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How Useful is the Widal Test in Modern Clinical Practice in Developing Countries? A Review

Abiodun Christopher Jemilohun[1*], Adebukola Temilade Adeyanju[2] and Modupeola Maria Bello[2]

[1]Department of Internal Medicine, Babcock University Teaching Hospital, Ilisan-Remo, Ogun State, Nigeria.
[2]Department of Medicine, LAUTECH Teaching Hospital, Ogbomoso, Oyo State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author ACJ designed the study, wrote part of the initial manuscript, managed literature search, and performed a critical review of the whole manuscript. Authors ATA and MMB performed literature search and wrote part of the initial manuscript. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Background: It has been over a century since the Widal test was developed for diagnosing typhoid fever. Yet, the test remains the major means of diagnosing the disease in many of the developing countries where it remains endemic. This review appraises the Widal test in regard to its performance techniques, its various drawbacks and the available alternative diagnostics methods.
Methods: The study was a non-systematic review. A literature search was conducted for relevant original and review articles primarily in MEDLINE database through PubMed. Relevant references in the articles at hand were searched manually with Google search engine. Related articles during the manual search were also reviewed. Inclusion criteria were the date of publication from 2,000 to 2017, original research conducted on human subjects and publication in the English Language. All articles that did not meet these criteria were excluded.
Results: The Widal test is a relatively cheap and readily available test in developing countries where more sophisticated tests like culture and polymerase chain reaction are either not available

**Corresponding author: Email: chrislohun2010@hotmail.com, chrislohun@gmail.com;*

or unaffordable where available. It is, however, difficult to interpret the result because of various reasons that may cause either a false positive or a false negative result. Although several alternative rapid diagnostic tests are now available, there is still no sufficiently reliable one that can replace the traditional diagnostic gold standard, which is culture isolation of the organism. **Conclusion:** The Widal test is grossly inadequate to be relied upon as a diagnostic tool for typhoid fever in an endemic area, culture isolation of the causative organism remains the gold standard for diagnosing the disease, and the quest to develop highly effective rapid diagnostic tests for the disease should continue.

Keywords: Widal test; typhoid fever; enteric fever; typhoid rapid diagnostic tests.

1. INTRODUCTION

Typhoid fever continues to be a major global health problem, most especially in the developing countries of the world where the annual incidence could range from 10/100,00 to above 100/100,00 per annum [1].

The Widal test was developed by Georges-Fernand-Isidore Widal, a French physician and bacteriologist, in 1896 [2,3]. It is a serological agglutination test which has been used for over a century in the diagnosis of typhoid fever [2,3]. Although the Widal test played a major role in the diagnosis of typhoid fever in the past in the developed nations, it is no longer relevant as a diagnostic tool because of the low prevalence of typhoid fever, availability of safe drinking water, good sewage facilities, improved laboratory facilities to isolate the organism, and the low diagnostic accuracy of the test in such countries [1,4–6]. However, the test continues to be the commonest diagnostic method for the disease in many of the poor developing countries where it is endemic [7–9].

Ideally, a fourfold rise of antibody in paired sera is considered diagnostic but the test is often used on a single acute-phase serum sample despite several reports that it lacks adequate diagnostic accuracy in regions where typhoid is endemic when used in this way [10]. Medical practitioners in endemic areas often use of Widal test to diagnose typhoid fever because it is relatively cheap, easy to perform and requires minimal training as compared to more sophisticated tests like culture and polymerase chain reaction (PCR) [5]. Inadequate knowledge and sheer force of habit may also be contributory to this practice.

This review, therefore, appraises the Widal test, the alternative diagnostic methods and their applicability in modern clinical practice with the intent to bring to light the various pitfalls and limitations of the test. We do hope that this review will serve as an educational material for the clinician, the laboratory worker and the general public at large.

