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COMPREHENSIVE ANALYSIS OF SALMONELLA ANTIBODY PREVALENCE IN PATIENTS USING WIDAL TITER AT A TERTIARY CARE INSTITUTION IN NORTH-EAST DELHI

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Abstract— **Introduction: Enteric fever continues to pose a significant public health challenge in India. The detection of enteric fever through laboratory diagnosis relies predominantly on serological tests. Among these tests, the widal test stands out as a cost-effective, readily accessible, and widely employed method for serologically diagnosing enteric fever.**
Objective: This study aimed for comprehensive analysis of Salmonella antibody prevalence in patients using widal titer at a tertiary care institution in north-east Delhi.
Method: This is a cross-sectional observational study, that employed the widal tube agglutination test to 9,665 blood samples over a period of eighteen months (January 2022 to July 2023) for the serodiagnosis of enteric fever.
Results: In a year and a half study, 11% of the population exhibited significant agglutination titre of ≥1:128, indicating exposure to infection. The estimated prevalence of enteric fever by Salmonella Typhi, paratyphi A and B detected by the widal test was 10.98%, 0.54%, and 0.052%, respectively. Notably, two distinct surges in enteric cases were observed during the late winter to early spring and during monsoon. Enteric predominantly affected females (55.7%) compared to males (44.2%). The age group most affected was 11 to 30 years, with a prevalence of 51.9%, followed by young children aged between 1-10 years (30.5%).
Conclusion: Enteric fever remains a significant healthcare concern in India, particularly among the younger demographic. The diagnosis of enteric fever still predominantly relies on serological testing, such as the widal test, in developing nations

Keywords— **Salmonella Typhi**, **widal test, Enteric fever**

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I. INTRODUCTION

Enteric fever, commonly attributed to Salmonella enterica serovar Typhi (S. Typhi), remains significant contributor to the major morbidity and mortality around the globe particularly in developing countries [1].

Enteric fever is an acute, life-threatening febrile illness. It has the potential to cause outbreaks and is primarily transmitted through ingestion of contaminated food or water. The burden of enteric fever exhibits substantial variation both within and among different countries. Factors such as rapid population growth, urbanization, climate change, and growing antimicrobial resistance carry the risk of escalating the prevalence of enteric fever. This poses a significant public health challenge, particularly in economically under-developing nations, especially in countries across Asia and sub-Saharan Africa. The majority of these nations have a large population that faces limited access to safe water, adequate sanitation, and proper hygiene facilities [2,3].Unfortunately, many countries particularly in the African and Asian continent face challenges in providing adequate healthcare and diagnostic facilities, resulting in limited access to quality medical care. Also, lack of population-based surveillance studies results in scarcity and uncertainty of current data on the disease burden in these regions [4]. Prompt and accurate diagnosis, treatment as well as identification of carriers and sources responsible for outbreaks, are crucial measures in managing enteric fever.

According to the World Health Organization (WHO), as of 2019, approximately 9 million individuals experienced enteric feveral illnesses, resulting in around 110,000 fatalities annually [5]. In India, the estimated annual incidence of enteric fever is relatively high, it accounts for more than half the estimated global burden of enteric fever with estimated 360 cases per 100,000 person-years, resulting in approximately 4.5 million cases and a case-fatality rate of 0.2% [6,7]. Over the past decade, there has been a noticeable rise in the incidence of para-enteric fevers, caused by Salmonella enterica serovar Paratyphi A and Salmonella enterica serovar Paratyphi B [1,8]. In certain regions of southern Asia, (India, Pakistan, Nepal, Bangladesh) the proportion of enteric fever cases attributed to S. Paratyphi A currently varies between 20% and 50% [9].

Blood culture remains the gold standard for diagnosis of enteric fever and para-enteric fever, and is most commonly used method, however it's sensitivity is only 40%–60% due to numerous constraints, including the need for a significant blood volume due to low levels of bacteremia, low yield, and the impact of prior antibiotic usage etc [4,10]. Moreover, in developing countries where a single blood sample is typically cultured, the sensitivity of blood cultures diminishes, resulting in their underutilization and suboptimal performance. These factors pose challenges in achieving a definitive diagnosis of enteric fever [11,12].

