



## Original Research Article

# Seroprevalence of *Salmonella typhi* in a Tertiary Care Facility in North India

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## ABSTRACT

### Keywords

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The Widal test is one of the most extensively used tests in the developing countries for the diagnosis of enteric fever due its cost effectiveness, along with sufficient sensitivity and specificity. Aim : To determine the seroprevalence of *Salmonella Typhi* infections in a tertiary care facility in North India over a period of 3 years. A retrospective study was carried out in the Serology Laboratory, Department of Microbiology, Pt B D Sharma, Rohtak over a period of 3 years, from Jan 2012 to December 2014. Serum samples of patients suspected of having enteric fever were subjected to Widal test and were analysed for the presence of antibodies against the O and H antigens of *Salmonella enterica* serovar Typhi. A total of 16,642 samples were tested in the study period. Of these 1794 (10.8%) were positive for the presence of antibodies. The study also revealed an increasing trend of seropositivity. Over the three year study period an increase in the seroprevalence of *Salmonella Typhi* was noted. This can be attributed to overcrowding, poor hygienic conditions and also an increase in the awareness of the endemicity of this disease.

## Introduction

Enteric fever which includes typhoid and paratyphoid fever is a systemic febrile illness caused by the bacterium *Salmonella enterica* serovar Typhi and *Salmonella enterica* serovar Paratyphi A, B or C, respectively. *Salmonella enterica* serovar Typhi is found to be associated with over 90% cases of enteric fever. (Pandey et al, 2012) Typhoid fever is a noted cause of morbidity worldwide with an estimated 21.7 million cases, the bulk of the burden being borne by India, South and Central America

and sub-Saharan Africa, all with growing population and poor sanitary conditions. There is a dearth of available epidemiological data to project the actual situation in India, though a few hospital and large population based studies have demonstrated substantial changes in the incidence of typhoid fever. (Banerjee al, 2014)

In this context, over 100 years since its introduction, the Widal test has been and is

still being widely used for the diagnosis of typhoid fever, simply owing to the fact that no other serodiagnostic test of sufficient sensitivity and specificity along with cost-effectiveness has been developed, especially in typhoid endemic regions. (Olopoenia et al., 2000). Several commercial rapid diagnostic tests namely Typhidot and Tubex have also shown sensitivity and specificity of 70 and 80 per cent, respectively in most of the surveillance studies worldwide, apart from being costlier than the agglutination tests. (Ley et al, 2010) In actual practice, most of the infections by *Salmonella enterica* serotype Typhi are diagnosed clinically without proper laboratory evidence and consequently treated presumptively with antibiotics. (Olsen, 2004). The real concern is that though the gold standard technique of culture isolation of *Salmonella* Typhi (*S. Typhi*) provides a definitive diagnosis in 73-97 per cent cases prior to medications, excessive antibiotic use has reduced this isolation rate to 40-60 per cent. At the same time, the developed countries with low prevalence of typhoid have more infrastructure and culture facilities, thus, here Widal test can be abandoned, as opposed to the developing endemic regions where culture facilities are far from reality. Hence, in these regions the slide and tube agglutination test against the O and H antigens of *S. Typhi* is perhaps the only feasible laboratory method, despite controversies regarding its use in diagnosis of typhoid fever. The present study was undertaken with the aim to determine the prevalence of seropositivity of *Salmonella* Typhi infection in a tertiary care centre in north India, over a period of 3 years.

### **Materials and Methods**

This study was conducted on the sera sample of patients suspected of having enteric fever, which were sent to the Department of Microbiology for

investigation. Over a span of 3 years of the study period, a total of 16,642 samples were received in the laboratory. The serum samples were tested by the Widal tube agglutination test using commercially available lipopolysaccharide “O” and protein flagellar “H” antigens of *S. Typhi* (Central Research Institute, Kasauli, India). The sera were tested by the standard doubling dilution method. In house, positive and negative controls were tested with each batch. The tubes were incubated at 37°C for two hours and subsequently kept at room temperature overnight. (Cruickshank et al., 1975). The examination of the tubes and interpretation of the results was done the next day. A titre of  $\geq 100$  for the O antibodies and that of  $\geq 200$ , in case of H antibodies, was considered significant. India remains a typhoid endemic region and the burden of the disease in a developing country as ours remains underestimated. Thus, the present study was undertaken with the aim to estimate the seroprevalence of enteric fever in our setting.

