

## MODULE

Microbiology



Notes

23

# SALMONELLA

## 23.1 INTRODUCTION

Salmonella consists of bacilli leading to Enteric fever, Gastroenteritis, Sepsicemia etc. The important member of the genus is *Salmonella typhi*, which causes Typhoid fever.



## OBJECTIVES

After reading this lesson, you will be able to:

- describe the morphology of *Salmonella*
- discuss the cultural characteristics of *Salmonella*
- explain the biochemical reactions of *Salmonella*
- demonstrate the Widal reaction.

*Salmonella* are of two groups;

- (i) Enteric fever group consisting of typhoid & Paratyphoid bacilli exclusively or primary human parasites
- (ii) Food poisoning group, which are animal parasite but may infect humans causing Gastrointestinal infections

### Morphology

*Salmonellae* are gram negative rods. They are motile with peritrichate flagella except for *S. gallinarum pullorum*

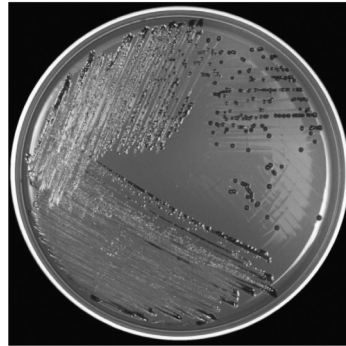


Fig. 23.1

### Cultural Characteristics

Salmonellae are aerobic and facultatively anaerobic bacteria growing readily on simple media over a range of pH 6-8 & temperature 15-41°C with optimum temperature of 37°C. Colonies are large, circular and smooth on MacConkey and Deoxycholate citrate media, colonies are colourless due to absence of lactose fermentation. Selenite F and Tetrathionate broth are commonly employed as enrichment media.

### Biochemical reaction

Salmonellae ferment glucose, mannitol and maltose forming acid and gas. Whereas *S. typhi* is an aerogenic i.e. it does not form fermentation of sugars like glucose etc. Lactose, Sucrose and Salicin are not fermented. Indole is not produced. They are MR positive, VP negative and citrate positive.

### Resistance

The bacilli are killed at 55°C in one hour or at 60°C in 15 minutes. Boiling or chlorination of water and pasteurization of milk destroy the bacilli. In polluted water it may survive for weeks and in ice for months.

### Antigenic Structure

Salmonellae possess the antigens and based on which they are classified as

- (i) flagella antigen H,
- (ii) Somatic antigen O and
- (iii) surface antigen Vi

### H antigen

This antigen present on flagella is heat labile protein. It is destroyed by boiling or by treatment with alcohols but not by formaldehyde.



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### O antigen

O antigen is a Phospholipid-protein-polysaccharide complex which forms an integral part of the cell wall. It is identical with endotoxin. This is unaffected by boiling, alcohol or weak acids



### INTEXT QUESTIONS 23.1

1. Salmonellae are gram ..... rods
2. Culturally salmonellae are facultative .....
3. .... & ..... broth are commonly used as enrichment media for salmonellae
4. Flagella antigen is ..... & somatic antigen is .....
5. .... antigen is unaffected by boiling, alcohol & weak acids

### 23.2 CLASSIFICATION AND NOMENCLATURE

Classification within the genus is on antigenic characterisation based on Kauffman-White scheme and this depends on identification by agglutination of the O and H antigens of the strains. Salmonellae are classified into serological groups based on the presence of distinctive O antigen factors and designated as 1, 2, 3 etc

Biochemically Kauffman proposed Salmonellae classification as

Subgenus I: Largest and medically most important group causing human and animal infections

Subgenus II: Species isolated from reptiles.

Subgenus III: Species isolated from reptiles and human beings

Subgenus IV: These are rarely encountered.

### Pathogenicity

Salmonellae cause the following clinical syndrome in human beings

1. Enteric fever
2. Gastroenteritis or food poisoning
3. Septicemia with or without local suppurative lesions

### I. Enteric Fever

This includes typhoid fever caused by *S. typhi* and paratyphoid fever caused by *S. Paratyphi A, B, C*. The infection is acquired by ingestion of contaminated food, on reaching the gut the bacilli attach to microvilli of the ileal mucosa and penetrate submucosa. They are phagocytosed by polymorphs and macrophages. Their ability to resist intercellular killing and to multiply within the cells is a measure of their virulence. They enter the mesenteric lymph nodes, where they multiply and via thoracic duct, enter the blood stream causing bacteremia.



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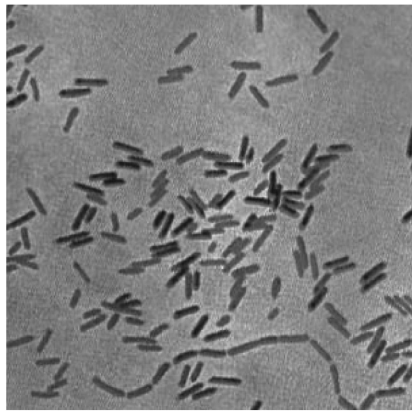


Fig. 23.2

As bile is a good culture medium for the bacillus it multiplies abundantly in the gall bladder and is discharged continuously into the intestine where it involves the Peyer's Patches and lymphoid follicles of the ileum, which ulcerate and may lead to intestinal perforation & haemorrhage as complication. The incubation period is usually 7-14 days but may range from 3-56 days.

