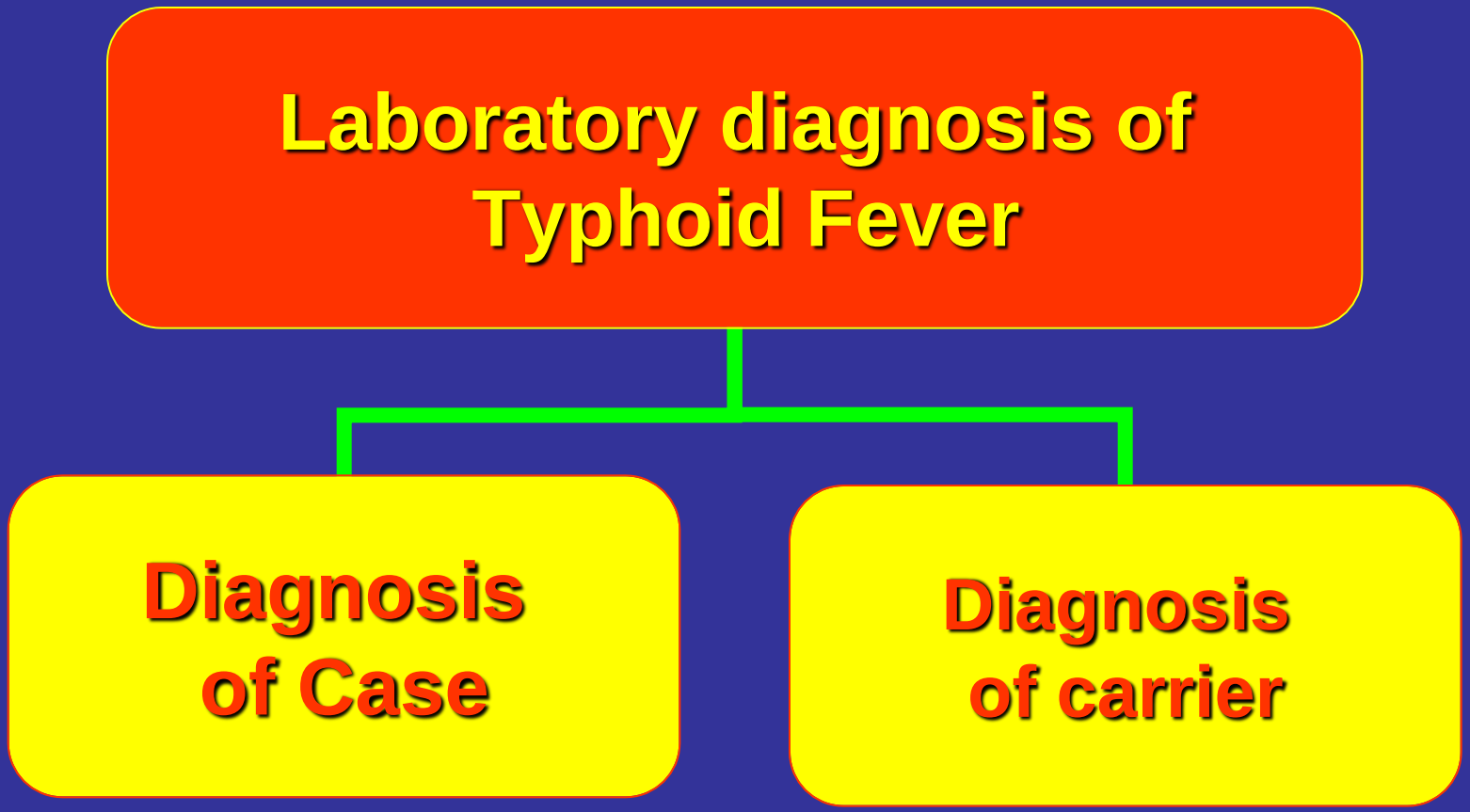
## Fate of the Disease

1. **Recovery:**
   - In the uncomplicated cases, the ulcers heal, the fever falls gradually, and the patient recovers.
   - This occurs during the fourth and fifth week.
2. **Complications:**
   - Hemorrhage from the ulcers.
   - Perforation of the small intestine with generalized peritonitis, the commonest cause of death in typhoid fever.
3. **Typhoid carriers:**
   - A small proportion of patients (2-5%) recover from the clinical disease.
   - They continue to harbor the organisms as a chronic infection in the gall bladder, and less often in the urinary tract.

