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Seroprevalence of Typhoid in Indian Regions: Tertiary Care Hospitals & Diagnostic Laboratories-Based Study

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ABSTRACT

Background: Typhoid fever is a systemic infection caused by Salmonella enterica typhi (*S. Typhi*). It is an important public health concern in developing countries like India. It is estimated that 17.8 million (95% CI: 6.9-48.4 million) cases of typhoid fever occur every year in low-middle income countries with highest incidence in Central Africa, followed by Central, South and Southeast Asia. The objective of this study is to understand the trend of typhoid by using diagnostic laboratories data across Delhi, Uttar Pradesh, Uttarakhand, Punjab, West Bengal, Maharashtra, and Kerala. **Methods:** It is an observational retrospective study. The study involves laboratories data of 1,01,977 subjects (males - 56,140, females – 45,837) who got tested for typhoid by Widal between Januray 2016-December 2017 across different regions of India. Positivity criteria for Widal test is taken as TO and TH titre > 80 and TO > 80 and TH > 160. **Results:** Out of the total subjects, 11.3% showed level of O and H agglutinins ≥ 80, while only 3% showed O and H agglutinin titre ≥ 160. North Indian states (Delhi, Punjab, and Uttar Pradesh) have the higher proportion of cases – estimated at 12-27% (at agglutinin level of >80 for H and O). Other states in South (Kerala), East (West Bengal) and West (Maharashtra) show lower proportion of subjects with significant titres. The highest typhoid burden is among young adults (18-30 years) contributing 32% of overall cases, followed by paediatric age group (0-18 years) contributing 27% of total burden. **Conclusion:** Lab based data analysis can play an imperative role in assessing the epidemiological characteristics of typhoid. In accord with the objective of the study, a large nation-wide surveillance program engaging various health institutions can help in understanding the epidemiology of infectious diseases and taking timely preventive steps.

Key Words: Typhoid, Widal test, Infectious disease, Seroprevalence

INTRODUCTION

Typhoid is a systemic infection caused by Salmonella enterica serotype Typhi (*Salmonella typhi*). It is an important cause of morbidity and mortality worldwide, but more specifically in tropical areas. Developing countries bear a high burden of typhoid because of poor sanitation and lack of clean drinking water.

An accurate estimation of typhoid burden in communities is a challenging task especially in developing countries such as India because of multiple factors. Broadly these factors are of two types, firstly because of inherent shortcomings of diagnostic tests and secondly due to external factors associated with diagnosis such as lack of culture facilities, rampant use of over-the-counter antibiotics before patient reach medical care facility which hampers culture yield.

There are multiple diagnostic tests for typhoid including culture (blood, bone marrow, urine, and stool) and serological tests (Tubex, Typhidot, Widal). Every diagnostic test has its own strengths and limitations. Multiple studies compared culture tests with serological tests to estimate sensitivity and specificity. Andualem *et al.*,[1] in a comparative study of Widal test with blood culture reported that Widal has 71.4% sensitivity, 68.4% specificity, 5.7% positive predictive value and 98.9% negative predictive value. High negative predictive value indicates that negative Widal test is good in ruling out disease. A prospective study [2] which compared bone marrow culture with serial blood cultures and agglutination test, found that *salmonella typhi* was recovered from marrow cultures in 95% of patients while blood culture was positive in 43.3 % patients only. Another study by Akoh[3], reported that among 64 proved typhoid patients 44% and 59% yielded *salmonella typhi* on blood and bone marrow culture respectively. Study by Gilman *et al* [4] suggested blood culture as less sensitive when compared to bone marrow culture in isolation of salmonella typhi from typhoid fever patients. Another study[5] among children also reported blood culture as less sensitive (44%) in comparison to bone marrow culture (84%) and stool culture (65%).

The culture tests have a definite advantage in being more precise than Widal in individual diagnosis, still they have operational limitations in resource-poor settings. Bone marrow culture is an invasive procedure and cumbersome to use in primary care settings. Blood culture has limitations in the form of time, cost, and availability.

Serological tests such as Widal need to be explored in depth as a surveillance tool in communities, where conditions do not permit other methods. Despite its limitations, it is among the most commonly used test for diagnosis of typhoid in low-middle income countries. The seroprevalence studies are an underrated tool in exploring the epidemiological behaviour of typhoid. A cross-sectional study[6] in Uganda analysed association between health education and other factors with seroprevalence of typhoid among febrile patients in the semi-urban area. This study estimated a seroprevalence at 26.5% (95 CI: 21.7-32.0) and revealed lower chances of typhoid among those who have received health education in the past two months in comparison to those reported no health education recently.