### Laboratory Diagnosis

Bacteriological diagnosis of enteric fever consists of isolation of the bacilli and demonstration of antibodies in serum. A positive blood culture is diagnostic; demonstration of antibodies is not conclusive of current infection. A third method is the demonstration of typhoid bacilli in blood or urine.

### Blood culture

Bacteremia occurs early in the disease and blood cultures are positive mostly in the first week of fever. About 5-10ml of blood is collected and inoculated into culture bottle containing 50-100ml of 0.5 percent bile broth. After incubation overnight at 37°C, the bile broth is subcultured on MacConkey agar, pale non-lactose fermenting colonies that may appear on this medium are picked up for biochemical tests and motility. Salmonellae are motile, indole and urease negative and ferment glucose, mannitol and maltose but not lactose or sucrose.



The typhoid bacillus will be anaerogenic, while the paratyphoid bacilli will form and gas from sugars. Identification of the isolate is by slide agglutination. A loopful of the growth from an agar slope is emulsified in two drops of saline on a slide. One emulsion acts as a control to show that the strain is not auto agglutinable.

If Salmonellae are not obtained from first subculture from bile broth, subcultures should be repeated every other day till growth is obtained. Cultures should be declared negative only after incubation for ten days.

### Feces culture

Salmonellae are shed in feces throughout the course of disease and even in convalescence, with varying frequency. A positive fecal culture, however may occur in carriers as well as in patients. The use of enrichment and selective media and repeated sampling increase the rate of isolation.

Fecal samples are plated directly on MacConkey, DCA and Wilson-Blair media. On MacConkey and DCA it appears as pale colonies. On Wilson-Blair medium *S typhi* forms large black colonies. *S paratyphi A* produces green colonies on this medium.

### Urine culture

Salmonellae are shed in urine irregularly and infrequently. Hence urine culture is less useful than culture of blood or feces. Cultures are generally positive in second and third weeks.

### Widal reaction

This is a test for measurement of H and O agglutinins for typhoid and paratyphoid bacilli in the patient's sera. Two types are generally used for the test—a narrow tube with a conical bottom (Dreyer's agglutination tube) for H agglutination and short round-bottomed tube (Felix tube) for O agglutination.

Equal volumes (0.4 ml) of serial dilutions of the serum and the H and O antigens are mixed in Dreyer's and Felix agglutination tubes and incubated in a water bath at 37°C overnight. Control tubes containing the antigen and normal saline are set to check for autoagglutination. The agglutination titres of the serum are read. H agglutination leads to the formation of loose, cotton woolly clumps, while O agglutination is seen as a disc-like pattern at the bottom of the tube.

### Salmonellae gastroenteritis

Salmonellae gastroenteritis or food poisoning is zoonotic disease, the source of infection being animal products and may be caused by any Salmonellae except *S typhi*.

## Salmonella

Human infection results from ingestion of contaminated food and most common source of food poisoning are poultry, meat, milk products. Salmonellae can enter through the shell if eggs are left on contaminated chicken feed or feces and grow inside.

Laboratory diagnosis is made by isolating the Salmonellae from feces and from the article of food which confirms the diagnosis.

### Salmonellae Septicemia

*S. choleraesuis* in particular, may cause septicemic disease with focal suppurative lesions such as osteomyelitis, deep abscesses, endocarditis, pneumonia and meningitis.



### INTEXT QUESTIONS 23.2

1. Enteric fever is acquired by ingestion of .....
2. .... & ..... are complication of Enteric Fever
3. .... is a good culture medium for salmonellae
4. .... tube is used for H agglutination
5. .... tube is used for O agglutination



### WHAT YOU HAVE LEARNT

- Salmonella belong to the family Enterobacteriaceae
- Salmonella are gram-negative, facultatively anaerobic, motile rods that are catalase positive and oxidase negative.
- Salmonella species are responsible for enteric fever spread only from human to human. Water contaminated with feces is a common source.
- The bacilli invade mucos cells in small intestine, transported through lymphatics and reach blood stream.
- For diagnosis, samples of feces or blood are plated on solid medium, such as MacConkey agar and Salmonellae-Shiella agar which yields gram-negative bacilli with flagella.
- The organisms are identified by biochemical characteristics and by slide agglutination tests using reference antibody to O, H, vi. antigens.

## MODULE

Microbiology



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**Notes****TERMINAL QUESTIONS**

1. Describe the cultural characteristics of salmonellae
2. Explain the laboratory diagnosis of enteric fever by Blood culture
3. Describe Widal reaction
4. Write about the prevention of Salmonella infection

**ANSWERS TO INTEXT QUESTIONS****23.1**

1. Negative
2. Aerobes
3. Selenite f & tetrathionate
4. H & O
5. O

**23.2**

1. Contaminated food
2. Intestinal perforation & Haemorrhage
3. Bile broth
4. Felix