



## ISOLATION AND ANTIBIOTICS PATTERN OF SALMONELLA TYPHI FROM FRUIT JUICE

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Article Received on 22/12/2017

Article Revised on 12/01/2018

Article Accepted on 01/02/2018

### ABSTRACT

Salmonellosis is a disease caused by a large group of bacteria of the genus *salmonella* that can affect human being throughout the world. It is among the most commonly isolated food borne pathogens associated with fresh fruits and vegetables etc. Outbreaks of Salmonellosis have been linked to a wide variety of fresh fruits and vegetables including apple, mango, tomato, melon and parsley. In the present study the isolation of Salmonella was carried by direct plate method from fruit juice, tap water and blood sample on SS Agar. The Salmonella shows small, circular, jet black colonies in fruit juice sample and further screened on differential media such as Bismuth sulphite agar shows silver black colonies and Brilliant green agar shows pinkish white colonies. The isolated strains was G<sup>-ve</sup>, rod shaped bacteria and shows positive MR, H<sub>2</sub>S, Catalase, Oxidase, Nitrate reduction test. The isolated strains shows maximum zone of inhibition against ciprofloxacin i.e.35mm, Chloremphenicol i.e.20mm and minimum against Ampicillin i.e. 5mm. The choice of the antibiotics for the treatment of salmonellosis are broad spectrum drugs.

**KEYWORDS:** Salmonella, Antibiotics, infection.

### INTRODUCTION

*Salmonella* are widely distributed in nature and they survive well in a variety of foods, Poultry, eggs and dairy products are the most common vehicles of salmonellosis. In recent years, fresh food like fruits and vegetables have gained concern as vehicles of transmission where contamination can occur at multiple steps along the food chain (Bouchrif *et al.*, 2009). Typhoid is transmitted mainly by the fecaloral route. In most cases an asymptomatic carrier of *Salmonella typhi*, or an individual who has recently recovered from the infection either through direct food handling, through transfer of bacteria by flies and other insects, or by contamination of potable water (Butler, 2011). However, *Salmonella typhi* and *Salmonella paratyphi* do not have animal reservoir, therefore infection can be happened by eating the improperly handled food by infected individuals (Newell *et al.*, 2010). *Salmonella* can enter the food chain at any point from livestock feed, through food manufacturing, processing and retailing as well as catering and food preparation in the home (Wong *et al.*, 2002). Approximately 10% of patients recovering from typhoid fever excrete *Salmonella typhi* in the stool for three months, and in the past 2-3% became permanent carriers (Zige *et al.*, 2013).

A number of symptoms of typhoid were also reported such as high fever, chill, nausea, headaches, malaise and

sometimes with delirium (Cook *et al.*, 2008) and others intestinal hemorrhage or perforation, pneumonia, myocarditis, hepatitis, acute cholecystitis and meningitis. The symptoms begin after an incubation period of 10 to 14 days. Enteric fever caused by *Salmonella typhi* may be preceded by gastroenteritis, which usually resolves before the onset of systemic disease.

The clinical diagnosis of typhoid is confirmed by culture of the organism from blood, bone marrow or urine (Abdoel *et al.*, 2007). Blood bacterial counts decline as the disease progresses. Bone marrow counts are approximately ten times higher than blood culture (Parry *et al.*, 2011).

Serological diagnosis is widely relied upon. Widal test which measures the antibody titres to the somatic O and flagella H antigens is relied upon widely, although there are very divergent views on its utility. Overall sensitivity is approximately 70-80% with specificity ranging from 80-95%. New IgM and IgG based rapid serological tests have proved useful in some areas (Baker *et al.*, 2010).

Antimicrobial therapy is the mainstay for the treatment of enteric fever and the complications associated with it. Penicillins such as amoxycillin and ampicillin, cephalosporins such as ceftriaxone and cefuroxime, aminoglycosides such as streptomycin and gentamycin,

macrolide such as erythromycin, fluoroquinolones such as ciprofloxacin, ofloxacin and perfloracin and tetracyclines are used for treatment of *Salmonella typhi* infection. The mechanism of drug resistance usually mediated by acquisition of R plasmids involvement. Plasmid resistance often code for enzymes that destroy or modify drugs. Plasmid associated genes have been implicated in resistance to aminoglycosides, chloramphenicol, penicillin, cephalosporin, erythromycin, tetracycline, sulphonamides and others (Willey *et al.*, 2013).

The assay based on nanotechnology would have high potential for rapid, on-site pathogen detection and also to avoid the distribution of contaminated food (Wang *et al.*, 2010).