In our study, we assessed the trend of typhoid by using Widal data from diagnostic laboratories across India. We analysed distribution of agglutinin O and H across various regions.

Objective: To assess the variation in seroprevalence of typhoid with the help of diagnostic laboratories data of Widal test across different regions in India.

METHODOLOGY

A retrospective, observational study is conducted to assess patterns of seroprevalence of typhoid across multiple Indian regions by using diagnostic laboratories data of Widal test.

Sampling and sample size: The analysis has been conducted on retrospective data from diagnostic laboratories and hospitals located in states of Maharashtra, Uttar Pradesh, Delhi, Punjab, Kerala, and West Bengal. For the present study, data was retrieved from diagnostic laboratories located in major metropolitan and tier-II cities in these states. In the study 20 diagnostic laboratories participated - 9 from Maharashtra, 1 each from, Kerala and West Bengal, 3 from Delhi, 2 from Punjab and 4 from Uttar Pradesh and Uttarakhand.

There were 1,01,977 subjects who got tested for typhoid by Widal, between January 2016 and December 2017 in the participating diagnostic laboratories. The Widal diagnostic cut off values vary across regions and different populations. Sensitivity and specificity depend on cut-off values. According to a community-based study in Kolkata, an agglutinin O titre of 1:80 was optimal with 58% sensitivity, 85% specificity, 69% positive predictive value, and 77% negative predictive value.[7] According to other Indian studies,[8] a cut off value for O and H agglutinin titres ≥80 has been considered as significant. Other two studies from Uttarakhand[9] and Maharashtra[10] have considered a level of 80 for O and 160 for the H as significant.

We divided levels of agglutinin titres in five categories to analyse agglutinin distribution:

1. < 40: Titre levels of O and H less than 40
2. ≥40: Titre levels of O and H equal to and more than 40 but less than 80
3. ≥80: Titre levels of O and H equal to and more than 80 but less than 160
4. ≥160: Titre levels of O and H equal to and more than 80 but less than 320
5. ≥320: Titre levels of O and H equal to or more than 320

Following are the two different diagnostic criteria used in the study:

Criteria 1: TO and TH titre ≥ 80 considered as significant

Criteria 2: TO ≥ 80 and TH ≥ 160 considered as significant

Statistical analysis: Descriptive statistics are summarized as mean ± standard deviation for continuous variables. Frequency distribution is presented in form of proportions for categorical variables. Data analysis was done by using MS Excel and R.

Ethical approval: Confidentiality of subjects has been maintained by de-identifying personal information and only anonymised data was used. Ethical approval for the study has been taken from the Max Healthcare Ethics Committee (MHEC), New Delhi.[11]

RESULTS

Total sample size was 1,01,977 with males accounting for 55.1%. Mean age of the sample population was 28 years (Table 1). The largest sample share was from Maharashtra (65%) followed by Uttar Pradesh / Uttarakhand (13%) and Delhi (11%).

Table 1: Descriptive statistics of sample population

Variable	Values
Total sample	1,01,977
Mean age	28 years (SD: 19.8)
Range (age)	2 months – 100 years
Duration	Jan 2016 – Dec 2017
Male proportion	56,140 (55.1%)
State-wise sample distribution	Maharashtra: 66,216 (65%)
	Uttar Pradesh/Uttarakhand: 13,514 (13%)
	Delhi: 10,887 (11%)
	Punjab: 7,257 (7%)
	Kerala: 2,612 (3%)
	West Bengal: 1,491 (1%)

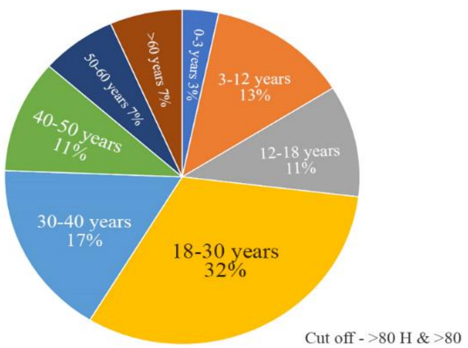
Table 2: Agglutinin 'O' and 'H' distribution in sample population

