

An epidemiological study to estimate the baseline titres of Widal test in apparently healthy children

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Abstract

Background: Enteric fever is one of the common infectious diseases of humans. It is a major health problem in developing countries and its diagnosis on clinical ground is difficult. Diagnosis in developing countries is still mostly done by Widal test. However, the value of the test has been debated. The following study was undertaken to determine the baseline Widal titre (the titre of the antibodies to the O and the H antigens of *S. typhi* and to the H antigens of *S. paratyphi A* and *B*) in normal healthy children. **Materials & Methods:** A total of 300 children satisfying the eligibility criteria were taken in the study after informed consent from their parents. A detailed history was taken from each participant with special reference to source of water and method used for purification. Participants were classified into various socio-economic classes as per modified Kuppuswami classification. Widal titres were done with tube agglutination method for all the patients. A Widal antigen kit (antigen suspension of salmonella enterica serotype typhi, paratyphi A and B) was used. Venous blood sample was collected from each participant; left to clot for 15 minutes in the room temperature then sera was separated by using micropipette. The separated sera was properly labeled and stored in -20°C for further study. **Results:** Most of the study subjects were between 6- 10 years of age with mean age of 7.8 +/- 3.2 years. More males were observed among study subjects with male to female ratio of 2:1 (67% males vs 33% females). Over half of the subjects (58.7%) were from middle socio-economic class as per modified kuppuswami classification. About one third were from lower class while only 7% were from upper class. Out of the total 300 subjects, 37% of the subjects were not using any purification method for water and were directly consuming water supplied from municipal sources. Positive agglutination in Widal test i.e titre \geq 1:20 was seen in 59% of the healthy subjects. Positive agglutination in Widal test was observed to be lowest among under-fives as compared to older children (46% vs 62%), but the results were not significant (p=0.06). No significant association was observed between positive agglutination in Widal test and gender (p=0.31). **Conclusion:** It is clear that Salmonella agglutinins are common among apparently healthy people and as endemicity of typhoid in an area may change over time, more studies should be carried out to determine Salmonella agglutinin titre in apparently healthy populations, so that a better judgment which is based on the prevailing agglutinin titres can be made.

Keywords: Typhoid fever, children, epidemiological study, Widal test, water purification

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Introduction

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Typhoid fever is an infectious disease caused by **Salmonella typhi**. Less commonly, strains of non-typhoidal *Salmonella* usually cause intestinal infections associated with diarrhoea, fever, and abdominal cramps that often last 1-week or less commonly longer, non-typhoidal *Salmonella* can cause extra intestinal infections such as bacteraemia and urinary tract infection [1]. In India, though *Salmonella enterica* serotype typhi remains the predominant *Salmonella* species causing enteric fever [2]. Enteric fever is an endemic disease in the tropics and sub-tropics primarily affecting children and young adults whereas in high-income countries it is mainly a disease of returning travelers [3]. The disease is most commonly acquired by ingestion of water and food contaminated with feces or urine of carriers. Human beings are the only reservoir host for enteric fever [4]. The gold standard for diagnosis of enteric fever rests on the recovery and identification of the causal organisms, from blood during the first few days of the illness, or from faeces during the second and third weeks of the illness or from urine during the third and fourth week [5]. Diagnosis of enteric fever may be delayed or overlooked and patients without enteric fever may receive unnecessary and inappropriate antimicrobial treatment due to the heavy dependence of rapid diagnosis using clinical features and serological methods. The detection of specific antibody response is only suggestive of enteric fever but not definitive [6, 7]. The Widal test is an agglutination reaction demonstrating the presence of lipopolysaccharide (LPS) somatic (O) and flagella (H) agglutinins to *Salmonella Typhi* in the serum of a patient using suspensions of O and H antigens. The test has been widely used for over a century for the diagnosis of typhoid fever [8]. The interpretation of the Widal test depends upon the baseline titre which is prevalent amongst the healthy individuals in a particular geographical area. The Widal titres among the healthy populations of different areas differ substantially and this depends upon the endemicity of typhoid in each area, which has been changing over time. Updating the baseline Widal titre is mandatory for the proper interpretation of the Widal test [9-11]. Hence, the

following study was undertaken to determine the baseline Widal titre (the titre of the antibodies to the O and the H antigens of *S. typhi* and to the H antigens of *S. paratyphi A* and *B*) in normal healthy children. The baseline titer calculation for widal test among apparently healthy children in a locality will give good information to categorize and treat the infected individuals in that particular locality and also this may be considered as the first test to screen out the patient and thus preventing unnecessary exposure to antibiotics among children. This baseline titer should also be monitored on regular intervals.

