

Comparative evaluation of rapid *Salmonella* Typhi IgM/IgG and Widal test for the diagnosis of enteric fever

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Abstract

Background: Accurate and early diagnosis of enteric fever is a diagnostic challenge where facility for blood culture is not available. As a result, Widal test is still used widely in resource limited settings. Recently, user-friendly rapid immunochromatographic tests (ICT) have been introduced for quick diagnosis of enteric fever. So, we evaluated sensitivity and specificity of an immunochromatography based *Salmonella* Typhi IgM/IgG test kit and Widal test compared to blood culture for the diagnosis of enteric fever.

Method: The study was conducted in the Department of Microbiology, Ibrahim Medical College (IMC) and Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM) from June 2017 to September 2017. Clinically suspected enteric fever cases were included. Blood culture, Widal and *Salmonella* Typhi IgM/IgG detecting ICT were employed for the diagnosis of enteric fever.

Results: Out of 71 suspected cases of enteric fever, blood culture was positive in 36 cases (50.7%) while 42 (59.15%) and 35 (49.29%) cases were positive by Widal test and ICT respectively. Widal and ICT had sensitivity and specificity of 100% and 89.9% and 82.9% & 91.4% respectively.

Conclusion: Findings of the study suggest that both Widal and immunochromatographic tests can be used interchangeably for rapid diagnosis of enteric fever.

IMC J Med Sci 2020; 14(1): 004. Epub date: 29 February 2020

Introduction

Enteric fever is a multisystem disease and its outcome can be fatal if not properly diagnosed and treated [1]. It is predominantly caused by *Salmonella enterica* serotype Typhi and less frequently by *Salmonella enterica* serotype Paratyphi A and B [2]. Lack of access to safe drinking water, unhygienic sanitation, and overcrowded population of underdeveloped countries may accelerate its feco-oral transmission [3]. Physicians often experience

diagnostic dilemma due to its protean clinical presentation which is quite similar to other febrile illness like dengue, malaria, chikungunya etc. in endemic area [4]. Prompt and accurate diagnosis of enteric fever is a pressing need albeit no such diagnostic test is currently available that can provide 100% sensitivity and accuracy. Diagnosis at an early stage can reduce indiscriminate antibiotic use; prevent unwanted life threatening complications and chronic carrier state [5]. Amidst available

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diagnostic test, isolation of organisms from blood, bone marrow, urine and stool is considered gold standard for diagnosis of enteric fever [6,7]. Culturing of organism from blood is frequently done in clinical setting which is insufficiently sensitive, laborious and time consuming and bone marrow culture, although more sensitive is not done routinely due to its high technical demand [8,9]. In spite of considering blood culture as a gold standard test, it is not available in every primary health care setting. Moreover, its turnaround time is longer, usually 2-3 days. As a result, diagnosis of enteric fever overlooked or delayed and based on clinical features, clinicians often provide unnecessary antimicrobial therapy or undertreat the patients when other differentials are considered [10,11]. Therefore, rapid, simple, convenient, easy to perform, sensitive serological test to identify *Salmonella* Typhi and Paratyphi is often considered as the only diagnostic tool that can guide clinicians [12].

Most routinely performed serological test is Widal which was developed by Georges Fernand Widal in 1890 based on the demonstration of agglutinating antibodies against lipopolysaccharide (LPS; O) and flagella (H) antigens of *Salmonella* Typhi and Paratyphi A and B. This test became obsolete in many developed countries due to its unsatisfactory results, low prevalence of enteric fever and availability of more sophisticated diagnostic tools [13]. Variable sensitivity and specificity of Widal test was documented in different studies and its role as a diagnostic tool is still debatable. However, some studies conducted in Tanzania, Vietnam, Bangladesh, India during different periods of time stated that Widal test could be relevant as a diagnostic tool and could be an alternative to blood culture [14-17]. On the other hand, findings of other studies conducted in Pakistan, Nepal, South Africa, Tanzania, and Ethiopia indicated that Widal test alone might not be suitable to diagnose enteric fever as it could produce false positive results [18]. But scenario is quite different in developing countries like Bangladesh where Widal test is still used widely as facility of culturing organism is limited to only in tertiary care hospitals, lack of trained personnel and prohibitively high cost of culture compared to serological test [16].

