



Original Research Article

Determination of baseline “Widal titre” amongst apparently healthy individuals at tertiary health care center



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ABSTRACT

Enteric fever is endemic in all parts of India. The term, ‘enteric fever’ includes typhoid fever which is caused by *S. typhi* and paratyphoid fever which is caused by *S. paratyphi A, B* and *C*. However, in countries like India, isolation of organism is often jeopardized by lack of facilities or inadequate and/or improper antibiotic use prior to culture and also, culture positive cases are very less and time consuming. Laboratory diagnosis of enteric fever still relies on serological tests such as the Widal test [2,3]. The test becomes reliable if at least two properly staged tests show about a four-fold rise in antibody levels [4]. Often specific antibiotics are administered based on the single Widal test. Interpretation of a single Widal test result needs to be based on the average baseline antibody titre which is seen among healthy individuals in the local area. So we aimed to find out the baseline antibody titer in our area. A single blood sample is collected from the healthy blood donors coming to the blood bank. Widal test is performed by tube agglutination method. The baseline titre found in our area for TO, TH, AH, and BH are 1:40, 1:40 & 1:20 respectively.

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1. Introduction

The bacteria genus *Salmonella* is bacilli that can infect the intestines of a large number of vertebrate species including human beings. Clinically the bacterial genus *Salmonella* leads to enteric fever, gastroenteritis, septicemia with or without focal suppuration and also carrier state. The most important species amongst the genus *Salmonella* is *Salmonella typhi*. is a causative agent of typhoid fever.

Salmonella presently comprise above 2000 serotypes or species and all of them are potentially pathogenic. Typhoid and paratyphoid bacilli that are exclusively or primarily human parasites and causative agents of enteric fever. Non typhoidal species of *Salmonella* can cause gastroenteritis, septicemia or localised infections in humans.

The term enteric fever includes typhoid fever caused by the species *Salmonella typhi* and paratyphoid fever caused by the species *Salmonella paratyphi A*, *Salmonella paratyphi B*, and *Salmonella paratyphi C*. Paratyphoid fever

is a very similar illness to typhoid fever, but usually far less severe.¹

Typhoid fever was prevalent all over the world and was not well demarcated from other unknown causes of pyrexia.

A detailed study of the disease was presented by the scientist named Bretonneau in the year 1826. He had identified the intestinal lesions.

Infection acquired by ingestion.² Person-to-person spread by fecal-oral route by ingestion of food or water contaminated with human excreta.³ In human ID50 is about 10³ to 10⁶ bacilli.⁴ Once the bacteria reaches to small intestine, they attach to microvilli of the ileal mucosa and penetrate to the lamina propria and submucosa. They are phagocytosed by macrophages and polymorphonuclear cells. Major virulence factor of the bacteria is their resistance to intracellular killing and their ability to multiply within these cells. Bacteria can enter in lymphatic circulation and blood circulation (transient bacteremia). Then they can be seeded in the liver, gall bladder, spleen, bone marrow, lymph nodes, lungs and kidneys. Bacteria can further multiply in these organs. After an incubation period of 10–14 days, fever,

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malaise, headache, constipation, bradycardia, and myalgia occur. The fever rises to a high plateau, and the spleen and liver become enlarged.⁵

Typhoid fever is virtually eliminated in the developed countries due to improvement in sanitation and water supply, but still the disease is endemic in the developing countries because of limited resources. Paratyphoid fever is not controlled successfully. Enteric fever is endemic in all the parts of India. An incidence of 500-980 per 100,000 population has been reported in different studies varying with age and geographical area. The proportion of typhoid to paratyphoid A is about 10:1. Paratyphoid B is rare and C very rare.

Typhoid fever can occur in two epidemiological types. One is “endemic typhoid” that occurs throughout the year though seasonal variations can occur. The second is “epidemic typhoid” which can occur in endemic or non-endemic areas.

Bacteriological diagnosis of enteric fever can be done by isolation of bacteria from the various clinical specimens of the patient and demonstration of antibodies in patient's serum. Definitive diagnosis of enteric fever depends on isolation of *Salmonella* from blood, stool, urine, bone marrow, bile or other body fluids.^{6–8}

The Widal test, serological test which was developed by Georges Fernand Isidore Widal in 1896, is an alternative to the microbial culture, which is commonly used for the diagnosis of enteric fever ever since its introduction 100 years back.⁹

However, in countries like India, the bacterial culture facilities are often unavailable or limited to teaching hospitals and accredited laboratories. For these reasons, laboratory diagnosis of enteric fever relies heavily on serological tests such as the Widal test.^{10,11}