Material and methods

A hospital based prospective observational study at Department of Paediatrics, Dr. Babasaheb Ambedkar Memorial Central Railway Hospital, Byculla, Mumbai between June 2014 to May 2016. Consecutive type of non-probability sampling was used for selection of study subjects. A total of 300 children satisfying the eligibility criteria were taken in the study after informed consent from their parents. Patil AM et al. [12] conducted cross sectional study in Devengere on 250 healthy children to determine baseline widal titre in year 2007. Gunjal SP et al. [13] conducted cross sectional study in Ahmednagar to determine baseline widal titre on 103 healthy individuals from 2011 to 2013. Ibekwe AC, et al. [14] studied 200 normal healthy population in Nigeria in 2008.

Inclusion criteria

1. Children with afebrile illness coming to the hospital
2. Apparently healthy children.
3. Age 1-12 years of age

Exclusion criteria

1. Persistent fever > 7 days in past 6 months
2. Febrile Patients
3. Children with grade iii, iv, malnutrition (IAP)
4. Children with immunocompromised status
5. Vaccinated with any available typhoid vaccine

A detailed history was taken from each participant with special reference to source of water and method used for purification. Participants were classified into various socio-economic classes as per modified Kuppaswami classification. Widal titres were done with tube agglutination method for all the patients. A Widal antigen kit (antigen suspension of *salmonella enterica* serotype typhi, paratyphi A and B) was used. Venous blood sample was collected from each participant; left to clot for 15 minutes in the room temperature then sera was separated by using micropipette. The separated sera was properly labeled and stored in -20°C for further study. The Widal test was

performed with serial serum dilutions: 1:20, 1:40, 1:80, 1:160 and 1:320 by using isotonic normal saline. Approx. 0.5ml of each of the antigen suspension was added to corresponding tubes, mixed well and incubated at 37^o C for 24 hours. The tubes were then examined visually for agglutination. A 50% agglutination is considered and recorded as the titer of antibodies present in the individual against salmonella.

Results

Table 1: Age and gender distribution among study subjects

Age group (yrs)	N	%
<= 5	63	21.0%
6 to 10	145	48.3%
> 10	92	30.7%
Gender		
Male	201	67.0%
Female	99	33.0%
Total	300	100.0%

Most of the study subjects were between 6-10 years of age with mean age of 7.8 +/- 3.2 years. In our study, numbers of males were more than females with male to

All the collected data was entered in Microsoft Excel sheet and then transferred to SPSS software ver. 21 for analysis. Qualitative data was presented as frequency and percentages and analyzed using chi-square test of fisher's exact test (in case of 2x2 contingency tables). Quantitative data was presented as mean and SD. P-value < 0.05 was taken as level of significance.

female ratio of 2:1 (67% males' vs 33% females) [Table 1].

Table 2: Distribution of subjects based on Socio-economic class

Socio-economic Class	N	%
Upper	21	7.0%
Middle	176	58.7%
Lower	103	34.3%
Total	300	100.0%

Over half of the subjects (58.7%) were from middle socio-economic class as per modified Kuppaswami

classification. About one third were from lower class while only 7% were from upper class [Table 2].

Table 3: Distribution of subjects based on water purification method used

Water Purification method Used	N	%
No	111	37.0%
Yes	189	63.0%
Total	300	100.0%

Out of the total 300 subjects, one or the other method of water purification was used in two third of the homes (63%) while 37% of the subjects were not using

any purification method for water and were directly consuming water supplied from municipal sources i.e. tap water [Table 3].

Table 4: Distribution of subjects based on for agglutination in Widal test

Widal Status	N	%
Agglutination (>= 1:20)	177	59.0%
No agglutination (< 1:20)	123	41.0%
Total	300	100.0%

Agglutination in Widal test with titre \geq 1:20 was seen in 59% of the healthy subjects [Table 4]. Positive agglutination in Widal test was observed to be lowest among under-fives as compared to older children (46% vs 62%), but the results were not significant (p=0.06).

The results can be attributed to the relatively lower frequency of eating outside food by under-fives, which makes one prone to salmonella infection. No significant association was observed between positive agglutination in Widal test and gender (p=0.31).