# The Genus *Salmonella*



# The Genus *Salmonella*

## Laboratory Diagnosis of Typhoid Fever case

### A During the First week of illness (septicemia)

1. **Blood Culture:**
   - 5-10 ml blood from the patient inoculated on 50-100 ml broth bottle ➔➔ incubated at 37°C.
   - Subculture on plate of MacConkey's agar.
   - Any pale yellow colonies are identified by:
     - Gram-stained film.
     - Biochemical reactions.
     - Slide agglutination test using specific O and H antisera.

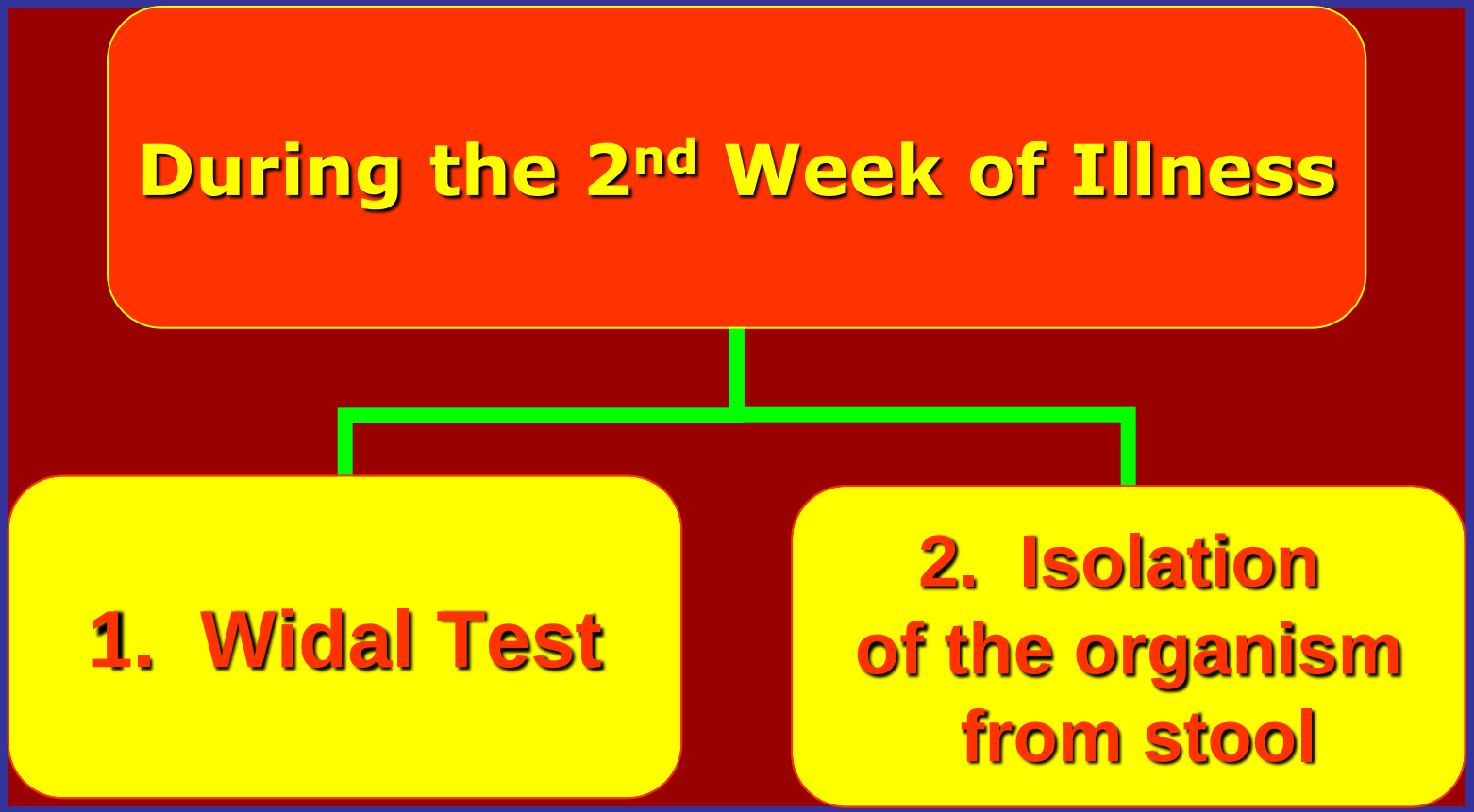
# The Genus *Salmonella*

## Laboratory Diagnosis of Typhoid Fever case

### A During the First week of illness (septicemia):

2. **Clot Culture:**
   - 5-10ml blood is collected in sterile tube.
   - Allowed to clot.
   - Serum is separated by centrifugation for performing (Widal test).
   - The blood clot is added to 15 ml nutrient broth containing streptokinase enzyme (to lyse the clot and release the Salmonella organisms).
   - Incubate for 24-48 hours.
   - Subculture on MacConkey's agar plates.
   - Pale yellow colonies are identified as before.

