



# Isolation and Identification of Salmonella Typhi from different Blood Samples

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## Abstract

Enteric fever continues to be a major cause of mortality and morbidity globally, particularly in poor resource settings. Lack of rapid diagnostic assays is a major driving factor for the empirical treatment of enteric fever. Salmonellae are food-borne pathogens transfer through oral ingestion of contaminated food or water and causing disease in both healthy and immune-compromised people.

Current study was designed for isolating and identifying *Salmonella typhi* from blood samples. This study was conducted on 39 patients from both genders suffering from typhoid fever. Their age range was 1 to 50 years. The samples were collected from different hospitals in Ujjain city. The effect of age, gender and residence on the frequency distribution of typhoid fever was also determined. The collected samples were tested by culture. The results showed that blood culture and serological techniques are used to isolate *Salmonella typhi* and *Salmonella paratyphi*

Key words: typhoid fever, ICT, *salmonella typhi*, blood culture

## INTRODUCTION

Bacteria are indeed the lowest, or at least the simplest, form of life. Their prokaryotic cell structure is significantly different from higher forms such as the single celled algae and protozoa, invertebrates, plants and animals[1]. Only the Archaea have a cell structure of similar simplicity. Bacteria are the most numerous of living organisms on earth in terms of number of species, number of organisms, and total mass of organisms. A concentration of 1000 organisms per ml is very significant in terms of protecting health, but is invisible to the naked eye. Bacterial growth or replication is in numbers and concentration rather than mass of a single organism[2].

Bacteria cause many human, animal, and plant diseases. Pathogenic bacteria, those that cause disease, are transmitted through direct contact with an infected host. The focus of this particular report is to render the pathogenic bacteria transmitted through water or associated with water in some manner. All waterborne or water-washed diseases are also associated with food and food preparation. In some cases the pathogenic bacteria are normal members of the intestinal flora of food animals and contamination results during processing[3]. Infection results from improper handling during preparation or from inadequate cooking. In other cases organisms may be transferred from a processor to the food directly or by washing the food with contaminated water. In most cases proper cooking and washing of preparation surfaces with soap and hot water will prevent infection.

A bacterial infection is a proliferation of a harmful strain of bacteria on or inside the body. Bacteria can infect any area of the body[3]. Typhoid is among the few illnesses that may be caused by harmful bacteria. Bacteria come in three basic shapes: rod-shaped (bacilli), spherical (cocci), or helical (spirilla). Bacteria may also be classified as gram-positive or gram-negative. Gram-positive bacteria have a thick cell wall while gram-negative bacteria do not.

Typhoid fever is a systemic human infectious disease caused by *Salmonella typhi*. Symptoms during the acute phase of infection comprise nausea, abdominal pain, headache and fever [4]. According to the World Health Organization (2014), the annual rate of typhoid fever reaches 21 million cases and 222,000 fatalities (1). This gram-negative enteric bacillus belongs to the family Enterobacteriaceae. It is a motile, facultative anaerobe and there is no animal reservoir that is susceptible to various antibiotics (2). Typhoid can be diagnosed with certainty only by isolation of *Salmonella typhi* from the blood. [4]. Complications of typhoid fever include intestinal hemorrhage or perforation, pneumonia, myocarditis, hepatitis, acute cholecystitis and meningitis.

## MATERIALS AND METHODS

Collection of Specimens Blood samples were collected aseptically from Patients suffering from typhoid fever who visited different hospitals of Ujjain for the period from (March 2020-February 2021). The age range of patients was 1 to 50 years. A total of 2 ml blood sample was drawn aseptically from each patient used for the culture and then incubated at 37°C. Blood culture was regularly examined for checking the turbidity and color change which referred to microbial growth. Culture should be incubated for at least 7 days before result is reported as negative. Nevertheless, bottle was discarded after 14 days[8]. Subcultures were performed as follows: from each positive blood bottle, a loopfull was transferred to MacConkey agar, streaked, incubated for 24 hours at 37 °C. The isolates were stained with Gram stain and examined under light microscope [9]. All culture media were prepared according to the information of the manufactures.

Biochemical test : Important relevant biochemical tests such as Oxidase test, Indol test, Urease test, Methyl Red/Voges-Proskauer test, Citrate utilization, Kligler test, Cytochrome oxidase tests and Catalase test were conducted according to (10,11).

## RESULTS AND DISCUSSION

Identification of salmonella on the culture media: The results of blood cultures of patients' samples showed that 45 isolates out of 190 cases were found positive for *Salmonella typhi*. Its sensitivity is poor, because a very small number of bacteria is needed to cause severe infection. The sensitivity of blood culture is maximum during first week of the illness and decreases with progression of the illnesses [12]. Bacteria may be found in bloodstream at any stage of the illness, but most commonly found in the first 7-10 days and during relapses [13]. If the patient is untreated, blood culture is usually positive in about 75% during first week and decreasing to 15%- 26% during later stages of the disease [14]. In current study, it was found that 50 ml of medium was sufficient for 5 ml of blood apparently because of very low degrees of bacteraemia in some patients [15].

Morphological properties: After 24 hrs of incubation at 37 C°, the bacterium showed convex, (2–4 mm) in diameter, and smooth colonies. On MacConkey agar, they looked pale due to their inability to ferment lactose. On Blood agar *S. typhi* produce grey white 2- 3 mm in diameter colonies. On nutrient agar; the colonies of most strains were moderately large, 2-3 mm, in diameter after 24 hours at 37 °C. Some strain produce mucoid colonies [16]. *Salmonellae* require enrichment of the minimal medium with one or more amino acids or vitamins e.g., cysteine or nicotinamide. Also, most *S. typhi* strains require tryptophan [17].

Microscopic Examination: Gram staining was done for morphological identification of *S. typhi*, where *S. typhi* was found to be Gram-negative short bacilli [18]. Microscopical appearance of *Salmonella typhi* as Gram-negative short bacilli

## Biochemical Reactions: Biochemical test Result

Indole – Methyl Red - Catalase +

citrate utilization - Voges–Proskauer – oxidase, and urease +

Production of H<sub>2</sub>S + (+) positive reaction, (– (negative reaction).

Results of biochemical test of API20E system Test result Test Result –

Urease - Tryptophan Deaminase Indol Production - H<sub>2</sub> S Production +

Acetone production - Citrate Utilization +

B–Galactosides - Gelatinase Liquefaction Glucose fermentation +

Arginine Dihydrolase + +

Lysine Decarboxylase +

Mannitol fermentation Inositol fermentation +

Decarboxylase + +

Melibiose fermentation Sorbitol fermentation +

Amygdalin fermentation +

Rhamnose fermentation +

Arabinose fermentation Sucrose fermentation.

In typhoid fever, however, a four-fold rise after 2 weeks is not always demonstrable even in blood culture confirmed cases. This situation may occur when the acute phase sample is obtained late in the natural history of the disease, because of high levels of probable background antibodies in an endemic region or because in some individuals the antibody response is blunted by the early administration of an antibiotic [19]. Moreover, results showed that the prevalence of typhoid fever, within the studied population, was higher among patients living in rural areas than among those living in cities. These findings could be attributed to life style of people living in rural areas where people drink water without any sterilization so that bacterial pathogens are easily and directly transmitted via water.

Further more, results of current revealed that the incidence of typhoid fever cases caused by *Salmonella typhi* was highest during May, 2020 and lowest during November, 2020

## CONCLUSION

In concluded, it was found that 39 out of 45 had positive blood cultures and the isolates belong to *salmonella typhi* test showed higher rate of typhoid cases.

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