2. METHODS

The study was a non-systematic review. A literature search was conducted for relevant original and review articles primarily in MEDLINE database through PubMed. Keywords employed for the search were 'Typhoid fever', "Widal test", "Widal agglutination", and "Typhoid fever" and "Rapid diagnostic" [Table 1]. Relevant references in the articles at hand were searched manually with Google search engine. Related articles during the manual search were also reviewed. Inclusion criteria were the date of publication from 2,000 to 2017, original research conducted on human subjects and publication in the English Language. All articles that did not meet these criteria were excluded.

Articles were selected to answer four principal questions: typhoid fever in relation to its etiology and epidemiology; Widal test performance, pitfalls, interpretation, and misuse; typhoid rapid diagnostic test; and the standard methods of diagnosing typhoid fever.

The references were stored in an electronic reference manager (Mendeley) and kept for subsequent use.

3. RESULTS

3.1 Typhoid Fever

Typhoid fever, also known as enteric fever, is a potentially fatal acute systemic illness characterized by fever and abdominal symptoms [11]. Though now rare in the industrialized countries of the world as a result of improved sanitation and food hygiene, the disease is still an important cause of morbidity and mortality in the developing countries [11].

The disease was initially called typhoid fever because of its clinical similarity to typhus fever

Table 1. Summary of the main search terms and results

Search	Search term	No. of papers	No. of papers included
1	"Widal test"	123	
2	"Typhoid fever"	2,529	10 extra (not in 1)
3	"Widal agglutination"	19	2 extra (not in 1 & 2)
4	"Typhoid fever" and "Rapid diagnostic"	29	4 extra (not in 1, 2 & 3)
5	Manual search	25	17
Total		2,725	52

[12–14]. The term "enteric fever" was proposed as a name for the disease in 1869 due to the anatomical site of the infection [13,14]. The two terms are, however, being used interchangeably.

Enteric fever is primarily caused by *Salmonella enterica serovar typhi* (*Salmonella typhi*). A less severe illness is caused by *Salmonella paratyphi* A and, less commonly by B and C [15]. *Salmonella typhi* is a gram-negative, non-encapsulated, facultative anaerobic, flagellated motile bacillus [16]. It has somatic (O), flagellar (H) and virulence (Vi) antigens [16]. The *S. paratyphi* also have similar antigens denoted by AH, BH and CH.

Typhoid fever is related to rapid population growth, increased urbanization, overcrowding, inadequate human waste treatment, limited water supply and overburdened health care systems. Majority of cases are sporadic, although large outbreaks do occur commonly resulting from breakdowns in water supplies and sanitation systems [16] In 2004, the WHO estimated the global burden of the disease at 21 million cases per annum, leading to an estimated 216,000–600,000 deaths annually [16]. The disease is endemic in the developing regions of the world: Indian subcontinent, South and Central America, Asia and Africa [11,16]. The world is divided into three regions according to the annual incidence of typhoid fever: high incidence (>100/100,000 cases/year) includes south-central Asia and south-east Asia; medium incidence (10-100/100,000 cases/year) includes the rest of Asia, Africa, Latin America and the Caribbean, and Oceania, except for Australia and New Zealand; and low incidence (<10/100,000 cases/year) includes Europe, North America, and the rest of the developed world [1].

Man is the main reservoir of the organism, largely current cases and chronic carriers who disseminate the organism in their feces [16]. The disease is acquired predominantly via the feco-oral route by ingestion of contaminated food or water [15]. Less common modes of transmission include sexual transmission (oral-anal, oral-penile, and receptive anal transmission) and transmission through the use of unsterilized instruments (endoscopes, polyvinyl duodenal tube or rectal tube) [17–19].

