Print ISSN: 2321-6379
Online ISSN: 2321-595X
DOI: 10.17354/ijss/2018/85

Original Article

Comparison of Three Different Tests for Diagnosis of Enteric Fever

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Abstract

Background: Enteric fever is a systemic illness caused by Salmonella Typhi and Salmonella Paratyphi. The different methods for diagnosis of enteric fever are blood, bone marrow, rarely stool and urine culture, nucleic acid detection, antibody detection by Widal test, and other rapid diagnostic tests.

Aim: The study was performed to evaluate the performance of tube Widal test, Typhiwell enzyme-linked immunosorbent assay (ELISA) test, and Typhifast, an immunochromatographic (ICT) test.

Materials and Methods: This study was carried out in the Department of Microbiology in a tertiary care center for 1 year (January–December 2015). The serum samples were collected from the patients with fever who had positive blood culture report. A total of 50 samples were included, of which 21 were positive for S. Typhi, 9 were positive for S. Paratyphi A, and 20 samples were positive for other organisms such as *Escherichia coli* (8 isolate), *Klebsiella pneumoniae* (8 isolate), and *Staphylococcus aureus* (4 isolate) by blood culture. The serum samples were used for doing the various tests for diagnosis of enteric fever such as tube Widal test, Typhiwell, ELISA test, and Typhifast, an ICT test.

Results: The three serological tests were performed and compared with blood culture, and it was found that Typhifast had a sensitivity of 70% and specificity of 100%, Typhiwell had a sensitivity of 90% and specificity of 75%, and Widal test had a sensitivity of 83.3% and specificity of 80%.

Conclusion: Widal test had a fairly good sensitivity and specificity, whereas Typhifast had a very good specificity but a lower sensitivity.

Key words: Blood culture, Enzyme-linked immunosorbent assay, Immunochromatography, Salmonella, Widal

INTRODUCTION

Enteric fever is a systemic infection caused by Salmonella enterica serovar Typhi (S. Typhi) and Salmonella enterica serovar Paratyphi (S. Paratyphi) . It is a common cause of morbidity in the developing countries including South and South-east Asia.[1] Typhoidal Salmonella is transmitted predominantly through water or food contaminated with human feces.[2] The diagnosis of enteric fever poses several problems due to the non-specific and wide array of clinical features. The common symptoms and signs are fever, vomiting, cough, anorexia, diarrhea, abdominal pain, hepatomegaly, splenomegaly, and coated tongue. Enteric fever should be considered in the differential diagnosis of febrile patients with abdominal symptoms.[3] The common tropical infections such as dengue, enteric fever, leptospirosis, typhus fever, and malaria having similar early presentations can cause confusion in decision-making. Recognition of these diseases is important to diagnose them and treat them early, to avoid potentially fatal complications.[4]

In endemic areas, diagnostic tests are needed to diagnose acute cases of enteric fever for clinical management, to detect convalescent and chronic fecal carriage, and for contact tracing. A suitable test may also allow an assessment of disease burden in a community to determine the need for vaccination programs.[5] The definitive diagnosis of enteric

Access this article online

www.ijss-sn.com

Month of Submission : 01-2018
Month of Peer Review : 02-2018
Month of Acceptance : 02-2018
Month of Publishing : 03-2018

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fever relies on the isolation of *Salmonella* species from blood and bone marrow. In untreated patients with enteric fever, the blood culture is positive in 80% of patients or more. In areas of endemicity where antimicrobials are frequently taken before evaluation, the yield from blood culture can be as low as 40%.[2] Although bone marrow cultures are more sensitive, they are difficult to obtain, relatively invasive, and of little use in public health settings.[6] In addition, Salmonella serovars that cause human infection can change over time and location. In certain areas of Asia, multidrug-resistant S. Typhi has been the main cause of enteric fever, but now S. Typhi is being displaced by infections with drug-resistant S. Paratyphi A.[7] Nucleic acid amplification tests, including conventional polymerase chain reaction (PCR) and real-time PCR, have been developed for the detection of both Salmonella serovars Typhi and Paratyphi A, mainly in blood.[2]