*Salmonella typhi* is a particular salmonella serovar, a major public health problem in developing countries (Oamar *et al.*, 2014). Although the global burden of typhoid fever has reduced emergence of multidrug-resistant *Salmonella typhi* is still a threat in public health.

## MATERIAL AND METHODS

### Collection of Material

For isolation of *Salmonella typhi*, different sample such as fruit juice, tap water and blood were collected from local area of Sudhowala, Dehradun. U.K.

### Isolation of *Salmonella typhi*

1 ml of water sample from different places were collected and pour in the sterile petri plates by pour plate method. Pour the sterile *Salmonella Shigella* Agar media in each petri plates and swirl in clockwise and anticlockwise direction. Solidify the plates and incubate in BOD incubator at 37<sup>o</sup>c for 48 hours. After 48 hrs

observe the plates for the growth of small, circular, translucent colonies of *Salmonella*.

### Screening of *Salmonella typhi*

The isolates from different sample such as fruit juice, tap water, blood were screened on differential media such as Bismuth sulphite agar and Brilliant green agar. The isolates shows jet black colonies of *Salmonella*.

### Identification and Biochemical characterization of *Salmonella typhi*

The selected isolates were identified by Gram staining and biochemical characterized such as IMVic, H<sub>2</sub>S, Fermentation, urease, catalase, nitrate Reduction and Oxidase test confirms as *Salmonella typhi*.

### Antibiotic sensitivity of different antibiotics against *Salmonella typhi*

To check the Action of different antibiotics such as ciprofloxacin, Erythromycin, Amoxycillin, Ampicillin, Chloramphenicol and Doxycyclin against selected isolates of *Salmonella typhi* by Kirby-Bohr method. To the sterilized Muller Hilton agar media place a disc of antibiotics at one corner of the media and another disc as a control. Diffuse the antibiotics with the media and incubate at 37<sup>o</sup>c for 24-48 hrs. After incubation, observe the plates for zone of inhibition against the *Salmonella typhi* in (mm) dia.

## RESULT

### Isolation of *Salmonella typhi*

From Three different samples such as Fruit juice, tap water and blood, the fruit juice sample shows small, circular, translucent colonies of *Salmonella typhi* (STFJ) on SS Agar. The other sample tap water and blood were not shows the colony of *Salmonella*.

Table no. 1: Isolation of *Salmonella typhi* from different samples.

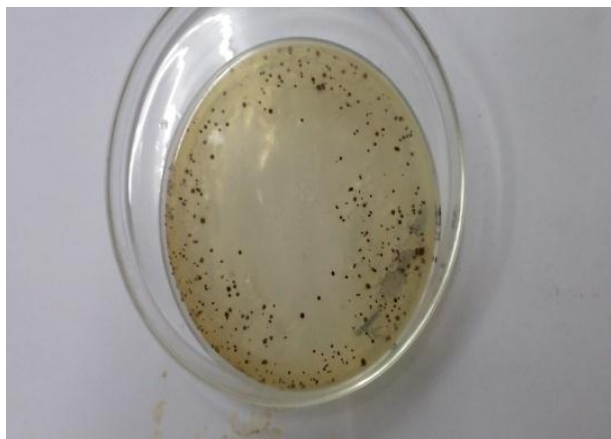
SL.NO.	SAMPLE NAME	MORPHOLOGICAL CHARACTERISTICS			
		COLOR	SHAPE	SIZE	GRAM STAINING
1.	Fruit juice	Black	Circular	Small	Gram -ve,Rod
2.	Tap water	Black	Circular	Transparent	Gram +ve
3.	Blood	Black	Circular	small	Gram -ve,Rod



Fig no. 1: Isolates *Salmonella typhi* from fruit juice in *Salmonella Shigella* Agar media.

### Screening of *Salmonella typhi*

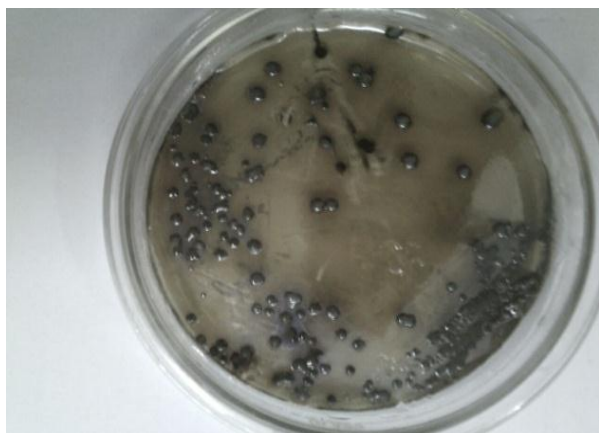
The selected isolates of *Salmonella typhi* from fruit juice (STFJ) was screened on differential media such as Bismuth sulphite agar, shows the small, circular jet black colonies and large, circular pinkish white colonies on Brilliant green agar. This shows the positive growth of *Salmonella typhi*.