In resource-limited settings, widal test is often the most readily available alternative tool for diagnosis of enteric fever [13]. Though the diagnostic value of the test has been debated, widal test is still extensively used in developing countries. Since enteric fever is endemic in most of the regions, the widal test serves as a rapid, affordable, and practical tool for diagnosing the disease in such areas. The widal test is based on detecting specific agglutinins (antibodies) in the serum of infected individuals, against the O (somatic) antigens of S. Typhi, H (flagellar) antigen of S. Typhi, Paratyphi A & Paratyphi B [13]. In the present study, the comprehensive analysis of Salmonella antibody prevalence in patients attending various clinics of a tertiary care institution in north-east Delhi was determine by using widal test.'

II. MATERIAL AND METHODS

A. Study area and duration –

A cross-sectional observational study was conducted in a government tertiary care hospital situated in the North-East Delhi, catering to the population from North-East Delhi and NCR region. The study was conducted over a period of eighteen months (January 2022 to July 2023) at serology laboratory, Department of Microbiology, University College of Medical Sciences & associated Guru Teg Bahadur (GTB) hospital, Delhi.

B. Samples processing

A total of 9,665 blood samples for the diagnosis of enteric fever were received. The blood sample was allowed to clot at room temperature. After this serum was obtained by centrifuging the sample at 1500 rpm for 5 min. The supernatant serum was collected for widal test. The widal tube agglutination test was performed by using commercial kit TYPHOCHEK®reagents procured from Tulip Diagnostics (P) LTD. India as per manufacturers instruction.

C. Method

A master dilution (1:16) of test serum was prepared by mixing 0.2 ml of the test serum in 3 ml of normal saline. Four rows of Felix tubes were set for each serum sample to be tested. Serial 2-fold dilutions were made from the master dilution. To each set of serum dilutions, 0.5 ml of antigen (S. Typhi O antigen and H antigens of S. Typhi, S. Paratyphi A and B) was added to achieve final dilution of 1:32, 1:64, 1:128, 1:256 and 1:512 for each set of antigens. The tubes were then incubated at 37° C in a water bath overnight.

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D. Interpretation

The highest dilution at which agglutination was observed (chalk-like deposit for "O" antigen and cotton wool or floating cloud-like deposit for "H" antigen) was considered to be the significant titre (≥1:128 for O antigen & ≥1:256 for 'H' antigen). With each batch of the test, positive control antisera (provided within the kit) and in-house quality control were included.

E. Data analysis

To analyze the data, Microsoft Excel SPSS 2.0 was employed, and the results were interpreted to generate percentages and tables.

III. RESULT

A total of 9665 samples were processed in the study duration, among which, 1062 (10.98%) exhibited a significant agglutination titre (TO ≥1:128 and TH or AH or BH ≥ 1:256). The estimated prevalence of enteric fever cases detected by widal across the study period was 10.98% in the population attending GTB hospital situated in the North-East Delhi region. The prevalence of para-enteric fever A was about 0.54%, and for para-enteric fever B, it was 0.052% among the population.

Figure 1 displays the month wise distribution of samples along with the percentage of positive samples. There was a significant spike in the number of samples received in May 2022 to November 2022 and from March 2023 to July 2023, while the highest rate of positive cases was recorded in January 2023 (17.8%) and July 2023 (17.2%). In the year 2022, the peak was documented in the month of March (13.6%).

