



Prevalence and different laboratory diagnosis methods for the determination of typhoid fever in Kashmir region of Jammu and Kashmir, India

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Abstract

The study of Prevalence and different laboratory diagnosis methods for the determination of typhoid fever in Kashmir region were studied with 676 samples. The samples of patients were classified into three groups, males, females and children from the age group of 8 to 60 years. Out of 676 patients, 250 were males with 146 found positive and 104 were found negative for typhoid. In 350 females samples collected, out of which 239 were found positive for typhoid and 111 has shown negative results. Whereas 76 children sample collected revealed that 28 were found to be positive and 48 were negative for typhoid. The overall results of Widal test showed that out of 413 sample 219 were positive for Antigen O, 165 were positive for Antigen H, 17 were positive for Antigen AH and 12 were positive for Antigen BH. Out of 413 sample, it was found that 146 were both IgM and IgG positive, 35 were both IgM and IgG negative, 192 were IgM positive and 40 were IgG positive. This study revealed that the highest cases were reported with IgM positive whereas as the lowest percentage was found with IgG positive. Out of 192 positive cases (IgM positive for typhidot), 189 were found positive for IgM and 3 were negative by ELISA method. Similarly, out of 40 positive cases (IgG positive for typhidot), 38 were found positive for IgM and 2 were negative by ELISA method. Thus the study found that the prevalence of typhoid was found higher in females than in males and children.

Keywords: *Salmonella typhi, Enteric fever, Widal test, Typhidot assay, ELISA.*

Introduction

Salmonella typhi is a Gram-negative facultative intracellular anaerobe responsible for causing typhoid disease throughout the globe which accounts 1.3 billion cases annually. Over 2500 serovars of *S. enterica* have been identified belonging to six subspecies.¹⁻² Serovars are differentiated by their characteristic features of

flagella, carbohydrate and lipopolysaccharide (LPS) structures. *Salmonella typhi* species (*S. enterica*) are a major cause for symptoms of enteric fever (typhoid), enterocolitis, diarrhoea, bacteraemia and chronic asymptomatic carriage.³ Typhoid fever is an infectious disease of global distribution.⁴ The outbreaks of typhoid are mainly due to improper sewage disposal, contamination of drinking water,

contaminated food.⁵ Typhoid fever patient develop symptoms like digestive hemorrhages, ileocecal perforation, encephalitis, myocarditis, enterobacterial superinfections, ileal perforation, pancreatitis, acute pancreatitis, intestinal perforation, bowel perforation, infarction of spleen, typhoid glomerulonephritis, bleeding perforation, osteomyelitis of the spine, cholecystitis, and cholangitis.⁶⁻¹⁵ Since paratyphoid fever is indistinguishable from typhoid fever in its clinical course, *S. Enterica serovars typhi*, *Paratyphi A*, *Paratyphi B* and *Paratyphi C* are collectively referred to as typhoidal *Salmonella serovars*.

Typhoid is a systemic infection caused by *Salmonella enterica* serotype *typhi*, remains an essential worldwide cause of morbidity and mortality.¹⁵ This disease is a prolonged febrile illness and continues to be a health problem in developing countries where there is poor sanitation, poor standard of personal hygiene and prevalence of contaminated food and water. The disease endemic in many parts of the developing countries, and as global travel increases, illness can and do occur around the world in the span of a day.¹⁶ The prevalence of typhoid Fever is increasing tremendously in Kashmir region because of poor sanitation, contaminated food, and water, lack of medical awareness and hygienic education. The aim of this study is to find out the prevalence of Typhoid Fever in Kashmir valley sampling were carried out from different categories of patients. Including Males, Females, and Children with the age group between 8 years to 60 years and were examined in the laboratory using Widal as a baseline test.

Materials and Methods

This study was carried out at Department of Microbiology, MGR College and Research Institute, Hosur. The Study period was August 2016 to July 2018.

Collection and processing of samples

The samples from males, females, and children with the age group between 8 to 60 years were collected for epidemiological investigations from Kashmir

(Baramulla, Srinagar and Budgam districts). A total of 676 samples were collected from various patients from hospitals and clinical laboratories. The typhoid positive samples were further confirmed by using antibodies such as IgG and IgM antibodies. 5 ml of blood were collected and inoculated into blood culture bottle containing 45 ml of bile broth and incubated at 37°C overnight. After incubation bile broth was subcultured on MacConkey Agar plates and Wilson and Blair media was used to isolate *S. typhi*.

Identification

The standard Gram Staining, Slide test, and Tube test were performed for identification of *Salmonella* as per standard methodology.