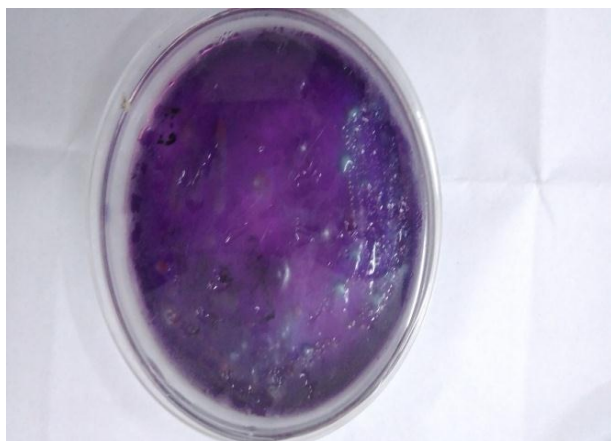
**Fig no. 2: Isolates Salmonella typhi from blood.**



**Fig no. 5: Morphology of Isolated Salmonella typhi under Microscope in 45 X.**



**Fig no. 3: Screening of Salmonella typhi in Bismuth sulphite agar.**



**Fig no. 4: Screening of Salmonella typhi in Brilliant green agar.**



**Fig no. 6: Biochemical Test (IMVic) of Salmonella typhi.**

#### **Identification and Biochemical characterization of Salmonella typhi**

The selected isolates of *Salmonella typhi* (STFJ) was identified by Gram staining shows G-ve, rod shaped, arranged in singly. It shows positive H<sub>2</sub>S, MR, Citrate, catalase, nitrate reduction and Oxidase test where as fermentation and urease test are negative. Therefore it confirms the *Salmonella typhi*.

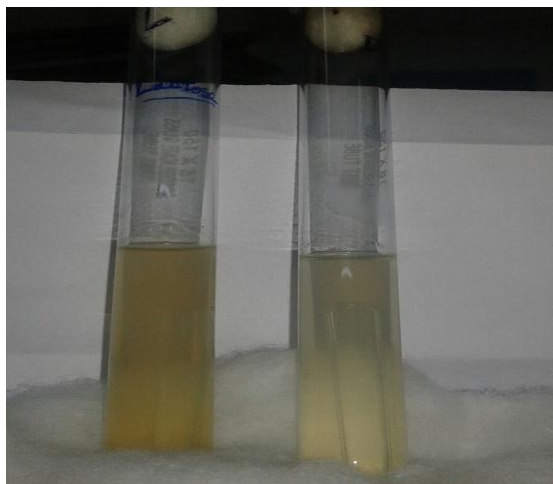


Fig no. 7: Fermentation Test of Salmonella typhi.



Fig no. 8: Catalase Test of Salmonella typhi.



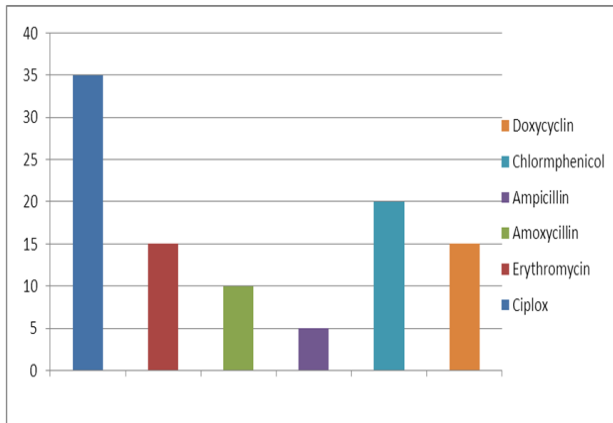
Fig no. 9: Nitrate Reduction Test of Salmonella typhi.