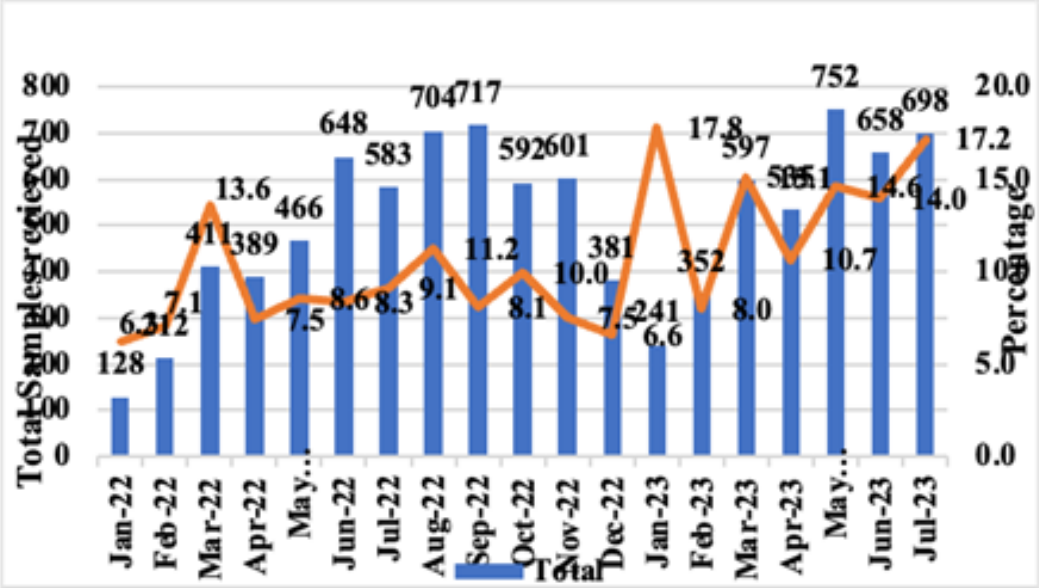

Fig. 1. **Distribution of total and positive samples over study period**

Figure 2 shows the month wise distribution of anti-TO, anti-TH, anti-AH, and anti-BH antibodies directed against he different Salmonella antigens. Throughout the entire study period, the levels of anti-TO antibodies and anti-TH antibodies consistently stayed above 40%. In contrast, the percentages of anti-AH and anti-BH antibodies remained consistently below 12% and 4%, respectively. There was a significant increase in the number of cases due to paratyphoid fever A during the month of April 2022 and June and July 2023 as compared to the rest of the study duration.

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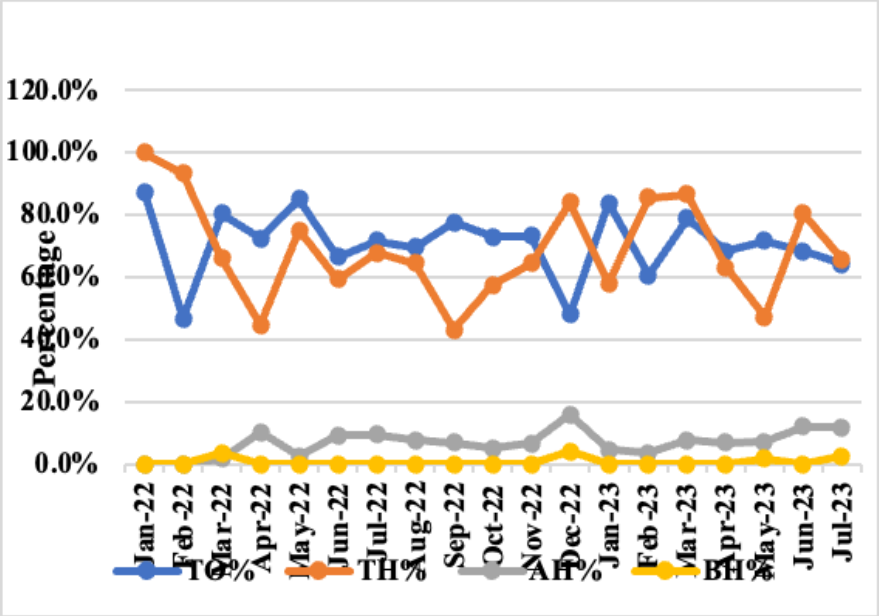

Fig 2: **Month wise distribution of antibodies against Salmonella antigens**

The presence of antibodies against S. Typhi was highest in the positive samples, with 49.3% (758/1537) antibodies against the TO antigen and 44.8% (689/1537) antibodies against the TH antigen. In contrast, the presence of antibodies against the AH antigen was much lower at 5.3% (82/1537) and those for BH were found to be extremely less 0.52% (8/1537). Figure 3 reflects the distribution of antibody titres in the positive sample.

