A comparative study of blood culture and immunochromatographic assay for rapid diagnosis of typhoid fever

Farjana Akter1, Nasima Akter2, Shakeel Ahmed3, Chusung Ching Marma4, Syed Mohiuddin5

1Department of Microbiology, Abdul Malek Ulkal Medical college, Noakhali, Bangladesh, 2Department of Microbiology, Chattogram Medical College, Chattogram, Bangladesh, 3Department of Microbiology, Bangladesh Institute of Tropical and infectious diseases (BITID), Chattogram, Bangladesh, 4Department of Clinical pathology, Shaheed Suhrawardy medical college Hospital, Dhaka, Bangladesh, 5MATS, Noakhali, Bangladesh.

Abstract

Background and objectives: Typhoid fever, caused by Salmonella Typhi, is an important cause of morbidity and mortality in many developing countries including Bangladesh. As early diagnosis of disease and prompt treatment are essential for optimal management, a rapid method is urgently needed. Blood culture is the gold standard for diagnosis of typhoid fever, but it is time consuming. Immunochromatographic test (ICT) is easily accessible, cheap and simple method for diagnosis of typhoid fever, especially in the resource poor countries like Bangladesh.

Methodology: 180 samples were collected from suspected typhoid fever cases. This study was conducted to isolate the S. Typhi by blood culture and to detect IgM and IgG antibody to S. Typhi by ICT method.

Results: Out of 180 samples, 105(58.33%) were positive in ICT and 24(13.33%) were positive in blood culture. Considering blood culture as gold standard, the sensitivity, specificity, positive predictive value of ICT was 91.67%, 46.79%, 20.95% and 97.33% respectively.

Introduction

Typhoid fever is a major cause of death worldwide with a major part of the disease burden in developing regions such as the Indian subcontinent1. The highest disease burden of typhoid fever caused by Salmonella Typhi is seen in young children. The relative risk for children compared with older persons was 8.92. Since all the sign symptoms of typhoid fever are non-specific, a definitive diagnosis of the disease depending on the clinical presentation alone is difficult. Therefore, laboratory-based investigation are essential for supporting the diagnosis of typhoid fever. Blood culture is the Gold standard method of diagnosis of typhoid fever3. Rapid and sensitive laboratory method for diagnosis of typhoid fever is essential for prompt and effective therapy. An early diagnosis and prompt treatment are mandatory not only for diagnosis but also for prevention of the spread of the organism by chronic carriers to the community. However, Blood culture is insufficiently sensitive, technically demanding and bone marrow culture although more sensitive, is infrequently performed4. Organisms can usually be detected in 75-90% of patients during the first ten days of infection5. Blood culture is 100% specific for diagnosis of typhoid fever6. Therefore, a fast, reliable and easy to perform serodiagnostic test with a higher sensitivity and specificity than Widal test is required for diagnosis of typhoid fever7. The rapid immunodiagnosis of typhoid fever can be done by the detection of anti-salmonella antibodies by immunochromatographic test which does not require any specialized laboratory or highly skilled personnel and can be done in field areas also. It is a simple and rapid diagnostic test. In ICT, S. Typhi specific antihuman immunoglobulin against IgG and IgM fixed in the strip can detect both IgM and IgG anti-salmonella antibodies4. The test simultaneously detects and differentiates the IgG and IgM antibodies to S. Typhi specific antigen in whole blood or serum8.

Correspondence:
Dr. Farjana Akter
Mobile: 01675180006
E-mail: farjanamuna1990@gmail.com
Rapid and sensitive laboratory method for diagnosis of typhoid fever is essential for prompt and effective therapy. Keeping these areas of concern in mind, this study has been designed to compare conventional blood culture with serological test (ICT) for the rapid and reliable diagnosis of typhoid fever.

Materials and Method
This cross sectional study was carried out at Department of Microbiology, Chattogram Medical College, Chattogram, Bangladesh over a period of one year from July 2018 to June 2019.

A total of 180 blood samples from patients clinically suspected to have typhoid fever were collected for both culture and serological test.

The blood culture was performed by inoculation of 30 ml of tryptcase soya broth with 3 ml blood in children and 50 ml of tryptcase soya broth with 5 ml of freshly collected blood in adult. The bottles were examined visually daily. Growth was indicated by haemolysis of red blood cells, gas bubbles in the medium or turbidity in the broth. In addition to daily visual examination, blind subculture from conventional bottle after the first 24 hours of incubation was performed by aseptically removing a few drops of the well-mixed medium and spreading the inoculums into MacConkey agar and Blood agar plates. Plates were incubated at 37°C aerobically for 24-48 hours. Culture negative bottles were reincubated for 5 to 7 days5. Presumptive identification of the colonies of S. Typhi on MacConkey agar media and Blood agar media were done by colony morphology, oxidase test and Gram staining from growth. S. Typhi was confirmatively identified by using triple sugar iron (TSI) medium, citrate utilization test and test on MIU media10.