# The Genus *Salmonella*



# The Genus *Salmonella*

## Laboratory Diagnosis of Typhoid Fever

### B During the second week of Illness

#### 1. Widal Test

- It is agglutination reaction.
- Used to detect serum antibodies against Salmonella (agglutinins).
- These antibodies rise during the second and third weeks of illness.
- Two serum samples will be collected with 10-15 days interval between them to detect rising titer in antibodies.
- In this test ➔➔ serial dilutions of patient's serum (1/10, 1/20, 1/40, 1/80, 1/160, ….)are tested against:
  - Salmonella O-antigen (common salm. Antigens).
  - Salmonella typhi (specific H-antigen).
  - Salmonella paratyphi A (specific H-antigen).
  - Salmonella paratyphi B (specific H-antigen)
- The end titer: is the highest dilution of serum giving agglutination.

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## Interpretation of the Widal Test Results

a) If the test is done in the first week ➔➔ give negative result because antibodies starts to appear during second week.

b) A titer below 1/80 ➔➔ non-significant and it indicates previous subclinical infection.

c) Agglutination with O-antigen indicates recent infection.

d) Recent vaccination with TAB vaccine gives low agglutination titer with more than one type of Salmonella antigens.

e) Early administration of chloramphenicol inhibits antibody formation and gives rise to false negative Widal test.

f) False positive results may occur in other conditions than enteric fever e.g. liver disease.

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## Laboratory Diagnosis of Typhoid Fever

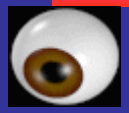
### B During the second week of Illness

2. Isolation of the Organism from Stool:
   - Stool specimen is inoculated on MacConkey's agar, SS or DCA.
   - After incubation, non-lactose fermenting colonies identified as previously done in blood culture.

### C During the third week of illness

1. Widal test.
2. Isolation of the organism from stool and urine:
   as described in the diagnosis during second week of illness.

# The Genus *Salmonella*



## Laboratory diagnosis of Salmonella Carriers

- Isolation of the organism from the stool and urine.
- Stool or urine deposit is inoculated onto tubes of selenite broth.
- Incubated at 37°C for 18 hours.
- Subculture on DCA plates for 48 hours.
- Non-lactose fermenting colonies identified as previously done.
- Repeated stool examination is necessary to exclude Salmonella carriers.

# The Genus *Salmonella*

## Prophylaxis against enteric fever

1. **TAB vaccine:**
   - Heat killed vaccine.
   - Contains 1000 million S. typhi organisms.
     750 million S. paratyphi A organisms
     750 million S. paratyphi B organisms
     Per ml
   - It is given in two doses separated by one month interval, 0.5 ml and 1 ml subcutaneously.
2. **Oral typhoid vaccine:**
   - It contains avirulent mutant (non-capsulate) of Salmonella typhi.
   - It has been given significant protection in some endemic areas.

# The Genus *Salmonella*

## Treatment

### 1. Case:

- Chloramphenicol is the drug of choice.
- Trimethoprim-sulfamethoxazole for sensitive strains to this drug.
- Resistant cases are treated by quinolones e.g. norfloxacin.

### 2. Carrier:

- Ampicillin, concentrates in gall bladder and kidneys.
- Cholecystectomy is indicated for chronic carriers with gall bladder diseases.

# The Genus *Salmonella*

## Salmonella food poisoning

### "Salmonella enterocolitis"

- **Causative organism:**
  - Salmonella typhimurium , salmonella enteritidis.
- **Mode of transmission:**
  - Ingestion of meat of naturally infected cattle or birds or their eggs.
  - Food may be contaminated from excreta of rats or mice.

# The Genus *Salmonella*

- **Clinical Picture:**
  - Incubation period : 12-36 hours.
  - Symptoms : nausea , vomiting , abdominal pain, diarrhea and mild fever.
- **Pathogenesis:**
  - Multiplication of the organisms in the intestine and colon.
  - No enterotoxin secretion or blood invasion.
  - Recovery from the disease occurs within one week.