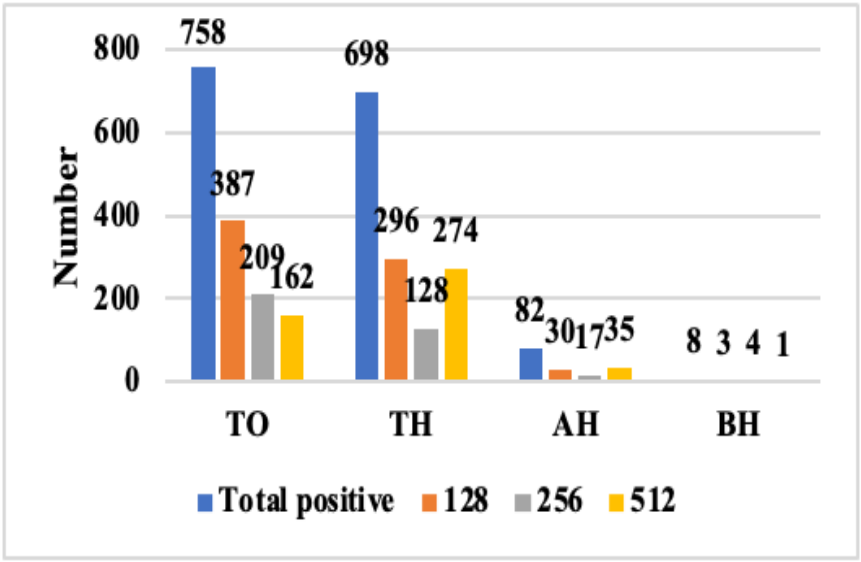

Fig 3: **Distribution of antibodies titre against Salmonella antigens**

Table 1 represents the socio-demographic characteristics of positive cases. The age of the widal positive cases ranged from 3 months to 90 years. Among the different age groups, the most significantly impacted by enteric fever were the younger individuals, aged 11 to 30 years, accounting for 51.9% of the cases followed by 1- 10 year age group accounting for 30.5% of total positive cases. Among positive cases, 44.2% (470/1062) were males, and 55.7% (592/1062) were females. The majority of positive cases, 71%, sought medical attention through outpatient services (OPD), while 29% of cases required inpatient care (IPD).

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Table.1. Socio-demographic characteristics of widal positive cases based on age-group

Age group	Location		Sex		Total
	OPD	IPD	Male	Female	1062
<1 Yr	4 (44.4%)	5 (55.5%)	6 (66.6%)	3(33.3)	9
1-10yr	256 (79%)	68(21%)	164(50.6%)	160(49.4%)	324
11-20yr	218 (71.7%)	86(28.3%)	145(47.7%)	159(52.3%)	304
21-30yr	162 (65.6%)	85(34.4%)	87(35.2%)	160(64.8%)	247
31-40yr	45(59.2%)	31(40.8%)	20(26.3%)	56(73.7%)	76
41-50yr	28 (66.7%)	14(33.3%)	19(45.2%)	23(54.8%)	42
51-60yr	29 (78.4%)	8(21.6%)	16(43.2%)	21(56.7%)	37
61-70yr	11 (64.7%)	6(35.3%)	9(53)	8 (47%)	17
>70yr	2(33.3%)	4(66.7%)	4(66.7%)	2(33.3%)	6