The immunochromatographic test was carried out as manufacturer’s instruction (Acro, Biotech, INC, USA). 1 drop of serum was put to the sample well of the test unit, then 1 drop of sample diluents added immediately. The test unit was kept for 15 minutes. Results were read after 15 minutes. Positive result was visible within 1 minute.

Results
From the 180 clinically suspected cases, 108 (60%) were male and 72 (40%) were female. Out of 180 clinically diagnosed cases, 24 (13.33%) cases were blood culture positive for Salmonella Typhi and the remaining 156(86.67%) cases were negative. Among culture positive cases, most cases belongs to 6-10 years of age which is 25% followed by 20.56% cases belonging to 1-5 years of age. Of 180 suspected cases of typhoid fever, ICT was positive by 105 (58.33%) cases whereas only IgM were positive for 76 cases and both IgM and IgG were positive by 29 cases. Here, only IgM positive and both IgM and IgG positive were considered as positive.

To calculate the diagnostic validity of ICT for the diagnosis of typhoid fever taking blood culture as a gold standard, it was found that, of 180 cases, 22 cases were positive by both blood culture and ICT whereas, out of 156 culture negative cases, 83 (46.11%) cases were only positive by ICT.

In this study, 22(12.22%) cases were true positive and 02(1.11%) were found false negative.

Here, out of 180 cases, 73(40.56%) clinically suspected typhoid fever cases found were both blood culture and ICT negative.

After calculation it was found that the sensitivity, specificity, positive predictive value and negative predictive value of ICT were 91.67%, 46.79%, 20.95% and 97.33% respectively.

Table 1: Diagnostic validity of ICT

<table>
<thead>
<tr>
<th>Test results</th>
<th>ICT</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture positive =24(13.33)</td>
<td>22(12.22)</td>
<td>91.67%</td>
<td>46.79%</td>
<td>20.95%</td>
<td>97.33%</td>
</tr>
<tr>
<td>Culture negative =156(86.67)</td>
<td>83(46.11)</td>
<td>73(40.56)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total = 180(100)</td>
<td>105(58.33)</td>
<td>75(41.67)</td>
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</tbody>
</table>

Figures in parenthesis indicate percentage
(p value=0.000003743, p <0.05 by chi-square test)

Discussion
Typhoid fever is an acute systemic infection caused by the bacterium Salmonella enterica serovar Typhi and a human host restricted organism11. Typhoid fever is transmitted by the feco-oral route via the contaminated food and water and is therefore common where sanitary conditions are inadequate and access to clean water is limited12. Although, blood culture is the Gold Standard for diagnosis of typhoid fever, but isolation of the organism by blood culture is quite time consuming and blood culture facility
are not well established in most of the areas. On the other hand, ICT is simple, rapid and easy to perform for diagnosis of typhoid fever.

In the present study, 180 suspected cases of typhoid fever of all age of both sexes irrespective of antibiotic therapy were enrolled for the detection of typhoid fever. Of them, 24(13.33%) cases were found positive for S. Typhi. Similar findings were also reported from India, 14% blood culture positive and 12.22% culture positive. The relative low rate of isolation from blood may be because of factors such as the bacteriostatic effect of antimicrobials which were already administered to some patients before blood culture was done, the time of sample collection, the type of culture medium used, the host immune response and the intracellular characteristics of S. Typhi. In contrast to our study, 24 (57.14%) culture positive cases among 42 suspected typhoid fever cases were found. Differences may be due to small sample size.

In present study, 105 (58.33%) cases were found positive for ICT among 180 clinically suspected cases of typhoid fever where we considered only IgM positive and both IgM and IgG positive as positive. Similar study from Pakistan was observed where 55.2% were positive by ICT. In contrast to our study, another study from Bangladesh found only 24.7%.

In our study, among 24 culture positive cases, 22 (91.67%) were positive by ICT. Another study from Bangladesh found 54 (93.1%) ICT positive cases out of 58 culture positive cases. ICT negative result in culture positive cases was probably due to the fact that antibodies did not yet reach the detectable level.