# The Genus *Salmonella*

- **Laboratory Diagnosis:**
  - **Specimen: stool, vomitus, or remnant of food.**
  - **Inoculate the specimen on tetrathionate broth tubes.**
  - **Incubate for 12 – 18 hours.**
  - **Subculture on plates of DCA medium.**
  - **The plates are incubated for 1 – 2 days.**
  - **Suspected colonies (non-lactose fermenters) identified by:**

    **morphology, B.R., agglutination with specific O and H Salmonella antisera.**
- **Treatment**: usually supportive treatment.

# The Genus *Salmonella*

## Salmonella Bacteraemia with Focal Lesions

- **Causative organism**: Salmonella cholera suis.
- **Mode of transmission**: oral infection.
- **Pathogenesis**:
  - **Early invasion of the blood stream with focal lesions in lungs (pneumonia), meninges (meningitis), and bones (osteomyelitis in sickle cell anemia patients).**
  - **There is no intestinal manifestations.**
- **Diagnosis**: blood culture (as in typhoid fever).
- **Treatment**: as typhoid fever.

# Genus Shigella

# The Genus *Shigella*

- **Family**: Enterobacteriaceae
- **Genus**: Shigella.
- **species** (subgroups):
  1. Subgroup A (Shigella dysenteriae): 12 serotypes.
  2. Subgroup B (Shigella flexneri): 8 serotypes.
  3. Subgroup C (Shigella boydii): 18 serotypes.
  4. Subgroup D ( Shigella sonnei): one serotype that can be divided into 17 colicine types.

  - ***All Shigella species are similar in morphology, culture characters.***
  - ***They differ in biochemical reactions and antigenic structure.***
  - ***All Shigella species can cause bacillary dysentery.***

# The Genus *Shigella*

- **Morphology:**
  - Gram-negative bacilli.
  - Non-motile, non-capsulate, and non spore-forming.

- **Cultural Characters:**
  - Aerobes, facultative anaerobes.
  - Can grow on ordinary media.
  - Selective media for Shigella e.g. MacConkey's agar, DCA, SS.
  - The colonies are non-lactose fermenters except Shigella sonnei (late lactose fermenters 3-8 days).

# The Genus *Shigella*

- **Biochemical Reactions**

- All shigellae ferment glucose with acid production only (⊥).

- Mannite Fermentation is used to classify Shigella into:
  1. Non-mannite fermenters (Shigella dysenteriae).
  2. Mannite fermenters which is subdivided into:
     a) Non-lactose fermenters (Shigella flexneri and Shigella boydii).
     b) Late lactose fermenters (3-8 days incubation: Shigella sonnei

- All Shigellae are → M.R. positive.
  → V.P., citrate, urease, gelatin and indole negative.

# The Genus *Shigella*

- **Antigenic Structure**
  - **There are no H-antigens (the organisms are non flagellate; non motile)**
  - **They carry O antigens (lipopolysaccharide somatic antigens) used for differentiation of Shigella into serogroups and serotypes.**