3.2 The Widal Test

The Widal test is an agglutination test that measures specific antibody titers in the serum. It involves the detection of *Salmonella* antibodies in the patient's serum by the use of bacterial suspensions of *S. typhi* and *S. paratyphi* A and B that have been treated to retain only the O (somatic) and H (flagellar) antigens [3]. The test, therefore, operates on the premise that patients with typhoid fever have antibodies in their sera that can agglutinate homologous antigens in killed *Salmonella* suspensions. The O agglutinins which are immunoglobulin M (IgM) antibodies appear early and represent the initial serological response in acute enteric fever while the H agglutinins which are immunoglobulin G (IgG) antibodies usually develop slowly and persist for a longer duration [3]. Killed colored suspensions of the *S. typhi* O antigen, *S. typhi* H antigen, *S. paratyphi* AH antigen and *S. paratyphi* BH antigen are routinely used for the test.

3.2.1 Widal test performance and methods

Two methods can be employed to perform the Widal test: the slide agglutination test and the tube agglutination test [3,20]. The tube agglutination test has better accuracy, but the slide test is commonly used because it is easier and faster to conduct [21].

3.2.1.1 Slide agglutination Widal test

The slide agglutination test could either be a qualitative or a quantitative test [3].

The qualitative test is used as a rapid screening test to determine the presence of *Salmonella typhi* and *paratyphi* antibodies in the serum of patients. A drop of the patient's serum is added

to an equal drop of each of the standard bacterial antigens (O, H, AH, BH) on a slide and thoroughly mixed with an application stick [3,20]. The slide is rotated gently and observed for agglutination. Physiologic saline and a previously reactive serum are used as negative and positive controls [20]. The appearance of agglutination to any of the antigens infers a positive test while non-agglutination implies a negative test. Agglutinations are visualized as clumps.

The quantitative test is usually done after the qualitative test (rapid screening test) has been performed. Only the previously detected *salmonella* antibody/antibodies in the patient's serum are then tested to determine their titers. A drop (0.03 ml) of the antigen suspension which previously showed agglutination is added to aliquots of 40 µl, 20 µl, 10 µl, and 5 µl of the patient's serum on a slide and thoroughly mixed with an applicator stick. The slide is then rotated gently and observed for reactions. Tests' results are scored from 0 to 4+ [0 (no agglutination), 1+ (25% agglutination), 2+ (50% agglutination), 3+ (75% agglutination) or 4+ (100% agglutination)] [3,22]. The smallest quantity of serum that shows a 2+ or 50% agglutination is chosen as the antibody titer of the test sample [3,22]. The presence of agglutination in 40 µl corresponds to 1:40, 20 µl to 1:80, 10 µl to 1:160 and 5µl to 1:320 titers respectively.

3.2.1.2 Tube agglutination Widal test

This is a more accurate but more cumbersome and time-consuming quantitative test in comparison to the quantitative slide test. It can be used to clarify ambiguous agglutination reactions gotten by the more rapid slide test [3].

Four sets of 8 test tubes are prepared for each antigen (O, H, AH, BH). The first test tube of each antigen set is then filled with 1.9 ml of physiological saline [20]. Next, 1 ml of physiological saline is added to the other tubes (tubes 2-8). To the first tube, 0.1 ml of the test sample (serum) is added and properly mixed. From tube 1, 1 ml of the diluted sample is transferred to tube number 2 and mixed properly. The dilution is continued serially up to the 7^{th} tube in each set of the four antigens. From tube 7 of each set, 1 ml of diluted serum is discarded. Hence, the dilution of the serum sample from tubes 1-7 respectively in each antigen sets are: 1:20,1:40,1:80,1:160,1:320,1:640,1:1280 [20]. Tube 8 serves as a negative control. To one set of test tubes, 50 µl of the O antigen is added (i.e. tubes no 1-8). Then, 50 µl of the H, AH and BH antigens are added to the 2nd, 3rd and 4th set of test tubes respectively and thoroughly mixed. The tubes are then covered and incubated overnight at 37°C for up to 20 hours [3,20]. After incubation, the sediments are dislodged and observed for agglutination. The control tubes are firstly examined and they must contain no agglutination. The results are scored from 0 to 4+ agglutinations as described above for the slide test. The highest dilution of serum to produce agglutination is taken as the titer. The titer for each of the antigens is noted and recorded.