Widal test

The Widal tube agglutination test was done on all sera collected from different laboratory diagnosis methods using commercially available antigens (Tulip diagnostics, India). 0.4ml of two-fold serially diluted patient's sera (1:20 to 1:320) in 0.9% normal saline were tested by adding an equal volume of antigen. A negative control was included in each batch of the test. The tubes were incubated at 37°C for 24 h and then examined for agglutination¹⁷ and higher for anti TH antibodies were taken as a cut of value to indicate a recent infection of typhoid fever.

Typhidot assay

TYPHIDOT IgG/IgM (Medsorce ozone bio medicals, India) is an indirect solid phase immunochromatographic assay designed for the qualitative detection and differentiation of specific IgM and IgG antibodies against specific *Salmonella typhi* antigen in human whole blood, serum or plasma. Around 40µl of blood was collected and inserted in the well of the test cassette and observed for colour change. This test is based on the principle of antigen-antibody complexes formed with the conjugated antigens present. Antibodies and reagents for the capture of anti-*S. typhi* IgM and IgG are immobilized onto cellulose nitrate membrane as test lines. When the test sample is added to the

sample pad, it migrates upwards together with dye conjugated to *S. typhi* antigens.

Salmonella typhi IgG and IgM by ELISA method

The 1:101 diluted patient serum was added to wells coated with a purified antigen with IgG and IgM antibody. The unbound substances were washed away and enzyme conjugate was added as per manufacture instructions to bind antibody-antigen complex (Medsorce ozone bio medicals, India). The excess enzyme conjugate was washed by adding the substrate and the plate was incubated for the hydrolysis of the substrate by the enzyme. The colour generated is proportional to the amount of IgG and IgM antibody and absorbance were taken at 450nm (Biorad, USA).

Results

Effect on the prevalence of typhoid fever

The total sample size was 676 comprising of three categories with the age group of 8 to 60 years old. Among 250 males, out of which 146 were found positive and 104 were found negative and in 350 females, out of which 239 were found positive and 111 were found negative. Whereas in 76 children, out of which 28 were found positive and 48 were negative. The males which were positive for typhoid were in the age group of 30 to 40 years of age. The females which were positive for typhoid were in the age group of 35 to 45 years of age. The children who were positive for typhoid were in the age group between 8 to 13 years of age. The prevalence of typhoid fever was shown in Figure 1 and the prevalence of typhoid fever percentage was shown in Figure 2.

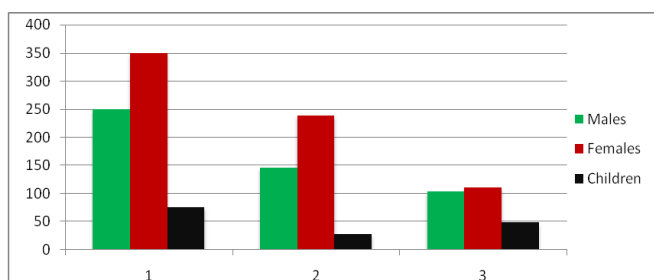


Fig.1 Prevalence of Typhoid fever: (1) Total number of samples, (2) Total number of positive samples and (3) Total number of negative samples.

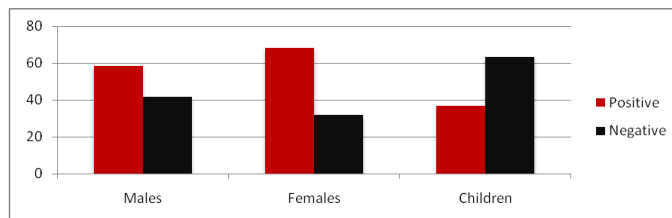


Fig. 2 Prevalence of Typhoid fever percentage in male, female and children

Morphological characters

Colonies were observed from Wilson and Blair medium, McConkey agar (MCA), Eosin methylene blue agar (EMBA) and Brilliant green agar (BGA) plates. In Gram’s staining, the morphological characteristics of the isolated Salmonella exhibited Gram-negative, small rod-shaped, single or paired in an arrangement under a microscope.

Effect of Widal test

The results of Widal test showed that out of 413 sample 219 were positive for Antigen O, 165 were positive for Antigen H, 17 were positive for Antigen AH and 12 were positive for Antigen BH (Table. 1). The highest percentage cases were with Antigen-O whereas the lowest percentage was found in Antigen-BH.