User friendly rapid diagnostic tests (RDT) for diagnosis of *Salmonella* Typhi are available commercially in

different methods and formats like ELISA or immunochromatography based tests (ICT) which can directly detect IgM and/or IgG antibodies against specific antigen of *Salmonella* Typhi [19]. It can also detect antibodies within 4-5 days of appearance of fever and ICT can provide results within 15-30 minutes. ICT is user-oriented, time saving and does not require highly skilled personnel to perform the test and to interpret the result which makes it an excellent choice for point of care service [20]. But these kits are still not widely acceptable due to its inconsistent sensitivity (73-95%) and specificity (68-95%) which have been documented in different studies conducted preliminarily in different Asian countries [21-25].

Therefore, the aim of the current study was to evaluate the sensitivity and specificity of both Widal test and rapid *Salmonella* Typhi IgM/IgG immunochromatographic test in comparison to blood culture for quick and accurate diagnosis of typhoid fever.

Materials & Methods

Study population, place and samples collection:

This study was conducted in the Department of Microbiology, Ibrahim Medical College (IMC) and Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM) from June 2017 to September 2017. Total 71 blood samples were collected from suspected cases of enteric fever for blood culture, Widal test and *Salmonella* Typhi IgM/IgG immunochromatographic test. From each patient 10 ml of venous blood was collected aseptically for blood culture and serological tests. Informed written consent was obtained from each patient prior to collection of blood.

Blood culture: An aliquot (8 ml for adult and 1.5 ml for children) of fresh blood was immediately processed for culture. Blood culture was done by lysis centrifugation method and inoculated on blood agar and MacConkey agar media and incubated for 48 hours at 37°C [25,26]. Suspected bacterial colony was identified by Gram staining and standard biochemical tests [25,27]. Serotype of *Salmonella* spp. was identified by slide agglutination test by specific 'O' (lipopolysaccharide), 'H' (flagella) antisera [27].

Widal test and Salmonella IgM/IgG ICT: Widal test was carried out by slide method using HiPer® Widal Test Teaching Kit (HiMedia Laboratories Pvt. Limited, India) according to the manufacturer's instruction. Test results were interpreted visually by demonstrating agglutinating antibody titres against 'O' (lipopolysaccharide) and 'H' antigen (flagella) of *Salmonella* spp. An antibody titre of 1:80 or more against 'O' and 'H' antigen was considered positive [28].

Enteroscreen-WB ICT kit Typhi manufactured by Zephyr Biomedical (Verna Industrial Estate, Verna, Goa, India) was used to detect *Salmonella* IgM/IgG antibodies against an outer membrane protein of *Salmonella* Typhi. Test was carried out as per manufacturer's instruction and reading was taken after 15-30 minutes based on appearance of coloured band in the control region and test region. The band in test region represented presence of either anti-*Salmonella* IgM or IgG. ICT was considered positive if any anti-*Salmonella* IgM, IgG or IgM+IgG band appeared positive in any sample. The result was compared with blood culture and Widal test.

Results

Total 71 suspected cases of enteric fever were included in the study. Out of 71 cases, blood culture, Widal and ICT were positive in 36 (50.7%), 42 (59.2%) and 35 (49.3%) cases respectively (Table-1). Out of 36 blood culture positive cases, *Salmonella* Typhi was isolated from 32 cases and *Salmonella* Paratyphi A was present in 4 cases (Table-2).

Widal test was positive in all *S. Typhi* and *S. Paratyphi A* positive cases. Out of 35 culture negative cases, 6 cases were Widal test positive as well. Widal test showed more than 1:80 titre of TO/TH in all *S. Typhi* culture positive cases and

higher titre of 'AO'/'AH' was observed in all *S. Paratyphi A* cases. Although TO/TH is specific to *S. Typhi*, higher titre was also observed in all culture positive cases of *S. Paratyphi A* and at the same time titre of AO/AH which was specific to *S. Paratyphi A* was raised in 11 cases of *S. Typhi*. ICT for *Salmonella* IgM/IgG was performed in all 71 cases. ICT was positive in total 32 (88.9%) out of 36 blood culture positive cases. Out of 32 *S. Typhi* positive cases, 29 cases were positive by ICT and 3 were negative by ICT. On the other hand among 4 *S. Paratyphi A* positive cases, 3 showed positive result in ICT (Table-2).