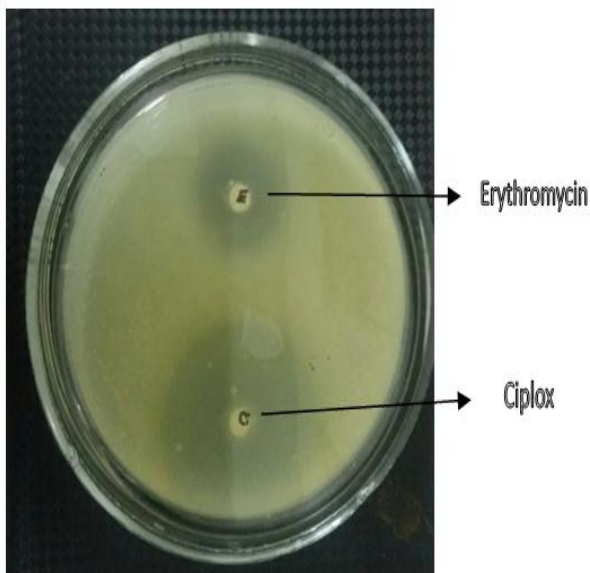
Fig no. 10: Oxidase Test of Salmonella typhi.

Table no. 2: Antibiotic sensitivity of different antibiotics against Salmonella typhi.

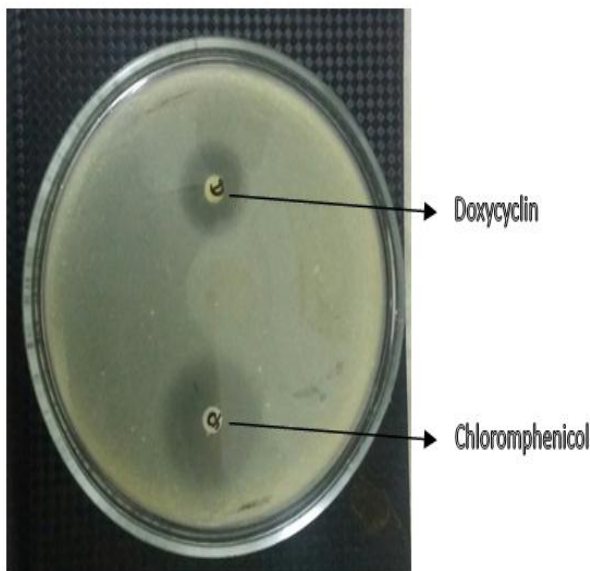
SL.NO	Name of Antibiotics	zone of Inhibition(mm) A	Dia of disc (mm) B	Effective zone of Inhibition(mm)= A-B
1.	Ciplox	40mm	5mm	35 mm
2.	Erythromycin	20mm	5mm	15 mm
3.	Amoxycillin	15mm	5mm	10 mm
4.	Ampicillin	10mm	5mm	5 mm
5.	Chlormphenicol	25mm	5mm	20 mm
6.	Doxycyclin	20mm	5mm	15 mm



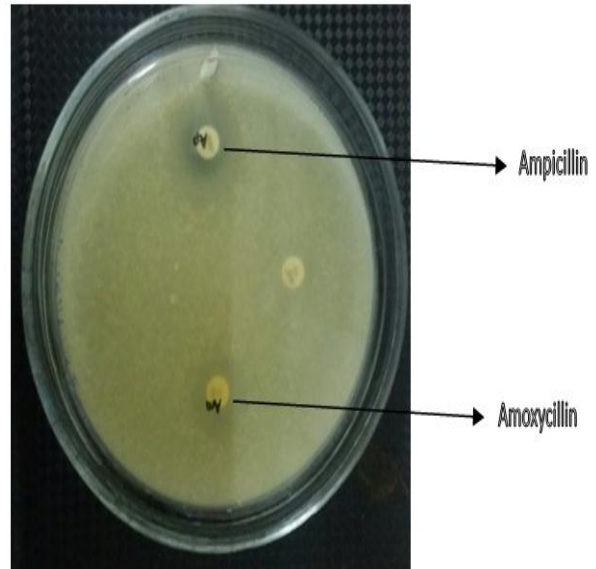
**Graph no. 1: Antibiotic sensitivity of different antibiotics against *Salmonella typhi*.**



**Fig no. 11: Antibiotic sensitivity by Erythromycin and Ciplox.**



**Fig no. 12: Antibiotic sensitivity by chloramphenicol and Doxycyclin.**



**Fig no. 13: Antibiotic sensitivity by Ampicillin and Amoxycillin.**

### CONCLUSION

It has been found that *Salmonella* was isolated from fruit juice sample on SS Agar, BSA and BGA media shows small, circular, jet black colonies. It is G-ve, rod shaped bacteria by Gram staining and biochemically characterized. The antibiotics such as ciprofloxacin are mostly effective against *Salmonella typhi*.

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