3.2.2 Pitfalls of the Widal test

The Widal test is a relatively cheap and readily available test, especially in developing countries where more sophisticated tests like culture and PCR are either not available or unaffordable. It is, however, difficult to interpret because of the various reasons that may cause either a false positive (Table 2) or a false negative result (Table 3) [3,23–26].

Table 2. Causes of false positive Widal test

1	Vaccination against *Salmonella typhi* or *paratyphi*
2	Previous enteric fever
3	Substandard quality reagent
4	Cross-reaction with non-*Salmonella* infections like malaria
5	Cross-reaction with non-typhoidal *Salmonella* antibodies
6	Infection with other *Enterobacteriaceae*
7	Anamnestic reaction
8	Non-infectious chronic illnesses like rheumatoid arthritis and ulcerative colitis
9	Laboratory error

Table 3. Causes of false negative Widal test

1	Antibiotics use before test
2	Wrong timing e.g. test before the end of first week of infection
3	Substandard quality reagent
4	Laboratory error
5	"Hidden organisms" in bone and joints
6	Poorly immunogenic strains of infecting organism

Salmonella are grouped into different serotypes (A – E) based on the somatic O antigen (the Kaufmann-White scheme) [27]. There are at least 78 organisms in the serotype D to which *S. typhi* belongs. While all the 78 group D organisms have O antigen 9, about 60 including

S. typhi possess O antigen 12 [27]. Hence, infection by any of the group D serotypes could lead to the production of antibodies that can agglutinate the O antigen used in the conduct of the Widal test. Also, because all the groups A and B organisms possess O antigen 12, they could produce O antibodies that can agglutinate the Widal test O antigen. This cross-reaction with non-typhoidal *Salmonella* antibodies significantly reduces the specificity of the test and is a cause of false positive results. Several other non-Salmonella infectious diseases in the typhoid endemic regions like malaria, infective endocarditis, chronic viral hepatitis, dengue, brucellosis, miliary tuberculosis etc. have been demonstrated to exhibit this cross-reactivity that increases the false positive rate of the Widal test [3,26,28]. Cross-reaction with other *Enterobacteriaceae* also exists [3,6].

A study was conducted by Olopoenia *et al* to determine Widal agglutinin titers among a Nigerian population who had no prior typhoid immunization [3]. Among participants who had a positive malaria smear and a negative *S. typhi* culture 85% had 1:40, 12% had 1:80, and 3% had 1:160 Widal titers respectively. Whereas, of the participants that had both malaria smears and *S. typhi* cultures negative results 45% had 1:40, 15% had 1:80, and 10% had 1:160 Widal titers respectively. In essence, all the participants in the former group had at least a 1:40 Widal agglutination titer while 70% of the latter group had a similar response. This study further corroborates the fact that patient with malaria fever tend to have a false positive Widal agglutination reaction.

Non-infectious chronic illnesses like rheumatoid arthritis and ulcerative colitis can also give a false positive result [24,29]. Patients who have received vaccines against *Salmonella* may have false positive reaction but this can be differentiated from true infection by a repeat test [24]. True untreated infection usually results in a titer rise whereas vaccinated individuals do not demonstrate any titer rise. Individuals who had suffered from repeated exposure to small inocula of *S. typhi* or to other *Salmonella* species that contains type 9 or 12 O antigens could have a false positive result [3,23]. This can also be differentiated from true infection by lack of any rise in titer on test repetition. It should be noted, however, that the mandatory repeat Widal test after the initial positive test is usually not done in many of the places where typhoid fever is endemic. Also, individuals who had suffered from enteric fever in the past sometimes develop anti-*Salmonella* antibodies during non-typhoidal fever states. This is termed anamnestic reaction [23].