Table-1: Results of blood culture, Widal and *Salmonella*-IgM/IgG ICT tests (n=71)

Test	Positive Number (%)	Negative Number (%)
^a Blood culture for <i>Salmonella</i> spp.	36 (50.7%)	35 (49.3%)
Widal Test	42 (59.2%)	30 (40.84%)
<i>Salmonella</i> -IgM/IgG ICT	35 (49.3%)	36 (50.71%)

Note: a = includes *S. Typhi* and *S. Paratyphi A*

Only anti-*Salmonella* IgM, IgG and both IgM and IgG were positive in 3 (4.2%), 18 (25.4%) and 14 (19.7%) cases respectively (Table-3). Total 32 cases (45.1%) were IgG positive, 17 (23.9%) were IgM positive and 14 (29.17%) were both IgM and IgG positive (Table-3). The sensitivity and specificity of Widal and *Salmonella* IgM/IgG ICT are shown in Table-4. The sensitivity and specificity of Widal test were 100% and 82.9% respectively while these were 88.9% and 91.4% for *Salmonella* IgM/IgG ICT. *Salmonella* IgM/IgG test had higher (91.4%) positive predictive value (PPV) compared to Widal test (85.7%).

Table-2: Comparative results of blood culture, Widal test and *Salmonella*-IgM/IgG ICT (n=71)

Blood culture result	No	Widal test		ICT for <i>Salmonella</i> -IgM/IgG	
		Positive	Negative	Positive	Negative
<i>S. Typhi</i>	32	32	0	29	3
<i>S. Paratyphi A</i>	4	4	0	3	1
Negative	35	6	29	3	32
Total	71	42 (59.2%)	29 (40.8%)	35 (49.3%)	36 (50.7%)

Table-3: Rate and pattern of *Salmonella* IgM/IgG antibodies by ICT in study cases (n=71)

ICT	Positive Number (%)
Only IgM	3 (4.2%)
Only IgG	18 (25.4%)
Both IgG and IgM	14 (19.7%)

Note: Total IgM positive: 17 (23.9%); Total IgG positive: 32 (45.1%).

Table-4: Sensitivity, specificity, PPV and NPV of Widal and *Salmonella* IgM/IgG tests

Test	Sensitivity	Specificity	PPV	NPV
Widal	100%	82.9%	85.7%	100%
<i>Salmonella</i> IgM/IgG ICT	88.9%	91.4%	91.4%	88.9%

Note: PPV: Positive predictive value; NPV: Negative predictive value.

Discussion

Isolation of *Salmonella* Typhi and Paratyphi A and B from blood for diagnosis of enteric fever is the current recommendation of WHO and considered as a reference while evaluating other tests [29]. Blood culture is highly specific but its suboptimal sensitivity after the first week of illness leads to the diagnostic difficulty and sensitivity [30]. In this study, it has been observed that blood culture for *Salmonella enterica* serotype Typhi and Paratyphi A was found to be positive in 50.7% cases which is quite similar to other study findings where 40-70% of presumptive cases were found culture positive [7,31-35]. In contrast to these study findings, rate of isolation of *Salmonella* spp. was found much lower ranging from 8.9-43% in many well documented studies [36-39]. This low rate of isolation may be attributed to negligence of seeking health care services at an early stage of fever, inappropriate use of antibiotic before blood culture and collection of inadequate amount of blood especially in case of children [40].

In this current series, both Widal test and ICT (Enteroscreen-WB) were performed in 71 clinically suspected cases of enteric fever to evaluate their sensitivity, specificity, positive predictive value

(PPV) and negative predictive value (NPV) compared to blood culture. Out of 42 Widal positive cases 36 cases were blood culture positive and 6 cases were blood culture negative. Sensitivity, specificity, PPV and NPV of Widal were noted as 100%, 82.9%, 85.7% and 100% respectively. In this study, the sensitivity of Widal was found higher though specificity was slightly reduced and was in accordance with the reported results of blood culture. Gopala kirshnan et al. [41] in 2002 reported sensitivity and specificity of Widal test as 98% and 76% respectively which closely resemble our study findings. Another study reported the sensitivity and specificity of Widal test as 71% and 62% [17]. In 2016, a study from Bangladesh reported the sensitivity, specificity, PPV and NPV of Widal test as 83.3%, 80%, 86.2%, and 76.2% respectively [42]. These study findings revealed fairly good diagnostic accuracy of Widal test for diagnosis of enteric fever.

In the present study raised titre of TO/TH of more than 1:80 was observed in all *Salmonella* Paratyphi A positive cases and AO/AH in 11 *Salmonella* Typhi positive cases. This may be due to cross reacting antigen between these serotypes. Moreover, lipopolysaccharide 'O' antigen also shared by other Enterobacteriaceae which results in false positive Widal test making the test less specific to detect *Salmonella* spp.

