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RESEARCH ARTICLE

SEROPREVALENCE OF SALMONELLA INFECTION IN A TERTIARY HEALTH CARE FACILITY IN NORTH INDIA

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ABSTRACT

Introduction: Enteric fever is a systemic febrile illness caused by Salmonella species, which includes Salmonella entrica serovar Typhi and Salmonella entrica serovar paratyhi A, B or C respectively.
Material and Method: This was a community based, retrospective study, conducted in Department of Microbiology, Jaipur National University Institute for Medical Science and Research centre (JNUIMSRC) from August' 2015 to July' 2016. A total of 268 samples were included in this study. The sample size of our study was 268 who were screened for the presence of anti-O, anti-H, anti-AH and anti-BH agglutinins by the Widal tube agglutination test.
Results: Eighty three (30.9%) samples were positive (i.e., showed a titre of ≥ 20) whereas 185 (69.1%) samples did not show agglutination.
Conclusion: We studied the baseline titres of anti-TO and anti-TH and found to be 40 and those of anti-AH& anti-BH 40. Baseline titres of our study were lower than other part of India.

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INTRODUCTION

Enteric fever is a systemic febrile illness caused by *Salmonella* species, which includes *Salmonella* entrica serovar *Typhi* and *Salmonella* entricaserovar *paratyhi* A, B or C respectively. Disease is transmitted by feco-oral route, especially by carrier handling food and water. Typhoid fever is a serious health issues in developing countries and India is an endemic zone of enteric fever (Crump, 2004; Bhutta, 2006; Ochiai, 2008; Ochiai *et al*., 2008; Chuttani *et al*., 1971; Chuttani *et al*., 1973; Chuttani *et al*., 1977; Sinha *et al*., 1999; Ochiai *et al*., 2005). In more than 90% of cases of enteric fever, *Typhi* is found to be the causative organism and reaming 10% of cases are caused by *paratyphi*. Worldwide, Typhoid fever is a major cause of morbidity with an estimated 21.7 million cases, the bulk of the burden being borne by many middle and low income countries due to an inappropriate level of personal hygiene, sanitation, rapid growth of population, increased urbanization, limited safe drinking water, health system and infrastructure (Aakanksha Sharma *et al*., 2015; Parry *et al*., 1999; Hosoglu *et al*., 2006; Parry, 2006; Pang, 1983; Harries *et al*., 1995). *Paratyphi* shows similar clinical picture but in milder form. Typhoid fever survivors may take several weeks to recover and to resume their work, an early and accurate diagnosis of typhoid fever at an early stage is important not only for diagnosis of etiological agent, but also to identify individual that may serve as a potential carrier, and may be responsible for acute outbreak. The definitive diagnosis of *Salmonella* infection requires the isolation of the organism from the blood, bone marrow, urine or other body fluids. In addition, ELISA, immunofluorescence are often used in developed countries but in under developed countries, where facilities for isolation and culture are often not easily available mainly in clinics, small Hospitals and diagnostic centers, diagnosis commonly relies upon the clinical findings and serological test. For serodiagnos is of typhoid, Widal test has been in use for many decade and still remains as one of the most important and specific investigation tool. Though organism can be isolated from blood during early stage of infection but in country like India, due to patient often receive antibiotics prior to medical diagnosis, isolation of microorganism for blood cultures varies from 40 to 60 % and facilities of blood culture are not

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available so often (Boyen *et al*., 2008; Manson-Bahr, 1987). As Enteric fever remains a serious problem in developing countries this present study brings out the prevalence of seropositivity of *Salmonella* infection in a tertiary health care centre in North India.

MATERIAL AND METHODS

This was a community based, retrospective study, conducted in Department of Microbiology, Jaipur National University Institute for Medical Science and Research centre (JNUIMSRC) from August' 2015 to July' 2016. JNUIMSRC Hospital is a tertiary health care, equipped with modern techniques for diagnostic and treatment. A total of 268 samples were included in this study.

Table-1: Distribution of positive and negative samples for agglutination in Widal Test

Widal reactivity	Frequency	Percentage
Positive (≥ 1:20)	83	30.9
Negative (<1:20)	185	69.1
Total	268	100

Table 2. Shows the age distribution

Age	Number	Percentage %
< 10 year	30	11.2 %
11– 20 years	67	25 %
21– 30 years	81	30.3 %
31– 40 years	40	14.9 %
41-50 years	22	8.2 %
51- 60 years	17	6.3 %
>61 years	11	4.1 %
TOTAL	268	100 %

Table 3. Shows the sex distribution

SEX	Number	Percentage %
MALE	151	56.3 %
FEMALE	117	43.7 %
TOTAL	268	100 %

Inclusion criteria: All the a febrile patients who attended JNUIMSRC hospital with other complained.

Exclusion criteria: Patientsof clinical symptoms similar to Enteric fever and were advised Widal test, patients who received vaccine.

Blood sample collection: A 2ml of blood was collected from each patient suspected to be suffering from Enteric fever from the median cubital vein, under aseptic conditions using 5 ml disposable syringes and alcohol swabs. The collected blood was then immediately transferred to sterile vial and labelled properly.

Widal test: Two ml of venous blood was collected from each patient with aseptic precautions. It was allowed to clot at room temperature for about 30-60 minutes. Serum was separated by centrifugation at 3000-5000 rpm for 5 minutes and when ever required was stored at 4^{o}C and tests were performed within 48hrs. Four rows were set for each serum sample to be tested. The first row consisted of 5 Felix tubes and the remaining 3rows of 5 Dreyer's tubes each. Serial 2-folddilutions of the test serum i.e., 1:10, 1:20, 1:40, 1:80 and 1:160 were prepared in all the rows so that each tube contained 0.5 ml of the diluted serum.