		Agglutinin 'H' Level					
		<40 (%)	**>=40-80 (%)**	**>=80 – 160 (%)**	**>=160 – 320 (%)**	**>=320 (%)**	**Total (%)**
Agglutinin 'O' Level	**<40 (%)**	66.2	1	1.1	0.9	0	**69.2**
	>=40 – 80 (%)	0.7	12.2	3.2	0.3	0	**16.4**
	>=80 – 160 (%)	0.6	1.3	4.5	2.1	0.1	**8.6**
	>=160-320 (%)	0.5	0.5	1.3	1.6	0.3	**4.1**
	>=320 (%)	0.1	0.2	0.3	0.6	0.5	**1.6**
	Total (%)	**68**	**15.2**	**10.5**	**5.4**	**0.9**	**100**

Table 3: Significant level of titres across different states

Region	**State**	**Positivity rate (≥80 H & O)**	**Positivity rate (≥160 H & ≥80 O)**
North	Delhi	12.30%	4.60%
North	Punjab	26.60%	12.10%
North	Uttar Pradesh/Uttarakhand	18.00%	4.70%
South	Kerala	4.20%	2.10%
East	West Bengal	2.10%	1.40%
West	Maharashtra	3.10%	1.60%

Figure 1: Proportional burden of typhoid across age categories



The Table 2 presents the distribution of H and O agglutinin titres in sample population. Out of the total subjects, 11.3% showed level of O and H agglutinins ≥ 80, while only 3% showed O and H agglutinin titre ≥ 160.

The Table 3 shows estimated typhoid positivity across states. North Indian states (Delhi, Punjab, Uttar Pradesh) have the higher proportion of cases – estimated at 12-27% (at agglutinin level of >80 for H and O). Other states in South (Kerala), East (West Bengal) and West (Maharashtra) show lower proportion of subjects with significant titres, estimated at 2-4%.

The Figure 1 suggests that highest typhoid burden is among young adults (18-30 years) contributing 32% of overall cases, followed by paediatric age group (0-18 years) contributing 27% of total burden.

The Table 4 presents age and state-wise distribution of typhoid. The table shows that states in north India (Delhi, Punjab, and Uttar Pradesh/Uttarakhand) have higher proportion of cases (at agglutinin level of >80 for H and O) among the age group >3-30 years.

The analysis (Table 5) revealed a higher proportion with significant level of agglutinins in paediatric age groups (3-12 and 12-18 years) among males, and in early adult age groups (18-30 and 30-40 years) among females at a cut-off of ≥ 80 for H and O and >160 H, >80 O.

DISCUSSION

Our study estimated seroprevalence at level of ≥ 80 and ≥ 160 for agglutinin 'O' as 14.3% and 5.7% respectively, while for agglutinin 'H' it was 16.8% and 6.3% respectively. The seroprevalence estimates were 11.3% and 3% considering levels of both 'H' and 'O' agglutinin titres together at levels of ≥ 80 and ≥160 respectively. There were 68% and 69.2% subjects with agglutinin titres <40 for 'H' and 'O' respectively.

According to a study conducted in Ethiopia,[12] at agglutinin titres of O ≥ 80 and H ≥ 160, sensitivity and specificity of Widal were found to be 75% and 95.7% respectively among febrile patients, while considering only O ≥80, sensitivity and specificity was 87.5% and 96.5% respectively.

A study by Kulkarni,[13] reported sensitivity and specificity of Widal at 76.7% and 90% respectively, at agglutinin O levels of 1:80, and 70% and 97% respectively, at a level of 1:160. At O titre level of 1:320, sensitivity and specificity were 60% and 100% respectively. These studies showed good sensitivity and specificity of Widal test and also revealed that increase in cut-off titre leads to decreased sensitivity and increased specificity.

A systematic review by Jacob *et al*[14] estimated the prevalence of laboratory-confirmed typhoid among individuals with fever across hospital studies as 9.7% (95% CI: 5.7-16%). A seroprevalence study conducted in Fiji reported seropositivity against Vi antigen of Salmonella typhi as 32.3% (95% CI: 28.2-36.3%) in a sample population comprising vaccinated and unvaccinated individuals.[15] This study also highlighted the role of improved hygiene such as the presence of

Table 4: Distribution of significant titres of H and O across age and state categories