# The Genus *Shigella*

- **Virulence of Shigella**

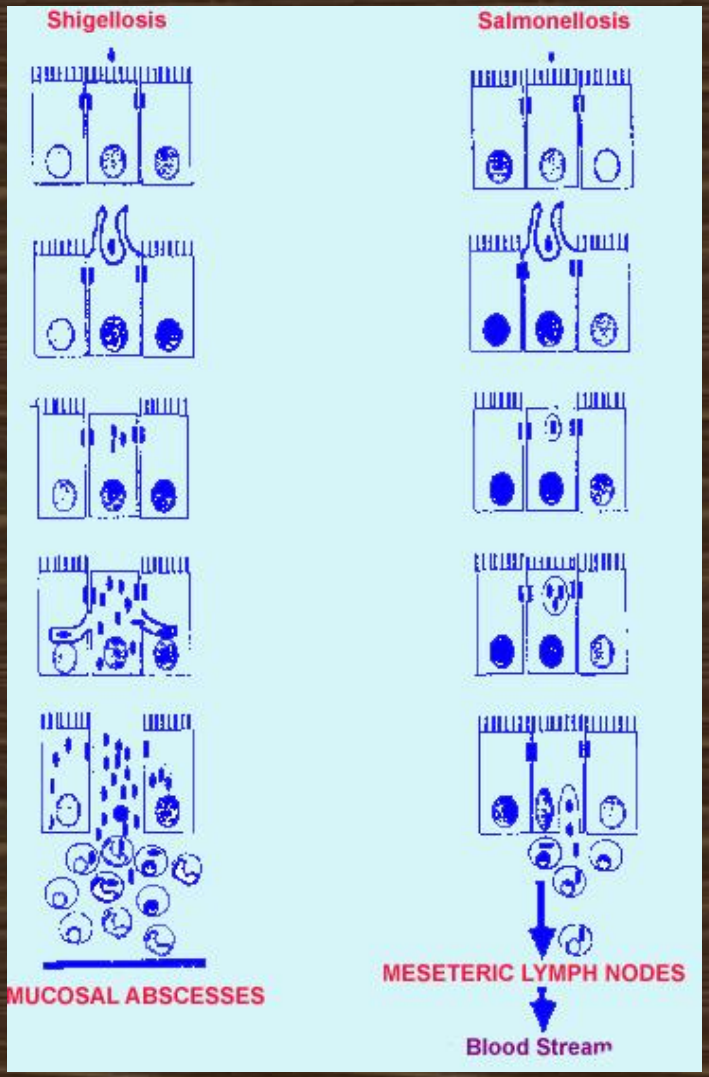
**Virulence factors include:**

1. **Invasion plasmid antigen**
   - **They mediate attachment and penetration of mucosal epithelial cells.**
   - **These antigens are products of genes located on a large plasmid common to Shigella**

2. **Intracellular spread proteins:**
   - **Mediate escape of the organism from phagocytic vesicles, where they multiply in the cytoplasm.**
   - **Mediate attachment to cytoskeleton proteins that facilitate transfer of bacteria to adjacent cells through membrane proteins.**

# The Genus *Shigella*

**Comparison of the invasion strategies of Shigella & Salmonella in the intestinal epithelium**



# The Genus *Shigella*

**3. Shiga toxin:**

- **Heat – labile cytotoxin.**
- **Produced by Shigella dysenteriae type 1.**
- **It inhibits protein synthesis by inactivating 60S ribosomal subunit of mammalian cell ribosomes.**
- **It is responsible for the haemolytic uremic syndrome (associated with bacillary dysentery caused by Shigella dysenteriae type 1).**

# The Genus *Shigella*

## Pathogenesis of Bacillary dysentery (Shigellosis)

- Causative organisms: Shigella bacilli
- Mode of transmission: (ingestion)
  - **Feco-oral route.** 
  - **Direct contact from person to person.**
  - **Contaminated food and water.** 
- There is no animal reservoir.
- Infection is limited to the colonic mucosa and submucosa with invasion and ulceration resulting in bloody diarrhea with mucus and pus.
- Systemic invasion is rare.