Yr: year

Table 2 depicts the distribution of antibodies categorized by their titres across different age groups. Antibody titre of 1:128 against the TO antigen was seen in 62.3% individuals in the age group 21-30 years followed by 50.2% in the age group 11-20 years and 42.6% in the age group 1-10 years. The TO antibody titres ≥1:512 were seen in 13.8% , 23.6% and 26.5% in the respective age groups (21-30 yrs; 11-20yrs; 1-10yrs).The antibody titre of 1:128 against TH antigen was seen in 51.3% of individuals of age group 21-30 years followed by 38.6% in the age group 11-20 years and 31.2% in the age group 1-10 years. The TH antibody titres ≥1:512 were seen in 27%, 48% and 48.5% individuals in the respective age groups (21-30 yrs; 11-20yrs; 1-10yrs).

Table 2: **Age-group wise distribution of antibodies against Salmonella antigens (n/%)**

Age group	TO				TH				AH				BH			
	128	256	512	Total	128	256	512	Total	128	256	512	Total	128	256	512	Total
<1 yr	6 (75)	1 (12.5)	1 (12.5)	8	1 (25)	0	3 (75)	4	0	0	0	0	0	0	0	0
1-10yr	116 (42.6)	84 (30)	72 (26.5)	272	72 (31.2)	47 (20.3)	112 (48.5)	231	3 (25)	1 (8.3)	8 (66.7)	12	0	2 (100)	0	2
11-20yr	117 (50.2)	61 (26.2)	55 (23.6)	233	75 (38.6)	26 (13.4)	93 (48)	194	9 (33.3)	6 (22.2)	12 (44.4)	27	0	0	0	0
21-30yr	104 (62.3)	39 (23.2)	23 (13.8)	167	78 (51.3)	33 (21.7)	41 (27)	152	10 (40)	6 (24)	9 (36)	25	1 (50)	0	1 (50)	2
31-40yr	24 (57)	13 (31)	5 (12)	42	26 (56.5)	9 (19.5)	11 (24)	46	2 (50)	0	2 (50)	4	1 (33.3)	2 (66.6)	0	3
41-50yr	6 (46.1)	6 (46.1)	1 (7.7)	13	19 (67.8)	5 (17.8)	4 (14.3)	28	2 (33.3)	2 (33.3)	2 (33.3)	6	1 (100)	0	0	1
51-60yr	5 (41.7)	3 (25)	4 (33.3)	12	17 (63)	4 (14.8)	6 (22.2)	27	2 (33.3)	2 (33.3)	2 (33.3)	6	0	0	0	0
61-70yr	6 (66.7)	2 (22.2)	1 (11.1)	9	6 (50)	3 (25)	3 (25)	12	1 (100)	0	0	1	0	0	0	0
>70yr	3 (100)	0	0	3	2 (50)	1 (25)	1 (25)	4	1 (100)	0	0	1	0	0	0	0

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IV. DISCUSSION

The widal test is quick and widely employed diagnostic tool for enteric fever, particularly in developing nations. Widal tube agglutination test was done to ascertain the prevalence of Salmonella antibodies in the study population. This study showed an overall infection rate of 10.98%, suggesting a significant disease burden among patients visiting a tertiary care hospital in North-East Delhi. The incidence of enteric fever (Paratyphoid fever A and Paratyphoid fever B) was noted to be approximately 9.6% lower than for enteric fever.

Various studies across India have reported enteric fever infection rates ranging from 17% to 40%. Singh et al. (2018) found a 23.4% positivity rate in Delhi, while Tewari et al. (2020) reported 38.6% in Meerut, Uttar Pradesh [14,15]. Chauhan et al. (2019) recorded 17.6% positivity in another study in Uttar Pradesh and Shinde et al. (2021) noted 32% positivity among blood donors in Maharashtra [16,17]. Bahl et al. (2021) observed 32% in Jammu district [18]. Shethwala et al. (2019) saw a reduction in enteric fever prevalence from 62% to 22.5% over a decade, while John et al. (2016) estimated that laboratory-confirmed enteric fever and paratyphoid fever prevalence among individuals with fever in Indian hospitals as 9.7% and 0.9%, respectively which aligns with the findings of our study [3,19].