The Widal agglutination test detects serum antibodies to the somatic and flagellar antigens of S. Typhi and S. Paratyphi A and B. The interpretation of the Widal test remains problematic to this day. In many places, instead of the standard tube agglutination test, a quantitative slide agglutination test is used, but this should always be interpreted with reference to clinical data. A rise in titer over time or a single high test, the result is diagnostically significant in Widal test. False negative results may occur if the blood is collected too early in the disease. False positive results may be associated with a history of immunization for typhoid fever, cross-reacting antibodies, or a host of infections and conditions.[8] Although commercial point-of-care rapid diagnostic tests (RDTs) for enteric fever are available as alternatives to the current reference standard test of blood or bone marrow culture, or to the widely used Widal test, their diagnostic accuracy is unclear.[9]

The objective of this study was to evaluate the performance of tube Widal test, Typhiwell enzyme-linked immunosorbent assay (ELISA) test, and Typhifast, an ICT test and to compare the diagnostic accuracy of these tests with the isolation of organism by blood culture for diagnosis of enteric fever.

MATERIALS AND METHODS

This study was carried out in the Department of Microbiology in a tertiary care center for 1 year (January–December 2015). After obtaining ethical clearance from the Institutional Review Board and consent from the patients, 4 ml of blood was collected in clotted vial. The patients included were those who had positive blood culture report. Blood culture was done by automated Bact T/ALERT three-dimensional (bioMérieux Inc., France). Serum was separated from blood by centrifugation at 1500 rpm for 10 min. A total of 50 samples were included, of which 21 were positive for S. Typhi, 9 were positive for S. Paratyphi A, and 20 samples were positive for other organisms such as *Escherichia coli* (8 isolates), *Klebsiella pneumoniae* (8 isolates), and *Staphylococcus aureus* (4 isolates) by blood culture which served as control for the study.

The serum samples were used for doing the various tests for diagnosis of enteric fever such as Widal test (Tulip Diagnostics Private Limited, Goa, India), Typhiwell (Anand Brothers and AB Diachem Systems Pvt., Ltd., New Delhi, India), and Typhifast (Anand Brothers and AB Diachem Systems Pvt., Ltd., New Delhi, India). Widal test was performed by semi-quantitative tube method using different antigens such as S. Typhi O (TO), S. Typhi H (TH), S. Paratyphi A H (AH), and S. Paratyphi B H (BH). The test was performed according to the manufacturer's instructions and positive was taken as titer ≥ 80. Typhiwell was an ELISA for the detection of immunoglobulin (IgM) antibodies specific to enteric fever in human serum. The test was performed according to the manufacturer's instructions, and positive was taken as optical density >0.5 after proper validation of the test. Typhifast was a rapid ICT test to detect specific IgM antibodies against S. Typhi. The test was performed according to the manufacturer's instructions, and reading was taken after seeing the control line showing the test to be valid. All data were entered in Excel spreadsheet (Microsoft, USA) and analysis was done. The performances of the tests were compared, and diagnostic accuracy (sensitivity, specificity, positive predictive value, and negative predictive value) of these tests were calculated.

RESULTS

Among the patients included in the study, there were 33 (66%) male and 17 (34%) female. The age of the patients was between 5 and 66 years (mean = 21.67, SD = 8.36). The serum samples were used for doing the various tests for diagnosis of enteric fever such as tube Widal test, Typhiwell ELISA test, and Typhifast ICT test, and the results obtained in the different tests are noted in Table 1.

Culture is the gold standard for diagnosing a Salmonella infection.[10] Using blood culture as the standard and reference test for diagnosis of enteric fever, the sensitivity, specificity, positive predictive value, and negative predictive value of the different tests were calculated from the samples having growth of *Salmonella* species as true positives and samples with growth of other organisms as true negatives. It was found that Typhifast has the lowest sensitivity of 70% but highest specificity of 100% while Widal test has sensitivity and specificity of both around 80% [Table 2].