Tests	Table. 1. Effect of Widal test		
	Observation	No. of patients	Percentage (%)
1	Antigen O	219	53.02
2	Antigen H	165	39.95
3	Antigen AH	17	4.11
4	Antigen BH	12	2.90

Effect of Typhidot assay

Out of 413 sample, it was found that 146 were both IgM and IgG positive, 35 were both IgM and IgG negative, 192 were IgM positive and 40 were IgG positive. The highest cases were reported with IgM positive whereas as the lowest percentage was found with IgG positive (Table. 2).

Tests	Table. 2. Effect of typhidot assay		
	Observation	No. of patients	Percentage (%)
1	Both IgM and IgG positive	146	35.35
2	Both IgM and IgG negative	35	8.47
3	IgM positive	192	46.48
4	IgG positive	40	9.68

Effect of ELISA assay

Out of 192 positive cases (IgM positive for Typhidot), 189 were found positive for IgM and 3 were negative by ELISA method. Similarly, out of 40 positive cases (IgG positive for Typhidot), 38 were found positive for IgM and 2 were negative by ELISA method (Table. 3).

Tests	Table. 3. Effect of ELISA assay			
	Observation	No. of total positive samples (Typhidot result)	ELISA method	Percentage (%)
1	IgM Positive	192	189	98.43
2	IgM Negative	-	3	1.56
3	IgG Positive	40	38	95
4	IgG Negative	-	2	5

Discussion

All isolates were found for negative to indole tests positive to MR and negative to VP. In the present study, the prevalence rate of *Salmonella* is 61.09%. So, the results of this study are more or less in agree with the findings of the previous workers who also conducted research investigation on *Salmonella* from human stool sources. The slight differences among the prevalence percentages might be due to the species differentiation, hygienic, environmental and geographic variation and technical limitations of the laboratory of the study.

After analysis, it was found that the prevalence of typhoid fever was found to be higher in females than that of males and children respectively in Jammu and Kashmir, India. In results it was found that females showed the highest positive cases with 68.28%, followed by males 62.4% and 36.84% in children respectively. In antigenic variation O antigen percentage was found higher as compared H, AH, and BH in the Typhoid positive samples.

The results of the Widal test showed that out of 413 sample, the Antigen-O showed 53.02%, Antigen-H showed 39.95%, Antigen-AH showed 4.11% and for positive Antigen-BH showed 2.90%. The results of TyphiDot test showed that out of 413 sample, both IgM and IgG positive was 35.35%, Both IgM and IgG negative was 8.47%, IgG negative was 46.48% and IgG positive was 9.68%. The Typhi Dot was evaluated in Pakistan and Singapore by using a variety of case definitions.¹⁸

¹⁹The sensitivity ranged from 59 to 93% for the

TyphiDot and 73 to 84% with the addition of the TyphiDot-M. Specificity was consistently higher when TyphiDot-M was used; 89% compared to 77% or lower with only the Typhi-Dot. An evaluation of the TyphiDot in India was 100% sensitive and 80% specific compared to a blood culture “gold standard”.²⁰

The tests for immunoglobulin G (IgG) and M (IgM) anti-lipopolysaccharide (LPS) of *Salmonella typhi* antibodies by ELISA showed the levels of all two classes of immunoglobulin anti-LPS of *S. typhi* were higher in typhoid patients than in healthy or febrile non-Typhoidal groups.²¹⁻²³ The results showed 98.43% positive for IgM and 95 positives for IgG by ELISA method respectively.

Follow-up studies of salmonellosis patients have demonstrated the persistence of LPS-specific IgG for up to 12 months after infection, while IgM and IgA disappear after 2–4 months in most patients.²⁴⁻²⁵ Thus it has been argued that an LPS-based ELISA which detects IgG, IgM and IgA responses provides a highly specific, sensitive, fast, easy and reliable assay for routine analyses of human sera.²⁵

The development of a standardized ELISA requires significant ground-work and an extensive production of new standards and controls that is logistically challenging and most likely not a priority for many countries which consider *Salmonella* a minor problem. From a broader perspective, widespread use of ELISAs for diagnosing bacterial infections can also create a more realistic estimate of disease burden as they can detect infection in patients who would otherwise not have been diagnosed.

Conclusions

The prevalence of enteric typhoid fever was more in females as compared to males and children. Females were found more susceptible to *S. typhi* than males and children in the temperate region of Kashmir. The different methods such as the Widal test, Typhidot and ELISA method found to be useful for the rapid diagnosis of typhoid fever. The Widal test was used for the determination of different antigens such as Antigen-O, Antigen-H, Antigen-AH,

Antigen-BH. The typhidot and ELISA method were found useful for the detection of IgM and IgG antibodies.

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