Table 5: Association between agglutination in Widal test and use of water purification method

Any purification method used for drinking water	Widal		Total
	Agglutination ($\geq 1:20$)	No Agglutination ($< 1:20$)	
No	78	33	111
	70.3%	29.7%	100%
Yes	101	88	189
	53.4%	46.6%	100%
Total	177	123	300
	59%	41%	100%

p- value < 0.05

A significant association was observed between agglutination in widal test and consumption of tap water supplied from municipal sources. Agglutination ($\leq 1:20$) in Widal test was observed in 70.3% such subjects as compared to 53.4% in those using any water purification method ($p < 0.05$) [Table 5].

Table 6: Association between agglutination in Widal test and socio-economic status

SES	Widal		Total
	Positive Agglutination($\geq 1:20$)	Negative Agglutination($< 1:20$)	
Upper	3	18	21
	14.3%	85.7%	100%
Middle	101	75	176
	57.4%	42.6%	100%
Lower	73	30	103
	70.9%	29.1%	100%
Total	177	123	300
	59%	41%	100%

p- value < 0.05

A significant association was observed between positive agglutination in widal test and lower social-economic class. Positive agglutination in widal test was observed in 70.9%, 57.4% and 14.3% subjects belonging to lower, middle and upper social class respectively ($p < 0.05$) [Table 6]. The agglutinins to *S. typhi* were the most prevalent among the sera of various dilutions (44.7% for the H antigen and 36.3% for the O antigen) which were tested. The levels of the agglutinins for *Salmonella paratyphi* AH and paratyphi BH were low (only 4.3% and 2% for the AH and the BH antigens respectively).

Table 7: Distribution of subjects as per *S. Typhi* – O antigen titres

<i>S. Typhi</i> O	N	%
1:20	27	9.0%
1:40	74	24.7%
1:80	6	2.0%
1:160	2	0.7%
Total	109	36.3%

Salmonella enterica serotype, Typhi showed that 9% sample had a titre of 1:20, 24.7% had a titre of 1:40 and 2% had a titre of 1:80, while only 2 samples (0.7%) had the titre of 1:160 for O antigen [Table 7/ Fig. 1].