Several studies have claimed that rapid diagnostic tests (RDT) provide better valid results than Widal test with regard to sensitivity and specificity [22, 43]. In our study with lateral flow rapid *Salmonella* IgM/IgG ICT, the sensitivity, specificity, PPV and NPV were recorded as 88.89%, 91.43%, 91.43% and 88.89% respectively. According to a study done by Sanjeev et al. [44], Typhi-dot performed better than Widal test and they found sensitivity and specificity of Widal and Typhi-dot as 100% and 76% and 78.78% and 58.82% respectively. They suggested that rather than using Widal test it might be more useful to use rapid test like Typhi-dot in routine diagnostic service besides blood culture. Studies from Bangladesh reported similar rate of sensitivity and specificity of a rapid ICT (SD Bioline) and TUBEX® for the diagnosis of typhoid fever [45,46]. This is in agreement with our findings. In contrary to these findings, Neheed et al.

demonstrated suboptimal performance of Typhi-dot and TUBEX® to diagnose typhoid fever among community populations [47]. Dissimilarity regarding the sensitivity and specificity observed in different studies could be due to the use of different format of rapid diagnostic test kit from different manufacturers. In addition, time elapsed from onset of symptoms and performance of test may affect sensitivity and specificity of ICT.

Among the available different RDT kit, diagnostic accuracy of Typhi-dot and TUBEX® were largely studied. Very limited studies were conducted where performance of Enteroscreen ICT (Bioline) was analysed. Prasad et al. included 2699 patients in their study to compare the diagnostic validity of two rapid *Salmonella* IgM tests with regard to blood culture [48]. Sensitivity, specificity, PPV and NPV of Typhi-dot and Enteroscreen were recorded as 97.29%, 97.40%, 98.18% and 96.15% and 88.13%, 87.83%, 92.03% and 82.27% respectively. Though Enteroscreen performed poorly in comparison to Typhi-dot, they recommend Enteroscreen during emergency situation due to its acceptable PPV and it takes less time to provide results [48]. Our data matched well with these values. On the other hand, another study mentioned sensitivity, specificity, PPV and NPV of Enteroscreen ICT in comparison to Widal as gold standard test as 50%, 96%, 66.66% and 92.30% respectively. Specificity and NPV of ICT were similar to our study while they found poor sensitivity and PPV of this particular ICT kit [49]. Although this ICT kit is meant to detect *S. Typhi* only, but in this study we observed that out of 4 *S. Paratyphi A* cases, 3 (75%) were positive by this kit. This could be attributed to cross reactivity between outer membrane protein of both *Salmonella* spp. Prasad et al. (2015) noted Enteroscreen ICT positive results in 22 cases of 46 blood culture positive *S. Paratyphi* cases and sensitivity was 47.83% in their study [48]. This cross reactivity provides extra advantages of diagnosing paratyphoid fever by Enteroscreen ICT kit.

The ICT kit that we have used is able to differentiate between IgM and IgG antibodies. This study demonstrated that only IgM became positive in 4.2% cases, only IgG in 25.4% cases and both IgM and IgG was found positive in 19.7% cases. Only

IgM (early phase) or both IgM and IgG positive (middle phase) indicates current infection and IgG without IgM usually denotes past, reinfection or late stage disease when sero-conversion has already been occurred. In this study we observed the percentages of IgG positive cases among culture positive group was high, but in other studies conducted through different ICT kit found more IgM positive cases [45]. This could be explained by the disappearance of IgM in the late stage of disease or masking of IgM by IgG [50].

In our study, Enteroscreen *Salmonella* IgM/IgG rapid test showed satisfactory results in terms of sensitivity and specificity compared to Widal test. Our results differ to some extent from above mentioned studies and this might be due to small sample size and collection of blood at different stage of fever. So, these serological tests may be used interchangeably with Widal test in suspected enteric fever where an adequate laboratory facility for blood culture is not available.

Development of convenient, rapid, highly sensitive, specific and robust diagnostic tool is a long felt need to diagnose enteric fever accurately at an early stage of disease. In this regard, rapid serological diagnostic tests in ICT format with considerable sensitivity and specificity can play a fundamental role, especially in resource-constrained rural settings of Bangladesh. At the same time, usefulness of Widal test in area with limited microbiology laboratory facilities cannot be ignored. Although clinical implication of Widal test is reducing day by day, but in some areas, it is the only available test in which clinicians have to rely to reach a diagnosis of enteric fever. Our work has led us to conclude that Widal test is justifiable as long as the results are interpreted in accordance with the clinical history indicative of enteric fever and background level of antibody titres of local populations are considered. At the same time introduction of ICT might be an important addition to serological test for more rapid and reliable diagnosis of enteric fever.

Acknowledgement

We are grateful to Tradesworth Ltd. Bangladesh for providing Enteroscreen-WB ICT kit.

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