# The Genus *Shigella*

## Pathogenesis of Bacillary dysentery

Ingestion of Shigella

↓

Transport to submucosa across mucosal cells

↓

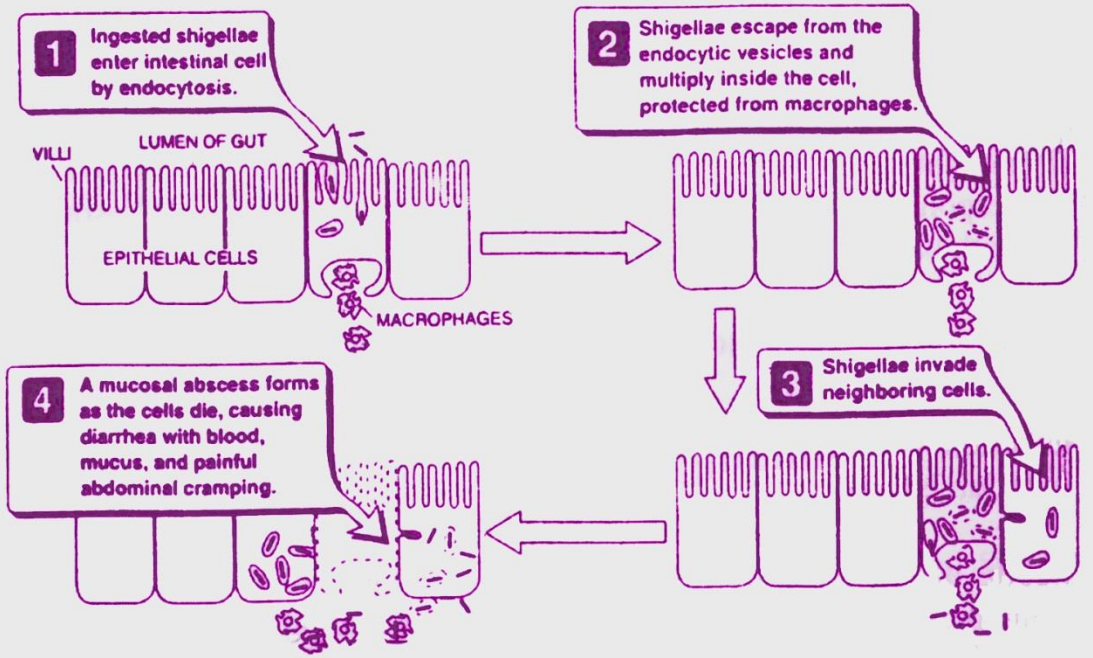
Invasion of Mucosal cells

↓

Ulceration

↓

Bloody diarrhea with mucus and pus



# The Genus *Shigella*

- **Clinical picture:**
  - incubation period: 1-9 days.
  - Symptoms: severe abdominal cramps, frequent painful passage of low volume stools containing blood and mucus.

# The Genus *Shigella*

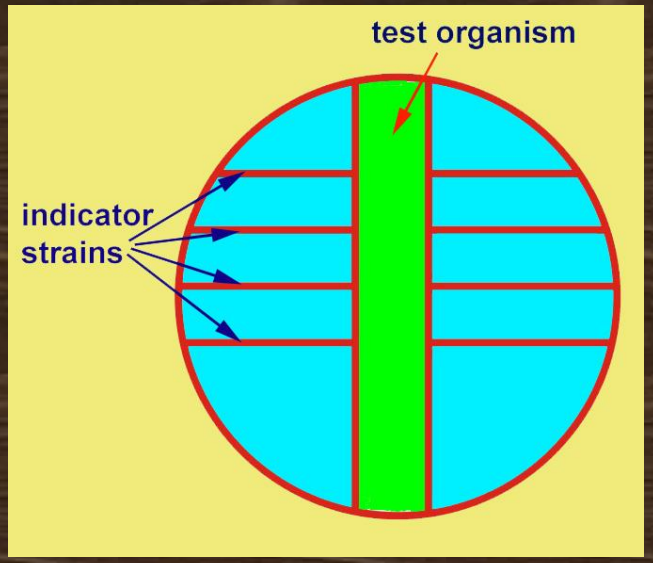
## Laboratory Diagnosis of Bacillary Dysentery (Shigellosis)

1. **Fresh examination of stool:** to exclude protozoa and parasites.

2. **Culture of stool on:**
   - MacConkey's agar or SS plates to isolate the organisms.
   - Non-lactose fermenting colonies identified by: Gram-stained films, B.R., and slide agglutination using specific O antisera.
   - In convalescent cases: enrichment on selenite broth tubes
   - Subculture on SS or DCA plates to isolate the organisms.

# The Genus *Shigella*

- **Colicin typing:**




  - Used for typing of Shigella sonnei isolatedfrom cases of diarrhea to trace source of infection.
  - The strain to be typed is cultured on agar plate as a single streak.
  - Incubate, then remove the growth by the edge of a glass slide and kill the organism by chloroform vapour.
  - Several indicator strains are inoculated on the plate in parallel streaks at right angle to the original growth.
  - After incubation, notice the pattern of growth inhibition of indicator strains which determine the type of the test strain.

# The Genus *Shigella*

## Treatment

1. Supportive therapy:
   - **Fluids and electrolyte replacement especially when the case is associated with watery diarrhea.**