The timing of the test is very important as the antibodies begin to rise in the first week and titers increase through the second to fourth week after which they gradually decline. Hence, the test may yield a negative result in the early part of the first week. Patients who have been treated with antibiotics in the early stages may have a false negative result [3,25,26]. Such patients may also not show any rise in titer when they initially tested positive, instead there may be a fall in the titer. Occasionally, “hidden organisms” in bones and joints and poorly immunogenic strains of infecting organism may cause false negative results [24,25].

There seems to be poor standardization in the manufacturing process of the Widal antigens as variability in quality exists [3,29]. Bakr et al. compared the *Salmonella typhi* O and H antigens obtained from four different manufacturers by testing them against the same serum samples obtained from patients suspected to have typhoid fever [29]. There was a considerably variability in the results obtained from the four Widal brands in terms of sensitivity and specificity at three cut-off values of 1/80, 1/160 and 1/320. The study showed a significant variability in the agglutination titers of the antigens. This kind of variability could lead to either false positive or false negative results.

A study was conducted by Enabulele and Nyemike in a Nigerian population to determine the validity of a single Widal test as a diagnostic tool for enteric fever among febrile adults using blood culture as the reference standard [30]. Malaria parasite test was also done on each sample. Of the 271 participants, 124 (45.76%) were positive for the Widal agglutination test, 60 (22.14%) blood cultures grew *salmonella* and 55 (20.30%) had a co-infection of enteric fever and malaria. Out of the 124 that were positive for Widal test, only 21(16.94%) of them had a positive blood culture. Whereas, of the remaining 147 that were negative for Widal test, 39 (26.53%) of them had blood culture confirmation of enteric fever. For Widal agglutination test, a sensitivity of 35%, specificity of 51%, positive predictive value (PPV) of 17% and negative predictive value (NPV) of 73% were obtained. These values are too low than for the test to be considered reliable.

Mengist and Tilahun conducted a systematic review of 16 published articles presenting data on the sensitivity, specificity, PPV and NPV of Widal test compared to other tests to determine the diagnostic value of Widal test [4]. They obtained a mean sensitivity, specificity, PPV and NPV of 73.5% (95% CI: 60.9% - 86.1%), 75.7% (95%CI: 55.5% - 95.9%), 60% (95% CI: 31% - 89%) and 75.2% (95% CI: 50.4% - 100%) respectively. All the mean diagnostic accuracy parameters were less than 80%. The authors concluded that the Widal test has a comparatively poor reliability and should not be used as a lone diagnostic tool.

From the foregoing, it is obvious that a single Widal test cannot be reliably used to diagnose typhoid fever in an endemic area.

3.2.3 Interpretation of the Widal test

While several reports from certain developing countries have suggested that a single Widal test is enough to make the diagnosis of typhoid fever [28,31–34], others have disputed the reliability of such a single test result [7,23,30,35–38] However, it is generally agreed that the reliability of the test improves if the interpretation is made against a local cut-off titer [3,6,27,39]. Theoretically, a single Widal test may have some diagnostic relevance in an “unexposed” febrile patient (unvaccinated or lack of active infection). In reality, however, such a single test’s result does not have any diagnostic significance in an endemic area because repeated subclinical exposures to *Salmonella typhi* may have occurred [3]. Even in cases of extremely high single Widal agglutination titers, the causal organisms may be other species of *Salmonella* and not *S. typhi* [3] A review by Olopoenia and King to determine the significance of the Widal test for typhoid fever diagnosis in modern medicine concluded that the test cannot be expected to give a reliable diagnostic result in endemic regions because of its several pitfalls; hence, its use should be discouraged [3]. Disregard for this fact probably accounts for the seemingly high rate of diagnosis and treatment of typhoid fever in the endemic areas which are mainly developing countries.