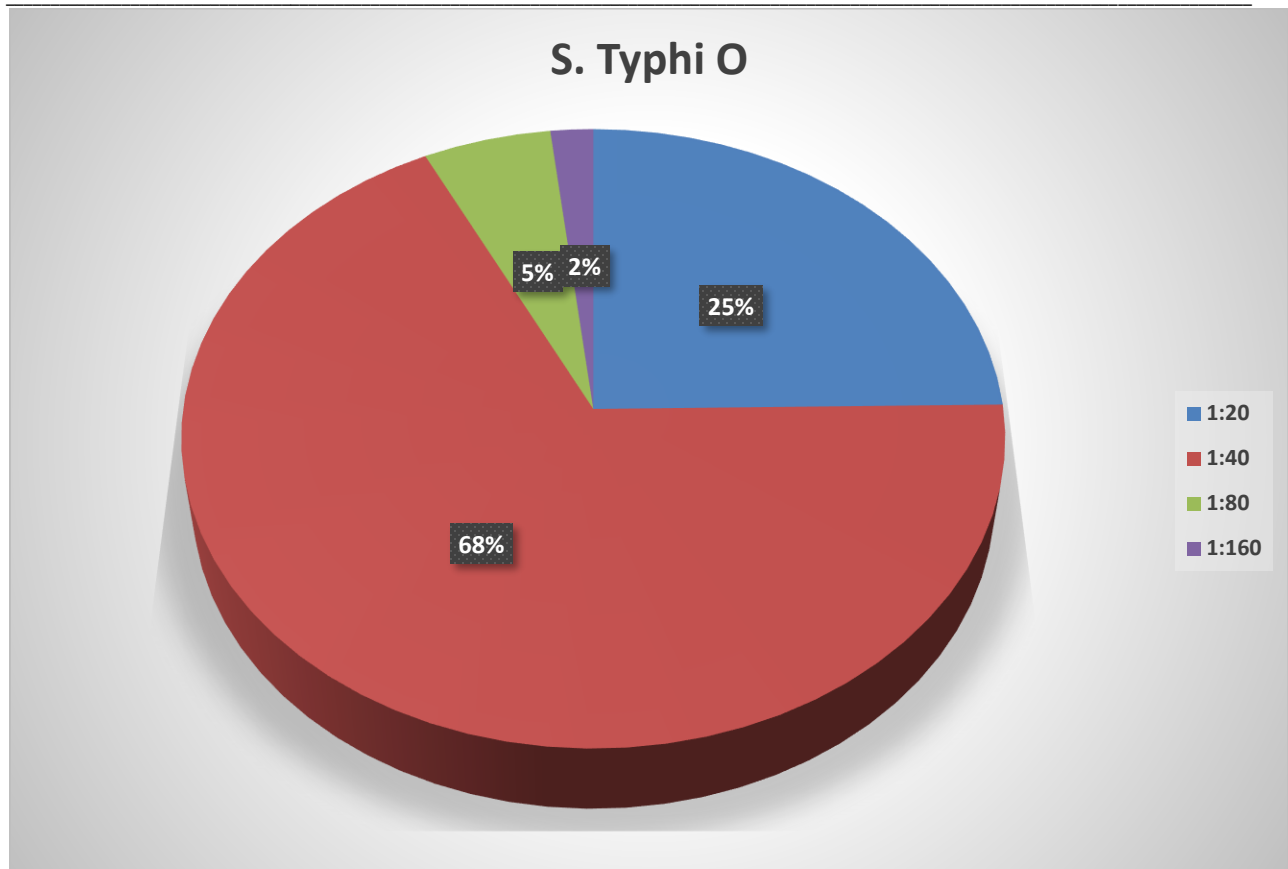


Fig 1: Distribution of subjects as per S. Typhi – O antigen titres

Table 8: Distribution of subjects as per S. Typhi – H antigen titres

S. Typhi H	N	%
1:20	30	10.0%
1:40	13	4.3%
1:80	81	27.0%
1:160	10	3.3%
Total	134	44.7%

Among the 134 samples with anti-H titre of $\geq 1:20$ to the Salmonella enterica serotype, Typhi, 30 samples (10%) had a titre of 1:20, 13 samples (4.3%) had a titre of 1:40 and 81 samples (27.0%) had a titre of 1:80. The titre of 1:160 was found in 10 samples (3.3%) [Table 8].

Table 9: Distribution of subjects as per S. Paratyphi A– H antigen titres

S. Paratyphi A - H	N	%
1:20	10	3.3%
1:40	2	0.7%
1:80	1	0.3%
1:160	0	0.0%
Total	13	4.3%

Altogether, 13 samples (4.3%) showed an agglutination titre of $\geq 1:20$ against the H antigen of the Salmonella enterica serotype, Paratyphi A, among which

10 samples (3.3%) had a titre of 1:20 and 2 samples (0.7%) had a titre of 1:40. Only 1 sample (0.3%) had an anti-H titre of 1:80 [Table 9].

Table 10: Distribution of subjects as per S. Paratyphi B– H antigen titres

S. Paratyphi B - H	N	%
1:20	6	2.0%
1:40	0	0.0%
1:80	0	0.0%
1:160	0	0.0%
Total	6	2.0%

A total of 6 samples (2%) showed an agglutination titre of $\geq 1:20$ against the H antigen of the Salmonella enterica serotype, Paratyphi B, and all of them had an anti-H titre of 1:20 [Table 10].

Discussion

A hospital based prospective observational study was conducted with the aim of determining the distribution pattern of agglutinating antibodies to Salmonella enterica serotype: typhi, paratyphi A and paratyphi B antigens in normal healthy individuals and individuals with afebrile illness in pediatric population coming to our hospital. Most of the study subjects were between 6- 10 years of age with mean age of 7.8 +/- 3.2 years. In our study, numbers of males were more than females with male to female ratio of 2:1 (67% male vs 33% females). Positive agglutination in Widal test was observed to be lowest among under-fives as compared to older children (46% vs 62%), but the results were not significant (p=0.06). The results can be attributed to the relatively lower frequency of eating outside food by under-fives, which makes one prone to salmonella infection. While, no significant association was observed between Positive agglutination in Widal test and gender (p=0.31). A significant association was observed between Positive agglutination in Widal test and consumption of tap water supplied from municipal sources and with lower social-economic class. Enteric fever has continued to pose serious challenge to public health especially in economically developing and under developed countries, where level of hygiene is below standards and sanitary conditions are poor [15, 16]. Ajayi et al in their study observed the influence of water source on typhoid fever prevalence in the study areas. The study revealed that patients who sourced their water from well had the highest frequency (76%) of typhoid fever while the least frequency (3%) was obtained from patients using treated water [17]. Similarly in another study by Udeze et al., significant titre of antibody to salmonella was detected among subjects who drank sachet water while the least was detected in subjects drinking treated water [18].