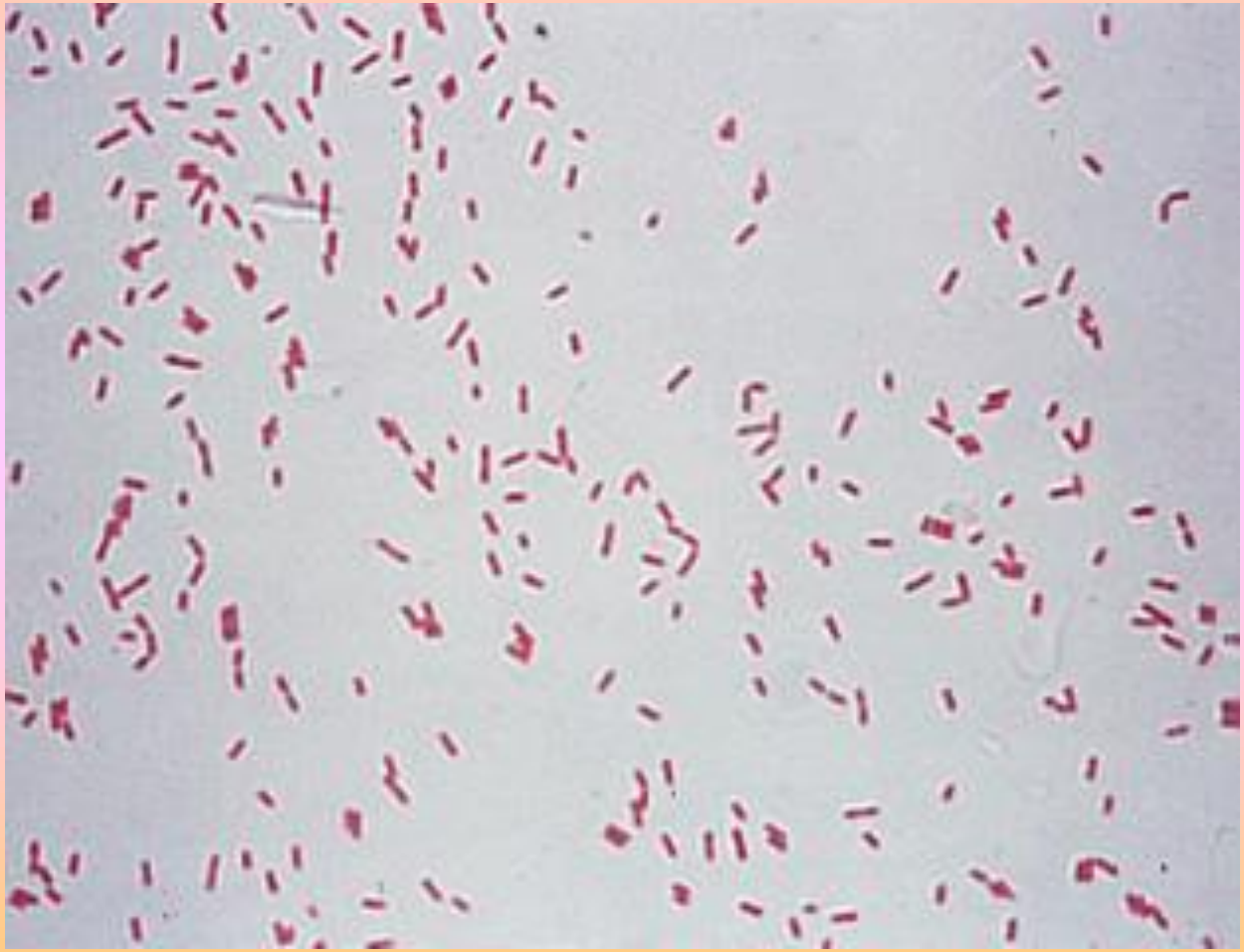
2. Antibiotics:
   - **Ampicillin, amoxycillin, or trimethoprim-sulfamethoxazole.**

# Genus pseudomonas

# Pseudomonas

- Gram negative bacilli.
- Present in the stool and skin of 5-10% of healthy population.
- Found in the hospital environment:
  - Growing in aqueous antiseptics e.g. dettol and cavlon.
  - Cleaning liquids.
  - Ophthalmic eye drops.
  - Water in flower vases.
  - Moist environment surfaces and ventilation equipments.
- Pseudomonas aeruginosa is the main species causing infections in man.

# Pseudomonas



# Pseudomonas

## Pseudomonas aeruginosa

- **Characters of the organism that differentiate it from members of Enterobacteriaceae family:**

1. It is strict aerobe.
2. Utilize glucose only oxidatively.
3. Oxidase positive.
4. Motile with single polar flagellum.
5. Naturally resistant to many antibiotics.
6. Ability to grow and survive on media containing antiseptic agents e.g. dettol.
7. Exopigment production:
   a) Pyocyanin which is blue pigment that diffuses into the agar medium.
   b) Pyoverdin which gives greenish colour to the agar medium.
   c) Pyorubrin which is dark red pigment.
   d) Pyomelanin which is black pigment.