It is generally considered that the Widal test is helpful in the diagnosis of typhoid fever in endemic regions only if the patient has a four-fold or more increase in the O or H agglutinin titers of serum specimens taken during the acute and convalescent period of infection (2-3 weeks apart) [3,27]. This again is a major obstacle to its use because antibiotic therapy often needs to be commenced at the early stage of the infection in order to prevent the patient from developing serious complications like gut perforation and gastrointestinal bleeding. Thence, no reasonable physician would wait for 2-3 weeks without treating a patient with suspected enteric fever. Again, once antibiotics have been commenced there may be no further rise in the agglutinin titer, rather, it may decrease.

3.2.4 Misuse of the Widal test

Misuse of the Widal test is rife in many of the developing countries of the world [3,27,40]. This is probably due to the fact that the test is cheap and readily available in contrast to the more reliable but sophisticated diagnostic methods. This is particularly so because patients often do not go to the appropriate medical facilities with competent hands for medical attention because of ignorance and poverty. A report from Libya shows that clinicians depend on the Widal test for the diagnosis of enteric fever despite that locally acceptable threshold titer has not been determined because their laboratories lack skilled and experienced personnel and appropriate facilities to detect and serotype *Salmonella* isolates [40]. Due to the weak nature of institutional regulation in these countries there is proliferation of quack medical and laboratory practices. The laboratories often perform the test, make the diagnosis and prescribe the antibiotics [3]. Many diagnostics centers use just a single positive result for diagnosis not taking into account the reality of cross-reaction between *S. typhi* and malaria parasites or other *Salmonella* species that are also endemic in their areas and the generally low diagnostic accuracy of the Widal test. In one study, the PPV and the NPV of the Widal test were 17% and 73% respectively [30]. In a systematic review of 16 studies, the mean PPV and the NPV of the Widal test were 60% (95% CI: 31%-89%) and 75.2% (95% CI: 50.4%-100%) respectively [4]. These studies indicate that a negative Widal test result may be more useful than a positive one. Despite this fact, medical practitioners in areas endemic for typhoid fever continue to use the test to diagnose the disease thereby contributing to unnecessary use of antibiotics.

It is imperative that laboratory personnel desist from usurping the role of the physician. Their role is to perform and report the test. It is the responsibility of the requesting physician to

utilize the information supplied by the laboratory in conjunction with the clinical information at his/her disposal to arrive at appropriate diagnosis. There is a need for close communication between the requesting physician and the laboratory so as to clarify ambiguous laboratory reports before use. Laboratories should desist from reporting test results in descriptive terms (negative or positive) as this may lead to wrong interpretation of results by the physician [3]. The results should rather be reported as either 'no agglutination' or in titers (1:20, 1:40, 1:80, 1:160 etc.) if agglutination is present [3].

3.3 Alternative Rapid Diagnostic Tests to the Widal Test

As a consequence of the drawbacks attendant to the Widal test, several alternative rapid diagnostic tests (RDTs) have been developed. These tests include the indirect hemagglutination assay (IHA), indirect enzyme-linked immunosorbent Assay (ELISA) for immunoglobulin M (IgM) and IgG antibodies to *S. typhi* polysaccharide, indirect fluorescence Vi antibody assay, counter immunoelectrophoresis (CIEP) and monoclonal antibody against *S. typhi* flagellin etc [39].