Widal test is easy to perform but testing method has some limitations, due to low antibody titre present in some individuals that react with *Salmonella* antigens. Cross reactivity with other *Salmonella* species may occur and the test cannot differentiate between recent and a past infection or if person is vaccinated for enteric fever. The test becomes reliable if at least two properly staged tests show about a four -fold rise in antibody levels.¹² In India, most of the patients present late to the hospital and they require an immediate diagnosis and a specific treatment and often, a single sample has to be relied upon, instead of paired serum samples.¹³ Single cutoff value is widely used for diagnosis.¹⁴

Widal test can be used as a diagnostic tool in endemic areas, if we know the baseline titre in a population. Interpretation of a single Widal test result needs to be based on the average baseline titre which is seen among healthy individuals. The planned study therefore, will be undertaken to establish the normal baseline titre of the antibodies to the ‘O’ and ‘H’ antigens of *Salmonella typhi* and the ‘H’ an

tigens of *Salmonella paratyphi* A and B amongst apparently healthy individuals and also to determine cutoff values for the Widal test.

2. Materials and Methods

The study was conducted over a period of one year from January 2017 to December 2017 in the Department of Microbiology, GMERS Medical College, Himmatnagar, Gujarat, India.

The test was done on 400 voluntary blood donors of both sexes. All the individuals are > 18 years of age. All the donors who were previously vaccinated against typhoid fever, those who were previously diagnosed with typhoid fever or those who gave a history of fever in the past 3 months were excluded from the study.

Widal test was performed by classical tube agglutination method from the serum samples of healthy individuals after taking their written consent. About 2ml of blood was collected for serology test from the healthy individuals. Serum was separated immediately, labelled and stored at 4°C for further processing.

Commercially available Widal tube agglutination test kits from span diagnostic were used. The tube agglutination test was carried out by using 0.5 ml of two fold serially diluted sera (Dilutions from 1:20 to 1:320). The test procedure was performed strictly as per the manufacturer's instructions. The test procedure followed is as follows.

1. Four sets of 7 test tubes taken and label led them 1 to 7.
2. Pipette 1.9ml of physiological saline in to tube number 1 of all sets
3. Add 1 ml of physiological saline to each of the remaining tubes [2 to 7 of each sets]
4. To tube number 1 of all sets add 0.1ml of serum sample to be tested and mixed well.
5. Transfer 1.0ml of the diluted serum sample from tube no.1 to tube no.2 and mix well
6. Discard 1.0ml of the diluted serum sample from tube no.6 of each set.
7. Tube no.7 in all the sets, serves as a saline control.
8. To all the tubes of the respective sets add 1 drop of the respective Vital Widal antigen suspension (the “O” and “H” antigens of *S. typhi* and “H” antigens of *S. paratyphi* A and B)
9. This will give final dilutions in tube 1 to 6 as 1;20,1;40,1;80,1;60,1;320,1;640
10. Cover and incubate at 37 °celsius over night.
11. Observe for agglutination macroscopically next day

The test results were interpreted as per standard guidelines.

3. Results

The purpose of this study was to find out the baseline antibody titre in the population and to develop a local

Table 1: Number and percentage of sera with end titres in the blood bank donors

| Antigen | No.& % of positive samples | Dilution (1:20) | Dilution (1:40) | Dilution (1:80) | Dilution (1:160) | Dilution (1:320) |
|------------------|----------------------------|-----------------|-----------------|-----------------|------------------|------------------|
| S. typhi "O" | 45(11.25%) | 11(24.44%) | 28(62.22%) | 06(13.33%) | Nil | Nil |
| S. typhi "H" | 37(9.25%) | 12(32.43%) | 21(56.75%) | 02(5.4%) | 02(5.4%) | Nil |
| S.paratyphi "AH" | 07(1.75%) | 02(28.57%) | 03(42.85%) | 01(14.28%) | 01(14.28%) | Nil |
| S.Paratyphi" BH" | 01(0.25%) | 01(100%) | Nil | Nil | Nil | Nil |

recommendation for interpretation of the Widal test results. A total of 400 sera were tested. Among these, 90 (22.5%) showed agglutinations at a titre of $\geq 1:20$ for the "O" and "H" antibodies against *Salmonella typhi*, *Salmonella paratyphi A* or *Salmonella paratyphi B*. The rest of the 310 did not show agglutination. The number and percentage of sera with corresponding end titres for agglutinins against *Salmonella typhi*, *Salmonella paratyphi A* and *Salmonella paratyphi B*, has been presented in Table 1 .

4. Discussion

The isolation of the various strains of *Salmonella enterica subspecies enterica* from blood remains the gold standard for the diagnosis of enteric/typhoid fever. In the modern time there is an alarming increase in the empirical use of broad spectrum antibiotics, the practice of self-medication and the lack of proper timing for the specimen collection, that attributes to the reduced productivity of the blood culture technique. Also, in the developing countries, such as the India many clinics and hospitals do not have easy access to the blood culture method, thus making the Widal tube agglutination test the most common alternative laboratory procedure for the diagnosis of enteric fever.