# Pseudomonas

## Pathogenesis in man

- **Pseudomonas aeruginosa infections involve:**

1. **Respiratory tract, urinary tract and skin infection in immunocompromised patients.**

2. **Otitis media, postoperative wound infection, burn infection, and meningitis.**

# Pseudomonas

## Laboratory diagnosis

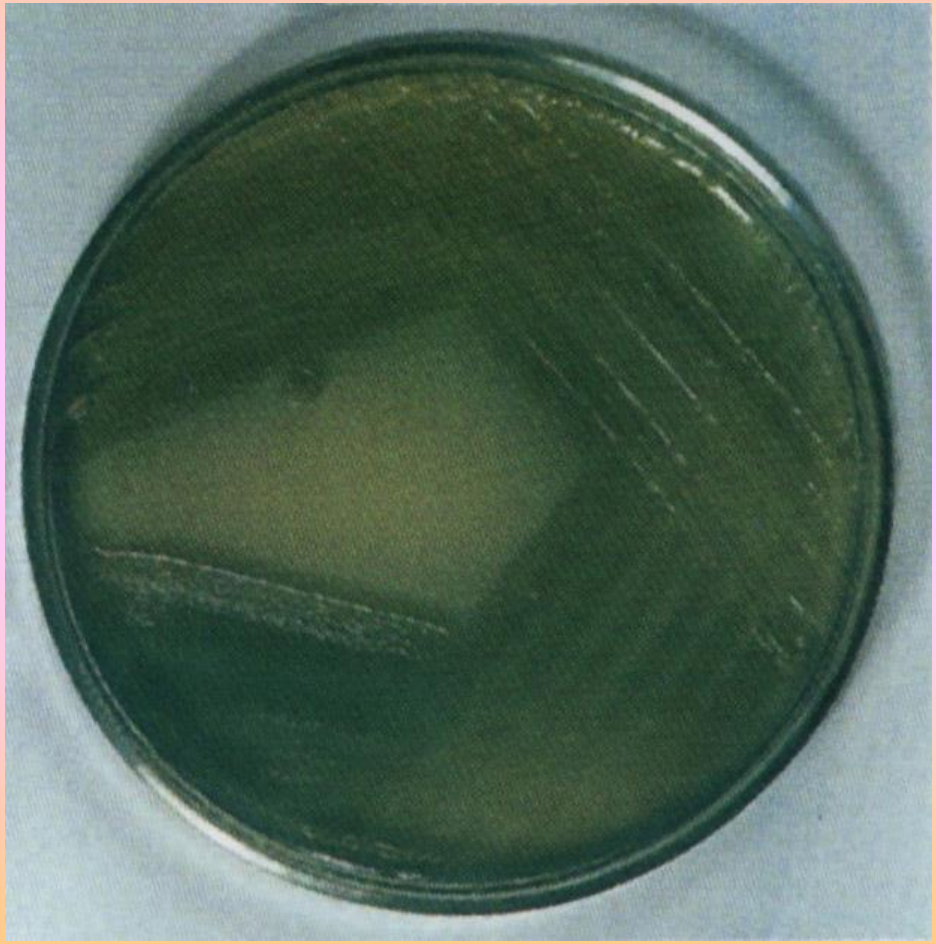
1. **Specimens: exudate, urine sputum, … etc.**

2. **Microscopic examination: gram negative bacilli.**

3. **Isolation:**
   - **Culture the specimen on N. agar and blood agar media → exopigment producing colonies.**
   - **On MacConkey's agar medium → non-lactose fermenting colonies.**

# Pseudomonas

**Pseudomonas aeruginosa culture on N. agar.**



# Pseudomonas

## 4. Biochemical Reactions:

- Oxidase positive.
- Utilize glucose oxidatively with acid production.
- Indole negative.
- Methyl red (M.R.) negative.
- Voges proskauer (v.p.) NEGATIVE.
- Citrate positive.
- Urease positive.
- $H_2S$ negative.
- Liquefy gelatin.

# Pseudomonas

## 5. Pyocin typing:

- Used for tracing source of Pseudomonas aeruginosa nosocomial infection.
- The principle and method is the same as in Colicin typing.

## Treatment of pseudomonas aeruginosa infections:

- A combination therapy is usually recommended e.g. carbenicillin and one of the aminoglycosides as amikin.
- Ciprofloxacin or norfloxacin: have enhanced activity against Pseudomonas.
- Polymyxin B: for topical applications e.g. infected burns.