S. Typhi Agglutination

The agglutinins to S. typhi O and H were the most prevalent among the sera which were tested at various

dilutions (36.3% and 44.7%). This is in accordance with the previous studies done by Pokhrel et al., Peshattiwar P et al., Pal et al. and Aruni et al. [19-22]. The most frequently recorded titre of the reactive samples for “O” agglutinins were found to be at 1:40 and this was considered as the cut off titre, as 24.7% of the study population had provided samples which were reacting at this level. For the “H” agglutinins, we observed that a majority of the study population (27%) had a titre of 1:80 and this was taken as the cut off titre. This study concludes that the current baseline titre for the diagnosis of typhoid fever in the Mumbai region is 1:40 for the anti-O agglutinins and that it is 1:80 for the anti-H agglutinins. Based on this finding, we propose laboratory guidelines for the H and O agglutinin Widal titres of 1:160 and 1:80 as being of diagnostic significance.

S. Paratyphi Agglutination

The agglutinins to S. paratyphi A & B - H were present in 4.3% and 2% of the sera which were tested at various dilutions. This is in accordance with the previous studies done by other authors [19-22]. The baseline anti-H agglutinin titre of the paratyphoid A and B groups was found to be 1:20 in 3.3% and 2% of the healthy population respectively, which suggested that the paratyphoid groups were less prevalent in this area as compared to S. typhi. Our results were in concordance with those of the studies which were reported by some researchers in other endemic states of India [12, 23, 24]. For the anti-TH antibodies, an agglutinin titre of up to 1:80 was discovered in the apparently healthy study population, whereas for the anti-TO antibodies, our result was lower (a titre of 1:40), which was in contrast to the reports other workers [12, 20, 23, 24] but it was in agreement with the reports of some previous studies [9, 20]. Several factors may have contributed to this discrepancy, because the differences in the antibody response may be due to the poorly standardized antigen preparation and the sharing of the antigen determinants with other Salmonellae [20]. A widespread antibiotic abuse can dampen the antibody response, giving a low titre in the Widal test and a previous immunization with the TAB vaccine and technical differences may be the other contributory factors. With respect to the last point, the

Widal test which was performed on the same serum specimen in four laboratories gave widely different results [9]. The countries which have enough resources to support highly developed national typhoid fever surveillance systems generally experience a low incidence of typhoid fever as compared to the developing countries and they contribute little to the global burden of typhoid fever [25, 26]. Proper hygiene and sanitation are the keys to a low prevalence of

enteric fever in the developed countries, which can result in a low antibody titre [27]. It has been evident from the various studies which have been conducted across our country that the baseline titre is subject to variations, depending on the geographical area and the sanitary conditions of the region. Hence, the baseline titre of a particular area should be known. The probable reason for the low titre in our study could be the better health and hygiene conditions.

Table 11: Comparison of Widal titre among different parts of India

Author	Place	Baseline Titre			
		Typhi-O	Typhi-H	Paratyphi - AH	Paratyphi -BH
Shukla S et al. [24]	Central India	1:80	1:80	<1:20	<1:20
Punia JM et al [23]	Chandigarh	1:80	1:160	1:20	1:20
Patil AM [12]	Karnataka	1:80	1:80	1:40	1:40
AJ Sneha [28]	Pondicherry	1:80	1:80	1:40	1:40
Pal et al. [21]	Assam	1:40	1:80	1:20	1:20
Gunjal et al. [13]	Maharashtra	1:40	1:40	1:80	1:80
Aruni et al. [22]	Kerala	1:40	1:80	1:40	1:40
Peshattiwari et al. [20]	Andhra Pradesh	1:40	1:40	1:20	0
Kogekar et al. [29]	Madhya Pradesh	1:20	1:80	1:20	1:20
Madhusudan et al. [30]	Karnataka	1:40	1:80	1:40	1:40
Bijapur GAM et al. [31]	Kerala	1:40	1:40	<1:20	<1:20
Present Study	Mumbai	1:40	1:80	1:20	1:20

Several studies have highlighted the limitations of using the Widal serological test in the laboratory diagnosis of Salmonella, the worst being its non-specificity. Despite this fact, considering the low cost and the absence of comparatively cheap tests, the Widal tube agglutination test is likely to remain the test of choice in many developing countries, as of ours, provided a baseline antibody titre of healthy individual in the population, is known.