The serological diagnosis depends on the demonstration of the rising titre of the antibodies in paired samples, 10 to 14 days apart. In typhoid fever, however, such a rise is not always demonstrable, even in the blood culture confirmed cases. This situation may occur because of the acute phase sample which is obtained late in the natural history of the disease, because of the high levels of the background antibody in a region of endemicity or because in some individuals, the antibody response is decrease due to early administration of an antibiotics. Furthermore, the patient treatment cannot wait for long. For practical purposes, the treatment decision must be made on the basis of the results which are obtained with a single acute phase sample. The cut off titre in a particular population depends on the background level of the typhoid antibodies and the level of the typhoid vaccination, which can vary with time.

Each country or region should have a baseline titre of their healthy population, which should be updated with time. This was the first study which was done in the Sabarkantha region of Gujarat India, to estimate the baseline antibody titre in the healthy population against various

species of *S almonella* by using the Widal tube agglutination test.

Ninety (22.5%) of the sera were found to be positive for agglutinins for the *Salmonella* serotypes. Our findings were quite lower than those which were reported by other investigators. A comparative analysis of samples which were positive for agglutinins for *Salmonella* serotypes has been presented in Table 2 .

None of the samples showed a titre of 1:160 for anti TO and only 5.4% of the samples showed a titre of 1:160 for anti TH. Only 14.28% of the samples showed a titre of 1:160 for AH. The highest dilution 1:320 showed by not a single sample. Only one sample is positive for anti BH and the highest dilution of it is 1:20

The most frequently recorded titre for O agglutinin (62.22%) was found to be 1:40 and for H agglutinin (56.75 %) 1:40 of *Salmonella typhi*. For H agglutinins of *Salmonella paratyphi A* & B most frequently recorded titre was 1:40 & 1:20 respectively. Thus the baseline titre for the O,H,A H and BH *Salmonella* agglutinins were assumed to be 1:40,1:40,1:40 and 1:20 respectively. A comparative analysis of baseline titres of O and H agglutinins has been presented in Table 3.

Table 2: Comparative analyses of samples positive for agglutinins for *Salmonella* serotype

| Author | Year | Percentage of positive samples |
|--------------------------------------|------|--------------------------------|
| Bharat et al., ¹⁴ | 2009 | 62% |
| Prashant Peshattiwari, ¹⁵ | 2012 | 55.12% |
| Achary et al., ¹⁶ | 2013 | 50.6% |
| Shekhar Pal et al., ¹⁷ | 2013 | 42.60% |
| Present study | 2017 | 22.5% |

5. Conclusion

The baseline titre for antibodies to "O" and "H" antigens of *Salmonella enterica* serotype *typhi* was 1:40 and hence, based on the above results; it could be recommended to use a cutoff level of $\geq 1:80$ for a single antibody test titre. Similarly, baseline titre for antibody to H antigen of *Salmonella enterica* serotype *paratyphi A* and *paratyphi B* was 1:40 and 1:20 respectively. The cut off level for antibody to *Paratyphi AH* is $\geq 1:80$ and for antibody to *paratyphi BH* is $\geq 1:40$ for a single antibody test titre.

Table 3: Comparative analysis of baseline titre of “O” & “H” agglutinins.

| Author | Place | Year | Baseline Titre | | | |
|------------------------------------|--------------------------|------|----------------|------|------|------|
| | | | TO | TH | AH | BH |
| Shekhar Pal et al ¹⁷ | Shrinagar (Ghrawal) | 2011 | 1:40 | 1:80 | 1:20 | 1:20 |
| Prashant Peshattiwar ¹⁵ | Andhrapradesh | 2011 | 1:80 | 1:80 | 1:40 | 1:20 |
| Saxena et al ¹⁸ | Hadoti region(Rajasthan) | 2012 | 1:40 | 1:40 | 1:20 | 1:20 |
| Sreenath K et al ¹⁹ | Kollam | 2014 | 1:40 | 1:40 | 1:20 | 1:20 |
| Vadsmiya et al ²⁰ | Ahmedabad | 2014 | 1:40 | 1:40 | 1:20 | 1:20 |
| Maulingkar et al ²¹ | Wayanad distict, Kerala | 2015 | 1:40 | 1:80 | 1:40 | 1:40 |
| Present study | Himmatnagar | 2017 | 1:40 | 1:40 | 1:40 | 1:20 |

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.

6. Source of Funding

None.

7. Conflict of Interest

None.

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