In the first and the second rows, 0.5 ml of *Salmonella typhi* – O and H antigens were added respectively. In the third and fourth rows, 0.5 ml of *Salmonella paratyphi*–AH and BH antigens (ARKRAY Healthcare Pvt Ltd) were added respectively. So the final serum dilutions obtained for each antigen were 1:20, 1:40, 1:80 1:160 and 1:320. Appropriate positive and negative controls were put up for each test. The test tube rack was placed in water bath at 37 °C for overnight incubation. The Widal anti-O agglutinin (TO) and the anti-H agglutinin (TH) titres were taken as the highest dilutions of serum with a visible agglutination. Based on the manufacturer manual, antibody titre of 1:80 and above for TO antigen and 1:160 and above for TH antigen were taken as the cut off value to indicate acute infection of Enteric fever.

RESULTS

The sample size of our study was 268 who were screened for the presence of anti-O, anti-H, anti-AH and anti-BH agglutinins by the Widal tube agglutination test. Eighty three (30.9%) samples were positive (i.e., showed atitre of ≥ 20) whereas 185 (69.1%) samples did not show agglutination

DISCUSSION

In the age of modern medical world the best practices of diagnosis are observed in developed countries where enteric fever is far less prevalent and in countries where the disease is much more prevalent and where such diagnostic tools are acutely needed, it often goes undiagnosed. Due to reasons

Table 4. Distribution of positive samples (with Antibody titres >20) against different serotypes of*Salmonella*

Serotype	Antibody type	Frequency of agglutinating sera (n=268)	Percentage (%)
Typhi Anti O antigen	Anti O antigen	83	30.9
Typhi Anti H antigen	Anti H antigen	54	20.1
Paratyphi A	Anti H antigen	19	7.1
Paratyphi B	Anti H antigen	7	2.6

Table 5. Number & percentage of reactive sera in various titres in the study population

Antigen	No. of +ve samples (%)	Dilution (1:20)	Dilution (1:40)	Dilution (1:80)	Dilution (≥ 1:160)
S. typhi O	83	83 (100%)	69 (83.1%)	50 (60.2%)	26 (31.3)
S. typhi H	54	54 (100%)	46 (85.2%)	28 (51.8%)	25 (46.3%)
S. paratyphi AH	19	19 (100%)	16 (84.2%)	13 (68.4%)	9 (47.4)
S. paratyphi BH	7	7 (100%)	6 (85.7%)	5 (71.4%)	4 (57.1%)

mentioned in the introduction, physicians in the developing countries have to make do with old procedures which are fraught with many inadequacies and which have been abandoned in most western world (Olopoenia, 2000). On the bases of previous studies regarding the clinical utility of Widal test, it is unscientific to base the diagnosis of enteric fever solely on the Widal test.

When there is no other confirmatory supportive test, such appositive blood culture particularly available, it can be used with benefit to suggest such a diagnosis. Interpretation of a single Widal test requires knowledge of the prevalence of baseline titres in the particular community. Based on baseline titre, acut-off titre can be assigned for the specific community where single Widal test in afebrile patient would serve as presumptive evidence of *Salmonella* infection. Thus, each geographical area needs to have its own baseline titre and significant titres.

To fulfill this need we undertook this study. We selected261 individuals belonging to age groups between 1-70 years representing the local population for the study. Inclusion and exclusion criteria were designed to ascertain they were not in a state of current ongoing infection of any kind or of artificial immunity to typhoid. Their sera were tested with the Widal tube agglutination test for the presence of antibodies to *S. typhi* and *S. paratyphi* serotypes A and B. The results showed a certain degree of seroprevalence (30.9%) of salmonella agglutinins among the members of the community. Butthe vast majority of the subjects (69.1%) were nonreactive. In those who had the antibody, the titres were not very high – only 50 (18.6% of the subjects) and 28 (10.4%) sera agglutinated TO and TH antigens respectively in dilutions> 1:80. The highest titre found for both anti-TO & anti-TH was 2 (2 subject each). Only19 (7%) subjects were reactive to A Hantigen and 7 (2.6%) to BH antigen. Among AH 13 (68.4%) and 5 (71.4%) BH sera agglutinated in dilution > 1:80. In our study we found maximum patients were from 21-30 years age group, this may be due to consumption of contaminated food or water on working area. Male (56.3%) were more infected than females (43.7%) as in India more numbers of females are housewife. According to a study conducted by Collard *et al*. (1959) significant Widaltitre of agglutinins to be considered of significance should be such as would not be expected in more than 5% of normal population (Punia *et al*., 2003). The highestvalue found in the remaining 95% of the studied population can still be taken as baseline titer. Applying the same toour findings, < 5% of the subjects had the highest titres of >80 for both anti-TO (31.3%) and anti-TH (46.3%) and >95% of the subjects had the titres of _40 for anti-TO and anti-T Hagglutinins. Hence the baseline titre for bothanti-O and anti-H Widal agglutinins in our population is 40 and any value found in Widal test for TO and TH antigens in titres of _80can be considered significant in the diagnosis of typhoid fever. Similarly in agglutinations with AH & BH significant titre for anti-AH and anti-BH is 40 along with a finding of significantly raised anti-TO and a clinically relevant situation.

Conclusion

Baseline Widal titres vary from community to community and with time to time. It is essential toknow the levels of prevalence of the specificcommunity to interpret the results of Widaltube test. In the local population in and around JNUIMSRC, we studied the baselinetitres of anti-TO, anti-TH, anti-AH & anti-BH found to be40. Anytitres obtained in a single Widal test over andabove these values i.e., ≥80 for anti-TO, anti-TH, anti-AH & anti-BHcan be considered to be significant andsuggestive of enteric fever.

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