### **Results and Discussion**

A total of 16,642 samples were received in the Serology Department during the study period, commencing from January 2012 to December 2014; 5328 samples in 2012, 5347 samples in 2013 and 5667 samples in 2014, respectively. An increase in the number of seropositive cases was noted with 9.7% in the year 2012 to 12.1% in 2014. Maximum of the positive cases were patients of the age group 15-30 years. Slight male preponderance was seen with a male to female ratio of 1.16, during the study period. (Table 1). More samples were positive for the presence of H agglutinins (986) as compared to those positive for the presence of O agglutinins (808) indicating an increased number of latent cases in the study population. However, this difference was not statistically significant. Similar to most

of the tropical diseases of infectious origin, seasonal pattern in distribution of seropositive titres were noted in our study, with maximum cases during rainy season (July-August).

Typhoid fever, caused by *Salmonella enterica* serotype Typhi, is a major cause of morbidity and mortality worldwide, causing an estimated 16.6 million new infections and 600,000 deaths each year.(Pang T, 1998) In Asia, the mean incidence of enteric fever is estimated to be 900 per 100,000 people per annum. (Ivanoff et al, 1994) Enteric fever is endemic in all parts of India and still constitutes a significant health hazard.

Isolation of *Salmonella enterica* serotype Typhi from blood, urine, or stool is the most reliable means of confirming an infection. However, this requires laboratory equipment and technical training that are beyond the means of most primary health care facilities in the developing world. Most serotype Typhi infections are diagnosed purely on clinical grounds and treated presumptively. As a result, the diagnosis may be delayed or

missed while other febrile illnesses are considered, and patients without typhoid fever may receive unnecessary and inappropriate antimicrobial therapy (Olsen S J et al, 2004)

The earliest serological response in acute typhoid fever is a rise in the titer of the O antibody, with a gradual elevation of the H-antibody titer, but persisting longer than the O- antibody cut off titer. Usually upto 70% of the adults show an early rise of antibody titer in the first week of infection. ( Mittal et al, 2014)

The Widal test has been used very extensively in the serodiagnosis of typhoid fever. In developing countries it remains one of the best, easily accessible, economic and simple method for the diagnosis of typhoid fever. (Kulkarni and Rego, 1993). This test is based on the demonstration of the agglutinin in the serum of the infected patient against the H (flagellar) and O (Somatic) antigens of *Salmonella* Typhi, during the acute and convalescent period of infection. (Washington and Henry, 1984).

**Table.1** Age-wise distribution of the seropositivity in males and females

Age Group	2012		2013		2014	
	Male	Female	Male	Female	Male	Female
< 15 years	71	68	91	57	90	72
15-30 years	114	88	92	121	146	127
30-60 years	57	61	89	54	83	71
> 60 years	36	23	41	46	56	40
<b>Total</b>	<b>518</b>		<b>591</b>		<b>685</b>	

An increase in the incidence of seropositivity was noted in our study with the seropositivity of 9.7% in 2012, 11.1% in 2013 and 12.1% in 2014. Similar findings

were also reported by other authors. (Kumar et al, 2008;Banerjee et al, 2014) This rise in seropositivity can be attributed to the increasing population and poor hygienic

conditions prevailing in the region. Since this is largely a preventable disease, health education must be imparted to people regarding safe hygienic practices and the available vaccination strategies.

The present study reinstates the importance of Widal test in diagnosing enteric fever infections in a developing country like India. A rise in the seropositivity rate was observed in the study, which could have been due to the overcrowding and poor sanitary conditions, in the endemic region. A drawback of this study was that the correlation with the blood culture was not done, as only the serum sample was analysed. However, this retrospective study provided an inexpensive way to estimate the burden of infection of enteric fever among the patients in a tertiary care hospital in North India.

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