Age category (years)	Delhi (%)	Kerala (%)	Maharashtra (%)	Punjab (%)	Uttar Pradesh/Uttarakhand (%)	West Bengal (%)	Overall (%)
0 – 3	9.1	4.3	3.7	9	10.5	4.9	**7**
>3 – 12	16.7	2.4	5.3	16.4	19.2	5.7	**10.9**
>12 – 18	15.2	7.1	4.4	28.8	18.5	2.3	**12.7**
>18 – 30	14	6.3	2.8	29.9	18.8	1.8	**12.8**
>30 – 40	10	2.9	2.5	31.6	21.3	1.3	**11.8**
>40 – 50	9.1	3.9	2.2	31.7	17.6	1.1	**10.4**
>50 – 60	6.9	1.7	1.7	26	14.9	0	**9.1**
>60	7.7	2.8	1.3	25.2	13.8	1.1	**9.9**

Cut off: >80 H and >80 O titration

Table 5: Age and gender-wise positivity (or significant titres)

Age category (years)	Positivity rate ->80 H & O (%)	Positivity rate - >160 H & >80 O (%)	Positivity rate (>80 H & O)	Positivity rate (>160 H & >80 O)
	Males		Females	
0 - 3	4.60%	1.40%	10.00%	5.00%
>3 - 12	11.80%	4.80%	9.60%	4.60%
>12 - 18	12.60%	5.20%	12.90%	5.50%
>18 - 30	9.50%	3.90%	16.80%	6.00%
>30 - 40	8.30%	3.60%	16.10%	6.10%
>40 - 50	7.00%	3.40%	14.20%	6.00%
>50 - 60	8.10%	2.80%	10.30%	3.70%
>60	9.30%	2.80%	10.70%	6.80%
Overall	**9.30%**	**3.70%**	**13.80%**	**5.60%**

septic tank or piped sewer than pit latrines, residential housing than settlements as important factors in reducing exposure to *Salmonella typhi*. Several other studies[16,17] have highlighted the association between poor sanitation and unclean water with a high incidence of typhoid. Our analysis showed higher seroprevalence in states like Uttar Pradesh where only 35% of households use improved sanitation facility as per the National Family Health Survey-4 (NFHS-4).[18] The seroprevalence was very low in states like Kerala which have 98.1% households with improved sanitation facility. Though this association was not observed in case of Punjab which has high seroprevalence but also a higher proportion of households with improved sanitation facilities (81.5%).

Our findings showed higher seroprevalence in children and young adults and estimated that these age groups (0-30 years) bear 59% of total burden at titre level of $\geq$ 80 for 'H' and 'O'. Studies [9,19] have shown that typhoid is a disease of children and young adults, with the highest rate of significant titres in early age groups (0-30 years). Sinha *et al.*,[20] showed a higher positivity rate in paediatric subjects. It is believed that higher exposure in early ages can be the reason for higher positivity. According to Ochiai *et al.*,[21] there is an inverse relation between typhoid incidence and mean age of cases.

There is a wide variation in reported prevalence in hospital-based studies. On comparison of studies conducted in Delhi[20] and Kolkata,[19] a higher rate of incidence rate is reported in Delhi than Kolkata. Our estimate also shows a higher proportion of subjects with significant titres in Delhi (7.7%) in comparison to West Bengal (1.1%).

This study helped in highlighting the differences in seroprevalence of typhoid across age, sex, and different geographical areas in India, which reflects the effect of environmental factors on the incidence of typhoid. A registry-based study [22] in Kelantan, found sex is associated with typhoid, and females are at higher risk. Our analysis reveals a higher proportion of females (13.8%) with significant titres in comparison to males (9.0%).

A recent data [10] from the Indian Council of Medical Research (ICMR) has given estimates of deaths due to typhoid. Uttar Pradesh shows high mortality (8.6/100000), while Kerala (0.17), West Bengal (1.68), and Maharashtra (3.0) show lesser mortality because of typhoid. These estimates are consistent with our findings of high agglutinin titres in Uttar Pradesh (and other North Indian states) in comparison to Kerala, West Bengal, and Maharashtra.

Limitation: This is a big data-based retrospective analysis and it is not possible to assure the quality of methods employed in Widal testing in different laboratories. There is a wide variation in living conditions and socioeconomic status even in a single state. As this data is from metropolitan and big tier-II cities, it might not be a representation of the overall community in a state. As data from only private laboratories were included, the analysis might be an underrepresentation of population with poor socioeconomic status living in rural and semi-urban areas. It is difficult to ascertain the clinical profiles of the patients and their association with agglutinin titre.

Conclusion: A country-wide analysis is useful to understand the detailed patterns of typhoid as well as regional variations in typhoid epidemiology. This analysis has consolidated several findings such as higher positivity rate in paediatric and young adults.

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