Conclusion

Widal titres were done with tube agglutination method for all the patients. The Widal test was started with serial serum dilutions: 1:20, 1:40, 1:80, 1:160 and 1:320 by using isotonic normal saline. A 50% agglutination is considered and recorded as the titre of antibodies present in the individuals against Salmonella. Most of the study subjects were between 6- 10 years of age with mean age of 7.8 +/- 3.2 years. More males were observed among study subjects with male to female ratio of 2:1 (67% males vs 33% females). Over half of the subjects (58.7%) were from middle socio-economic class as per modified kuppuswami classification. About one third were from lower class while only 7% were from upper class. Out of the total 300 subjects, 37% of the subjects were not using any purification method for water and were

directly consuming water supplied from municipal sources. Positive agglutination in Widal test i.e titre \geq 1:20 was seen in 59% of the healthy subjects. Positive agglutination in Widal test was observed to be lowest among under-fives as compared to older children (46% vs 62%), but the results were not significant ($p=0.06$). No significant association was observed between positive agglutination in Widal test and gender ($p=0.31$). A significant association was observed between positive agglutination in Widal test and consumption of tap water supplied from municipal sources. A significant association was observed between positive agglutination in Widal test and lower social-economic class. The agglutinins to *S. typhi* were the most prevalent among the sera of various dilutions (44.7% for the H antigen and 36.3% for the O antigen) which were tested. The levels of the agglutinins for Salmonella paratyphi AH and paratyphi BH were low (only 4.3% and 2% for the AH and the BH antigens respectively). Salmonella enterica serotype, Typhi showed that 9% sample had a titre of 1:20, 24.7% had a titre of 1:40 and 2% had a titre of 1:80, while only 2 samples (0.7%) had the titre of 1:160 for O antigen. Among the 134 samples with anti-H titre of \geq 1:20 to the Salmonella enterica serotype, Typhi, 30 samples (10%) had a titre of 1:20, 13 samples (4.3%) had a titre of 1:40 and 81 samples (27.0%) had a titre of 1:80. The

titre of 1:160 was found in 10 samples (3.3%). Altogether, 13 samples (4.3%) showed an agglutination titre of $\geq 1:20$ against the H antigen of the *Salmonella enterica* serotype, Paratyphi A, among which 10 samples (3.3%) had a titre of 1:20 and 2 samples (0.7%) had a titre of 1:40. Only 1 sample (0.3%) had an anti-H titre of 1:80. A total of 6 samples (2%) showed an agglutination titre of $\geq 1:20$ against the H antigen of the *Salmonella enterica* serotype, Paratyphi B, and all of them had an anti-H titre of 1:20. The baseline titre for antibodies to "O" and "H" antigens of *Salmonella enterica* serotype typhi was 1:40 and 1:80. Hence, based on the above results, it could be recommended to use a cut-off level of 1:80 for the S. Typhi anti-O antibodies and of 1:160 for the anti-H antibodies as diagnostic for enteric fever in the study area. Similarly, baseline titre for antibody to H antigen of *Salmonella enterica* serotype paratyphi A and paratyphi B was 1:20 and the cut-off level was $\geq 1:40$ for a single antibody test titre. It is clear that *Salmonella* agglutinins are common among apparently healthy people and as endemicity of typhoid in an area may change over time, more studies should be carried out to determine *Salmonella* agglutinin titre in apparently healthy populations, so that a better judgment which is based on the prevailing agglutinin titres can be made.

Recommendations

Baseline Widal titre study should be repeatedly done in different areas with different basic sanitation facility (both in urban and rural settings) and readings should be available to the clinicians so that evidence based medicine practice can ensue. Empirical treatment with antibiotics should not be given as it is increasing drug resistant in childrens. Blood C/S for S. typhi should be sent in all suspected cases of typhoid before the first dose antibiotics preferably. In addition to being gold standard test, it gives sensitivity to antibiotics and will help to treat MDR typhoid early and also will prevent complications of ineffective antibiotic therapy. Parents/primary caretakers should be sensitised to use good and safe sanitation practices in day to day activities. Boiling water before consumption is considered as best to prevent water borne diseases. Government should ensure safe drinking water practices in the population and it should provide sufficient funds for proper purification of water.

Limitations

The study sample was hospital based and not community based. Majority of railway population

enrolled in the study were urban population. Sample size was limited and a more extensive study would be more informative.

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