In our study, considering blood culture as “Gold Standard” the sensitivity, specificity, positive predictive value and negative predictive value of ICT were 91.67%, 46.79%, 20.95% and 97.33%. Another study carried out in India reported that the sensitivity, specificity, positive predictive value and negative predictive value of ICT were 96.43%, 54.4%, 25.96% and 98.92%. It is therefore, concluded that the ICT method is superior to blood cultures yielding very high sensitivity. Detection of antibody from whole blood by ICT method is easier, simple and non-invasive. ICT method is useful for early initiation of treatment. It should be adopted in routine clinical investigation for rapid diagnosis of typhoid fever in resource poor settings.

Acknowledgements
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Conflict of interest
There is no conflict of interest.

References
9. Kawano RL, Leano SA, Agdamag DMA. Comparison of serological kits for diagnosis of typhoid fever in the...
Typhoid fever is an acute systemic infection caused by the host-restricted organism Salmonella Typhi and a human host. It is transmitted by chronic carriers to young children. The highest disease burden of typhoid fever is seen in the Indian subcontinent. The disease is transmitted by contaminated food and water, and it is a public health problem in resource-poor settings. Blood culture is the Gold Standard method of diagnosis of typhoid fever, but isolation of the organism by blood culture is quite time consuming and blood culture facility is not available in many places due to lack of infrastructure. Therefore, laboratory-based investigation are essential for rapid and effective therapy. Rapid and sensitive laboratory methods for diagnosis of typhoid fever are non-specific, a definitive diagnosis of typhoid fever is essential for prompt and effective therapy. Immunodiagnosis of typhoid fever can be done by rapid and sensitive laboratory methods. Widal test is required for diagnosis of typhoid fever. The test simultaneously detects and differentiates the IgG and IgM antibodies against Salmonella Typhi. Although, blood culture is the Gold Standard for diagnosis of typhoid fever, but isolation of the organism by blood culture is quite time consuming and blood culture facility is not available in many places due to lack of infrastructure. Therefore, laboratory-based investigation are essential for rapid and effective therapy. Rapid and sensitive laboratory methods for diagnosis of typhoid fever are non-specific, a definitive diagnosis of typhoid fever is essential for prompt and effective therapy. Immunodiagnosis of typhoid fever can be done by rapid and sensitive laboratory methods. Widal test is required for diagnosis of typhoid fever. The test simultaneously detects and differentiates the IgG and IgM antibodies against Salmonella Typhi. Although, blood culture is the Gold Standard for diagnosis of typhoid fever, but isolation of the organism by blood culture is quite time consuming and blood culture facility is not available in many places due to lack of infrastructure. Therefore, laboratory-based investigation are essential for rapid and effective therapy. Rapid and sensitive laboratory methods for diagnosis of typhoid fever are non-specific, a definitive diagnosis of typhoid fever is essential for prompt and effective therapy. Immunodiagnosis of typhoid fever can be done by rapid and sensitive laboratory methods. Widal test is required for diagnosis of typhoid fever. The test simultaneously detects and differentiates the IgG and IgM antibodies against Salmonella Typhi.

**Materials and Method**

Keeping these areas of concern in mind, this study has been designed to compare conventional blood culture with the immunochromatographic assay (ICT) for diagnosis of typhoid fever. From the 180 clinically suspected cases, 108 (60%) were culture positive, 22 (12.22%) cases were true positive and 24 (13.33%) cases were found positive for ICT. In a study from Bangladesh, 54.4%, 25.96% and 98.92% of clinical cases were culture positive, ICT positive and ICT negative, respectively. In contrast to our study, another study from southern Bangladesh reported that the sensitivity, specificity, positive predictive value and negative predictive value of ICT among 180 clinically suspected cases of typhoid fever were 75.6%, 86.67%, 75.58% and 86.67%, respectively. In this study, 22 (12.22%) cases were true positive and 24 (13.33%) cases were found positive for ICT. In this study, 22 (12.22%) cases were true positive and 24 (13.33%) cases were found positive for ICT. In this study, 22 (12.22%) cases were true positive and 24 (13.33%) cases were found positive for ICT.

**Discussion**

It is therefore, concluded that the ICT method is superior in detecting typhoid fever. The ICT has the advantage of being simple, rapid and easy to perform. The ICT does not require any special equipment and is easy to use in the field. The ICT is a qualitative test and does not require any interpretation. The ICT is a rapid test and can be performed in 15 minutes. The ICT is also a cost-effective test and can be performed at a low cost. The ICT is also a specific test and only detects typhoid fever. The ICT is also a sensitive test and can detect even low levels of Salmonella Typhi.

**References**