

Modified Serological Method for Determining Significant Antibody Titre to *Salmonella* Infection in Endemic Area

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Abstract

Enteric fever is caused by antigenic variant group of bacteria known as *Salmonella* species. The disease is common in most developing countries being contacted through contaminated water, food and poor personal hygiene. The most significant challenge in the treatment of infected patients is misdiagnosis leading to high morbidity and mortality rate. We devised a time efficient technique which has been compared with tube agglutination method and culture and found it to be very efficient, sensitive and reliable. Blood samples from patients clinically diagnosed of enteric fever were collected and a 1:4 dilution of serum in saline was used for direct agglutination test. Results with visible agglutination were considered as significant and it compared favorably with tube agglutination and culture result. A total of two thousand two hundred and fifty specimens were tested. Among this, 1390 were positive for the modified serological method for significant antibody titre, 1380 were positive for only tube agglutination while 1372 were positive for stool culture. The modified serologic method for determining significant antibody titre to *Salmonella* infection had a sensitivity of 98.71%, specificity of 97.99% and an efficiency of 98.43%. The difference between the results of the modified method and stool culture for the diagnosis of enteric fever was statistically significant ($p < 0.05$).

Keywords: Typhoid; Enteric fever; Sensitivity; Efficiency; Specificity

Introduction

Typhoid fever is a disease condition caused by a group of antigenically related organisms of the genus *Salmonella*. It is a major disease condition in the tropical countries. The infection is accentuated by poor hygienic condition, degrading environment and lack of potable water. The disease manifestations include high grade fever, with temperature up to 39°C-40°C, vomiting, fatigue, and loss of cognition.

Enteric fevers are severe systemic forms of salmonellosis and the best studied enteric fever is typhoid fever. The causative organism of typhoid is *Salmonella typhi* and *Salmonella paratyphi* A, B or C of the paratyphoid fevers. There are three species of *Salmonellae* and only *Salmonella typhi* and *Salmonella enteridis* species are pathogenic to humans with or without having animal reservoirs [1].

Salmonella organisms penetrate the mucosa of both small and large intestine, coming to lie intracellularly where they proliferate. Rupture of infected cells occurs, liberating organisms into the bile thereby causing infection of the lymphoid tissue of the small intestine, particularly the ileum. It is this phase of heavy infection that brings the classical pathology of typhoid [2].

The pathology of typhoid fever occurs mostly in the Peyer's patches and has four phases which include; hyperplasia of lymphoid follicles, necrosis of lymphoid follicles during the second week involving both mucosa and submucosa, ulceration in the long axis of the bowel with the possibility of perforation and haemorrhage and finally healing which takes place from the fourth week onward. Typhoid perforations

are usually simple and involves the antimesenteric border of the bowel where they appear as punched out holes [3,4].

The laboratory diagnosis of enteric fever is very important mainly because most of the patients are treated empirically by the clinicians and when the fever does not subside, the cases are labeled as pyrexia of unknown origin [1]. The laboratory diagnosis of enteric fever is established by the presence of *Salmonella typhi* or *Salmonella paratyphi* which is detected by culture of the organisms or by demonstration of specific antibodies or antigen in the serum or urine. The organisms may be cultured from blood, stool, bone marrow or urine [4,5].

The current research is on the use of a modified method which combines the dilution and direct agglutination method to determine a significant antibody titre, this is aimed at establishing a standard for determining significant antibody titre for *Salmonella typhi* and *Salmonella paratyphi* agglutination tests.

Materials and Methods

Ethical clearance

Ethical clearance was sought for and obtained from the ethical committee of Federal Teaching Hospital, Abakaliki. Informed consent was also obtained from all the participants or from those participants that were children.

Study population

The study was conducted among two thousand two hundred and fifty patients attending Federal Teaching Hospital, Abakaliki. Relevant

clinical details and sociodemographic data was also obtained from all the subjects.

Eligibility criteria

Inclusion criteria comprised of patients showing clinical signs of enteric fever regardless of their age or sex and for whom Widal test had been required to be performed. Patients who did not present with clinical signs of enteric fever were excluded from the study.

Specimen collection

A volume of 3 ml of whole blood was collected from each informed and consenting subjects by venepuncture, this was dispensed into sterile plain containers and allowed to clot and retract then it was centrifuged at 4000 rpm for 5 minutes and the serum was separated into uniformly pre-labelled sterile plain containers. Stool specimens were also collected from each subject into sterile wide mouthed screw-capped containers.