Table 1: The result obtained by different tests for enteric fever

Test/result	Typhifast		Widal		Typhiwell	
	Positive	Negative	Positive	Negative	Positive	Negative
Blood culture						
Positive	21	9	25	5	27	3
Negative	0	20	4	16	5	15

Table 2: The performance of the different tests for diagnosis of enteric fever

Test	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Typhifast	70% (21/30)	100% (20/20)	100% (21/21)	68.9% (20/29)
Typhiwell	90% (27/30)	75% (15/20)	84.4% (27/32)	83.3% (15/18)
Widal	83.3% (25/30)	80% (16/20)	86.2% (25/29)	76.2% (16/21)

Among the 30 patients with enteric fever, the duration of fever was between 5 and 30 days (mean = 12.2 days). Among the 5 patients who were negative by Widal test but had blood culture positive, 3 had fever duration of 5–7 days and 2 had fever duration of 8–10 days. Among the 3 patients who were negative by Typhiwell but positive by blood culture, 1 had fever for 5–7 days and 2 had fever for 8–10 days.

DISCUSSION

The diagnosis of enteric fever currently depends on the isolation of Salmonella from a patient, most commonly by blood culture. This facility is not available in many areas where the disease is endemic. The other method is PCR-based amplification of DNA from the blood of enteric fever patients, but this technique requires expertise and a well-equipped laboratory. Antigen detection has not been investigated much and detecting an immune response specific for typhoid fever has been done only with antibody detection. Serodiagnosis depends on the age-old Widal test and other serological diagnostic tools.[8]

In a study done by Andualem and group among 270 febrile patients with symptoms clinically similar to typhoid fever, 7 (2.6%) cases of *of* S. Typhi and 4 (1.5%) cases of S. Paratyphi were identified with the total prevalence of typhoid fever 4.1%. The total number of patients who had indicative of infection by either of O and H antigens by Widal test was 88 (32.6%). The sensitivity, specificity, positive predictive value, and negative predictive value of Widal test were 71.4%, 68.44%, 5.7%, and 98.9%, respectively.[11]

The rapid test is emerging as a mode of diagnosis of enteric fever. Among the different rapid tests, the Typhi Dot is a DOT enzyme immunoassay that detects either IgM or IgG antibodies against a specific antigen on the outer membrane protein of serotype Typhi.[8] Application of a dipstick assay for the detection of S. typhi-specific IgM antibodies on samples collected from S. Typhi or S. Paratyphi culture-positive patients at the day of admission to the hospital revealed the presence of specific IgM antibodies in 43.5%, 92.9%, and 100% for samples collected 4–6 days, 6–9 days, and >9 days after the onset of fever, respectively.[12] The advantages of any dipstick assay are that the result can be obtained on the same day, allowing a prompt treatment; only a small volume of serum is needed; no special laboratory equipment is needed to perform the assay; and the reagents remain stable when stored at room temperature.[8] Hence, newer methods of RDTs are being developed.

A study was done by Sultana *et al.* in the Department of Microbiology, Mymensingh Medical College, Mymensingh, between 2010 and 2011, including 200 individuals, of whom 150 were clinically suspected cases of typhoid fever and 50 controls. Among 150 blood samples from the suspected cases, 106 (70.7%) were positive for IgM of S. Typhi by ICT and 67 (44.7%) were positive by Widal test. Whereas, among the 50 controls, 4 (8%) were positive by ICT and 6 (12%) were positive by Widal test. The sensitivity, specificity, positive predictive value, and negative predictive value of the ICT was found as 83.3%, 92.00%, 91.9%, and 83.6%, respectively. On the other hand, corresponding values for Widal test were of 44.4%, 88%, 80%, and 59.5%, respectively. The ICT (IgM) is rapid, easy to perform, applicable for field use, and highly sensitive and specific for the detection of antibodies in patients with typhoid fever.[13] Another ICT test devised by Preechakasedkit P *et al.* provided a lower detection limit and analysis time than a Dot blot immunoassay and was employed to detect S. Typhi in human serum, with high accuracy. This strip test offers great promise for a rapid, simple, and low-cost analysis of S. typhi.[14] In another study done in Bangladesh, it was found that a lateral flow dipstick assay had a sensitivity of 98% compared to blood culture results and a specificity that ranged from 78% to 100%. Unfortunately, microbiological culture of blood is only 30% to 70% sensitive although 100% specific.[15] In

the present study, the ICT test had a sensitivity of 70%, specificity of 100%, positive predictive value of 100%, and negative predictive value of 68.9%.