Some of the commercially available RDTs kits for typhoid include- Tubex TF (IDL, Sweden), Typhidot (Malaysian Biodiagnostic Research, Malaysia), Multi-Test Dip-S-Ticks (Panbio INDX, US), Typhidot-M (Malaysian Biodiagnostic Research, Malaysia), SD Bioline (Standard Diagnostics, Korea) and Mega *Salmonella* (Mega Diagnostics, US) etc [41]. Tubex TF and Typhidot are among the most commonly used of the recent generation of RDTs for typhoid fever [41]. These tests have mostly not been found to be sufficiently superior in performance to the Widal test to be recommend as lone reliable diagnostic tests for typhoid fever in endemic areas [42–45]. Thriemer *et al* conducted a meta-analysis to determine the performance of Tubex TF and Typidot in typhoid endemic countries [41]. A total of seven studies were included per test. The meta-analysis showed average sensitivity and specificity of 69% (95%CI: 45–85) and 88% (CI95%:83%–91%) respectively for Tubex TF. A formal meta-analysis could not be conducted for Typhidot because of data limitation but across the extracted studies, sensitivity and specificity estimates ranged from 56% to 84% and 31% to 97% respectively. It was concluded that the observed performance does not support the use of either of the RDTs exclusively as the basis for diagnosis and treatment of typhoid fever.

In another meta-analysis of 37 studies that evaluated three RTDs and their variants (TUBEX in 14 studies, Typhidot in 22 studies and Test-It Typhoid immunochromatographic lateral flow assay in 9 studies), Wijedoru et al obtained an average sensitivity of 78% (95%CI: 71%-85%) and specificity of 87% (95%CI: 82%-91%) for TUBEX; an average sensitivity of 69% (95%CI 59%-78%) and specificity of 90% (95% CI 78% to 93%) for all Test-It Typhoid and prototype tests (KIT); and an average sensitivity of 84% (95%:CI 73%- 91%) and specificity of 79% (95%CI 70%-87%) for Typhidot test [46]. The authors concluded that few of the 37 studies were at a low risk of bias, the three main RDTs and their variants had moderate diagnostic accuracy, the test did not show any evidence of superiority to one another in terms of their average sensitivity and specificity and there is need for more robust evaluations of alternative RDTs for enteric fever [46].

However, an immunodiagnostic assay for enteric fever, the typhoid/paratyphoid diagnostic assay (TPTest), that relies on the detection of anti-Salmonella enterica antibodies secreted by activated lymphocytes in the peripheral blood of acutely infected patients has been developed lately [47]. The test had a sensitivity of 100% compared to blood culture, and specificity that ranged from 78-97% (73-100, 95% CI), depending on definition of true negative among Bangladeshi patients [47]. The test also had a sensitivity of 96.0% (95% CI: 87.1%-99.8%) and specificity 96.6% (95%CI: 90.7%-99.2%). In a study that compared the diagnostic accuracy of TPTest to Tubex and Typhidot, the sensitivity of TPTest, Tubex and Typhidot were estimated at 96.0% (95% CI: 87.1%-99.8%), 60.2% (95% CI: 49.3%-71.2%), and 59.6% (95% CI: 50.1%-69.3%), respectively; while specificity was estimated at 96.6% (85%CI: 90.7%-99.2%) for TPTest, 89.9% ((5% CI 79.6%-96.8%) for Tubex, and 80.0% (67.7%-89.7%) for Typhidot [48]. Nevertheless, it takes 24-48hrs to obtain result at the moment and moderate laboratory capacity is required to conduct the test [48].

3.4 How Typhoid Fever is Diagnosed

The gold standard for the diagnosis of enteric fever remains culture isolation of the organism [3,39]. It is considered 100% specific [39]. The blood, bone marrow, stool, urine, rose spot,

gastric and intestinal secretions may be cultured. Culture efficacy varies with the type of specimen being tested. Bone marrow culture yield is about 90% sensitive at any point in the course of the disease to as long as five days after the initiation of antibiotic therapy [39,49]. The test is not the preferred option because the procedure is extremely painful. The sensitivity of blood culture approaches 90% in an untreated patient in the first week of infection but the figure drops to less than 50% by the third week. Large volume (10-30 ml) and multiple samples (>3 times) improve the diagnostic yield [39]. Stool culture is positive only in 30% of patients with acute typhoid fever [6]. A single rectal swab during hospital admission has a sensitivity of 30-40% [39]. Urine culture seems to be the least sensitive among all the cultures [50,51]. However, the high rate of antibiotics use by patients before hospital presentation in the developing countries tends to interfere with the isolation of infectious agents from clinical specimens, particularly blood culture. Though blood culture is currently the preferred diagnostic method, it is expensive; the result is only available after more than 48 hours; and requires elaborate laboratory equipment and rare technical expertise.