Throughout the study duration, the percentage of antibodies against S. Typhi (anti-TO and anti-TH antibodies) consistently remained above 40% at any given time. Within the study population, the highest titres of anti-TO antibodies were predominantly found to be at 1:128, whereas, for anti-TH and anti-AH antibodies, the majority of elevated titres exceeding 1:512 were observed. Khan et al. (2020) reported similar results, where they found that the percentages of anti-TO and anti-TH antibodies remained consistently above 45%. Notably, they observed higher antibody titres for anti-TH in comparison to anti-TO antibodies [20].

We observed two distinct surges in enteric fever cases, manifesting during the transitional periods from late winter to early spring (January to March 2023) and in monsoon season (July 2023). Whereas not much significant seasonal variation for para-enteric fever was seen over the study duration. Similar to our study, Singh et al. (2018) observed the widal test positivity rates began to increase during the summer months and further escalated during the monsoon season [14]. The seasonal transition to warmer temperatures promotes bacterial growth while the high humidity and heavy rainfall during the monsoon, favours the survival and growth of bacteria. The heavy rainfall can lead to flooding, thereby causing contamination of water sources and potential increased risk of enteric fever transmission explaining the surge observed in our study.

The female population was more affected by enteric fever compared to males. In concurrence with our observations, Wam et al. (2019) found that 64.3% of those affected were females, while 35.7% were males, mirroring findings by Shubbar et al. (2020) [21, 22]. In many societies, particularly in India, females play an active role in food preparation and caregiving, subsequently increasing their exposure to contaminated water and food items leading to possible transmission of enteric fever causing agents, exposing females to these common routes of enteric fever transmission as compared to males.

Among the analysed age groups, the younger individuals aged 11 to 30 years were notably the most affected age group. Abioye et al. (2017) reported the highest prevalence of enteric fever, at 86.36%, among individuals aged 21 to 25 years [23]. The increased susceptibility of young individuals can be attributed to more social activities, more consumption of street food and a probable less cautious approach to hygiene. In the study, second most affected age group consisted of children aged 1 to 10 years accounting up-to 30.5% of the cases. Arya et al. (2019) found that most cases of enteric fever in children were between 5 to 10 years old, making up 50% of the cases. Additionally, during the course of our study, there was an observed enteric fever prevalence of 0.85% among infants [24]. The immature immune system and no previous exposure to enteric fever or vaccination leave the young children with limited to almost no immunity against the disease. They often have tendency to put contaminated objects or hands in their mouths, increasing their risk of exposure to the possible etiological agents of enteric fever.

This study offers significant insights into the antibody titres for enteric fever and paratyphoid fever among different age groups and across different seasons. This laboratory-based data analysis holds great importance in comprehending the epidemiological traits of enteric fever in and around North-Eastern Delhi, India. In a country like India where enteric fever is widespread, the widal test is a commonly used method for diagnosing he disease. While this test is convenient and quick, it remains a valuable tool in resource limited settings where more advanced diagnostic methods may not be readily available. This study had limitations in terms of the lack of correlation between the widal test and blood culture, as well as the utilization of a single sample instead of paired samples.

V. CONCLUSION

Enteric fever continues to be a substantial health challenge in India, especially among the younger population. In developing countries like India, the ability to isolate the causative pathogen is frequently compromised due to insufficient facilities or the rampant use of antibiotics before attempting culture. Additionally, culture-positive cases are infrequent and require a significant amount of time. Consequently, the laboratory diagnosis of enteric fever continues to depend largely on serological tests, such as widal tests. Gaining insight into disease prevalence and seasonal variations in the area is essential for enhancing disease management and implementing more effective and timely preventive interventions.

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Conflict of interest: None.

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