Various studies have been done for evaluation of ELISA for the diagnosis of enteric fever. Enzyme-linked immunosorbent assays (ELISAs) have been used to study the normal antibody response during enteric fever to LPS, flagella, Vi capsular polysaccharide, or outer membrane protein antigens.[2] In a study done by Rastawicki *et al.* for detection of antibodies to S. Typhi lipopolysaccharide O and capsular polysaccharide Vi antigens in persons from outbreak of typhoid fever by ELISA, it was found that anti-LPS and anti-Vi antibodies were detected in 80% and 53.3% of sera obtained from patients with laboratory-confirmed typhoid fever, respectively.[16] In this study, the sensitivity, specificity, positive predictive value, and negative predictive value of ELISA for the diagnosis of enteric fever was found to be 90%, 75%, 84.4%, and 83.3%, respectively.

The Widal test measures agglutinating antibodies against LPS (O) and flagellar (H) antigens of Salmonella serovar Typhi in the sera of individuals with suspected enteric fever. Although usually discouraged due to inaccuracy, it is simple and inexpensive to perform and is still widely used. The performance of the method has been hampered by a lack of standardization of reagents and inappropriate result interpretation. The Widal test ideally requires both acute and convalescent-phase serum samples taken approximately 10 days apart, and a positive result is determined by a 4-fold rise or fall of antibody titer. However, antibody titers in infected patients often rise before the clinical onset, making it difficult to demonstrate the required 4-fold rise between initial and subsequent samples. In practice, the result from a single, acute phase serum sample is often used, but false negative and false positive results are common. Knowledge of the background levels of antibodies in the local population may aid interpretation of the Widal test, and performance is best among patients with a high prior probability of enteric fever.[2]

In a study done by Adhikari *et al.* among 1371 febrile cases, 237 were found to be S. Typhi positive by blood culture. Blood culture-confirmed patients had ≥1:40 anti-TH and anti-TO titer in 45.56 % (n = 108) and 43.88 % (n = 104) patients, respectively. The sensitivity and specificity of IgG (0.96 and 0.95) and IgM (0.95 and 0.94) at 95 % confidence level were significant compared to Widal anti-TH (0.72 and 0.58) and TO (0.80 and 0.51) test (P = 0.038) at titer level ≥1:200. Further, the PPV of Widal TH and TO (0.38 and 0.23) was low compared to IgG and IgM ELISA (0.78 and 0.77) (P = 0.045).[17]

In another study, 92 Bangladeshi patients with suspected enteric fever were categorized into four groups: S. Typhi bacteremic patients (n = 28); patients with a 4-fold change in Widal test from day 0 to convalescent period (n = 7); patients with Widal titer ≥1:320 (n = 13) at either acute or convalescent stage of disease; and patients suspected with enteric fever, but with a negative blood culture and Widal titer (n = 44), healthy endemic zone controls (n = 20), and Bangladeshi patients with other febrile illnesses (n = 15). of 28 S. Typhi bacteremic patients, 28 (100%), 21 (75%), and 18 (64%) patients were positive by TP test, Tubex, and Typhidot, respectively. In healthy endemic zone controls, the TP test method was negative in all, whereas Tubex and Typhidot were positive in 3 (15%) and 5 (25%), respectively. The sensitivity and specificity of all diagnostic tests were calculated using Bayesian latent class modeling. The sensitivity of TP test, Tubex, and Typhidot was estimated at 96.0%, 60.2%, and 59.6%, respectively. Specificity was estimated at 96.6% for TP test, 89.9% for Tubex, and 80.0% for Typhidot.[18] In this study, the sensitivity, specificity, positive predictive value, and negative predictive value of Widal test for diagnosis of enteric fever were found to be 83.3%, 80%, 86.2%, and 76.2%, respectively.

CONCLUSION

This study showed that immunochromatography test (Typhifast) has a very good specificity, but the sensitivity is low. However, as it is easy to perform and can be done in field setting, it may be used in certain places where other methods of diagnosis of enteric fever are not available or feasible. Widal test, an age-old test, has a relatively good sensitivity and specificity, especially from 2nd week of illness and can still be used for the diagnosis of enteric fever.

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How to cite this article: Sengupta M, Sengupta M. Comparison of Three Different Tests for Diagnosis of Enteric Fever. Int J Sci Stud 2018;5(12):65-69.

Source of Support: Nil, **Conflict of Interest:** None declared.