Stool culture

The stool specimens were inoculated onto strontium selenite broth, modified bismuth sulphate agar and shigella-salmonella agar and incubated for upto 24 hours. Isolates suspected to be *Salmonella* species were identified and characterized using standard methods which include culture characteristics of the isolates and their biochemical test reactions.

Tube agglutination

A total of six tubes were set up and a serial twofold dilution of the serum specimen was made from 1 in 20 to 1 in 640. Equal volume of the *Salmonella* antigen was added and incubated in a water bath at 37°C. The results were interpreted as the tube with the highest dilution which had agglutination. Agglutination results from the 1 in 160 tube up to the 1 in 640 tubes were taken as positive results.

Slide agglutination test

One drop of the serum was placed on a tile and equal volume of the antigen was added, this was mixed and the tile rocked gently and examined for visible agglutination within one minute.

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A 1:4 dilution of the serum sample was made with sterile normal saline. This was allowed to stand for 5 minutes to ensure complete dilution. One drop was then placed on a tile and an equal volume of *Salmonella* species antigens were added and then gently rocked for 1 minute and subsequently examined under bright light. Positive result was indicated by visible agglutination.

The sensitivity, specificity and efficiency of the method was calculated as follows Sensitivity=[(true positive/true positive+false negatives) X 100], Specificity=[(true negatives/false positives+true negatives) X 100] and efficiency=[(true positives+true negatives/true positive+false positive+false negative+true negative) X 100]. The true positive result of the serological test was taken as those which also had positive culture result, positive serological tests with negative stool culture result was taken as false negative result.

Data analysis

The data obtained was analyzed using simple descriptive statistics and Chitest. P value was considered significant at 95% confidence interval. Data analysis was done with the aid of Statistical Programme for Social Sciences (SPSS) version 18.0.

Result

A total of two thousand two hundred and fifty specimens were tested. Among this, 1390 were positive for the modified serological method for significant antibody titre, 1380 were positive for only tube agglutination while 1372 were positive for stool culture (Figure 1).

The sensitivity, specificity and efficiency of the modified serologic method was compared with stool culture and tube agglutination method using the results obtained from the stool culture as the gold standard for either positive or negative result for enteric fever.

The modified serologic method for determining significant antibody titre to *Salmonella* infection had a sensitivity of 98.71%, specificity of 97.99% and an efficiency of 98.43%. The difference between the results of the modified method and stool culture for the diagnosis of enteric fever was statistically significant (p< 0.05).

The results that were positive to the modified serological method correlated with titres equivalent to 1/160 and above. The results were comparable to other standard methods used at present for the laboratory diagnosis of enteric fever such as culture isolation of the aetiologic agent and the tube agglutination method of widal test.

The prevalence of typhoid fever among the subjects studied was 61.78%. Among those positive, there was a higher prevalence of the infection among subjects aged 0-5 years than among subjects of other age range (Table 1). The 1 in 640 tube did not show any positive agglutination result.

Age	1/20	1/40	1/80	1/160	1/320
0-5	14	13	28	286	138
6-10	17	21	25	73	53
11-15	13	24	22	79	25
16-20	09	34	28	52	21
21-25	03	58	34	143	62
26-30	10	11	31	39	45
31-35	12	04	03	12	18
36-40	16	43	29	41	22
41-45	19	09	53	09	08
46-50	34	26	91	06	46
51-55	00	24	11	15	10
56-60	00	15	29	13	63
61-65	12	00	03	22	26
66-70	03	05	24	14	39

Total	162	287	411	804	576
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Table 1: Reaction to different dilutions in tube agglutination method.

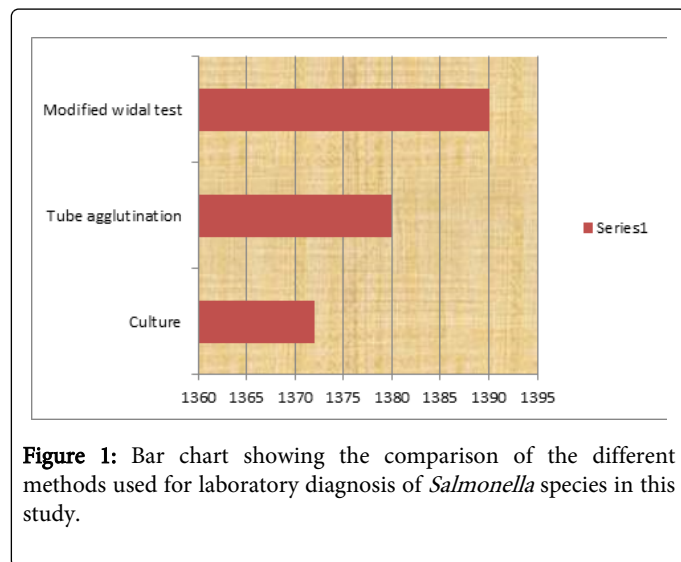


Figure 1: Bar chart showing the comparison of the different methods used for laboratory diagnosis of *Salmonella* species in this study.

Discussion

Typhoid fever remains a serious public health problem in developing countries [6]. However, scarcity of diagnostic facilities in areas of high typhoid endemicity has probably led to an underestimation of the burden of this disease worldwide. Population-based studies have demonstrated a wide variation in the incidence of typhoid fever globally [7-9].

The prevalence of typhoid fever among the test subjects was high (61.78%). The high degree of the infection among children aged 0-5 years is comparable to the results obtained from other studies [10].

A good number of in-patients in most hospitals are infected with this pathogen resulting to high level of morbidity and mortality. The most challenging aspect of the disease is the difficulty in diagnosing the infection due to its clinical manifestation which simulates other tropical infections. The lack of advanced technical diagnostic equipment in most poor resource countries accounts for misdiagnosis. In most cases, late diagnosis or misdiagnosis have often led to mortality.

Frequently, the use of serological method and stool culture remains the only available option in the laboratory diagnosis for most hospitals. This method relies on the subjective interpretation of result by the laboratory scientist. The stool culture method requires an expertise in bacteria identification and good handling to differentiate the *Salmonella* species from a milieu of other enteric pathogens. Similarly, the serological method is fraught with the difficulty of determining significant antibody titre especially considering the fact that most other enteric pathogens have shared antigens resulting in the production of blocking antibody titre.

The use of tube dilution method is laborious and time consuming. However, the screening method which relies basically on direct tile agglutination test is also unreliable because of blocking antibody titre and prozone phenomenon arising from lack of specific method to determine the equimolecular level of antibody and antigen in the test.

The current study has presented a modified method for the rapid diagnosis of typhoid fever with a high sensitivity, specificity and efficiency in poor resource settings as prevalent in most developing countries. The method is easy to perform, time saving and highly economical.

The 1 in 4 dilution of the serum in saline enhances the strength of the antigen-antibody interaction and reduces the effects of blocking antibodies hence it promotes the reaction such that visible agglutination in that dilution strongly indicates a positive reaction. This method correlates well with the standard culture method for detecting the presence of *Salmonella typhi* and *Salmonella paratyphi* (Figure 1). The method is important because it enables fast diagnosis and as such reduces the turnaround time for widal test. Besides, the low income which is required as against the high sensitivity, specificity and efficiency of the method, the accurate diagnosis of typhoid fever will reduce the morbidity and mortality and hence contribute immensely in the overall control and reduction of the disease to the barest minimum.

Most developing countries, Nigeria inclusive are plagued by poor economy but beyond that is the very poor attitude of the political leaders in putting in place effective measures necessary to tackle the health challenges in the country. The almost nonexistent potable water supply from government owned water resources across the entire country subjects the populace to depend on alternative sources of drinking water. Water is sold in bottles and in sachets popularly called "pure water" in the local parlance. This commercial water has been shown to harbor a host of pathogenic microorganisms. Hence, it is a source of contamination [11]. These factors contribute immensely to the high endemicity of typhoid fever in most developing countries like Nigeria.

Yet another factor which militates against the eradication or hitherto drastic reduction of the disease among the population is the practice of empirical therapy and the indiscriminate prescription of antibiotics which have been shown to cause resistance and hence increase the morbidity of typhoid fever in the population [10].

Conclusion

This study advocates the effective and accurate diagnosis of enteric fever based on the modified serologic method for the diagnosis of enteric fever which will contribute to the eradication of the disease and a drastic reduction in the burden of the disease. We therefore recommend the adoption of this method in hospital laboratories for the diagnosis of typhoid fever.

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