The polymerase chain reaction has varying success. The nested PCR has a sensitivity of 82.7% and a specificity of 100% when blood and urine assays are combined [51]. The test is not readily available in the endemic areas and where available, it is mostly used for research purpose because it is generally too expensive for patients.

Common nonspecific laboratory findings in patients with typhoid fever include moderate anemia, thrombocytopenia, relative lymphopenia and elevated erythrocyte sedimentation rate (ESR) [Table 4] [39]. Depending on the stage of the disease at the time of diagnosis, there could be slight elevation of the prothrombin time (PT) and activated partial thromboplastin time (aPTT), a reduction in fibrinogen levels and elevation in the levels of the fibrin degradation products. The liver transaminases and serum bilirubin values could rise to as twice the reference range.

The question then comes to mind: “How is enteric fever to be diagnosed in the absence of a rapid, reliable and cost-effective diagnostic method?” The fact is that accurate diagnosis of typhoid fever remains a challenge in resource-poor settings because the disease does not have specific clinical features. The diagnosis is often made by the exclusion of other common febrile illnesses in the locality. This practice inevitably leads to a significant disparity between clinical diagnoses and laboratory diagnosis of the disease [52]. In many of the areas where malaria is endemic, typhoid fever is usually suspected whenever a febrile patient gives a history of repeated standard malaria treatment without abatement of the fever. Features that may suggest the development of complications such as generalized abdominal tenderness with guarding and hematochezia are also sought for. Apart from fever and abdominal pain, nonspecific symptoms such as headache, diarrhea or constipation, anorexia, body weakness and muscle pains are often associated with the disease [11]. Neuropsychiatric manifestations (known as typhoid psychosis) ranging from confusion to frank psychosis could occur in 5% to 10% of patients with enteric fever [11]. Rose spots (red macules of 2-4 millimeters diameter) may be observed in light skinned persons. It is not uncommon for physicians to commence empirical antibiotic therapy while awaiting culture results once a clinical impression of typhoid fever has been made.

Table 4. Nonspecific laboratory findings in patients with typhoid fever

1	Moderate anemia
2	Thrombocytopenia
3	Relative lymphopenia
4	Elevated erythrocyte sedimentation rate (ESR)
5	Elevated prothrombin (PT) and activated partial thromboplastin time (aPTT)
6	Reduced fibrinogen levels
7	Elevated fibrin degradation products levels
8	Elevated liver transaminases and bilirubin

4. CONCLUSION

The Widal test cannot be relied upon to accurately diagnose typhoid fever due its glaring inadequacies. Therefore, its use should be discouraged by all means. The general public, laboratory workers and clinicians need to be adequately enlightened on the limitations of the Widal test as this will go a long way to reducing the rate of unnecessary diagnosis and treatment of typhoid fever in the endemic populations. The quest for the development of effective typhoid RDTs should continue until they could perform at levels comparable to the malaria RDTs. Since culture isolation of the causative organism remains the definitive diagnosis of enteric fever, there is an urgent need for the provision of

appropriate laboratory equipment and training of more laboratory personnel on microbiology culture techniques in the developing countries where the disease is endemic.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Crump JA, Luby SP, Mintz ED. The global burden of typhoid fever. Bull World Health Organ. 2004;82(5):346–53.
2. Agarwal Y, Gupta D, Sethi R. Enteric fever: Resurrecting the epidemiologic footprints. Astrocyte. 2016;3(3):153–61.
3. Olopoenia LA, King AL. Widal agglutination test - 100 years later: Still plagued by controversy. Postgrad Med J. 2000; 76(892):80–4.
4. Mengist HM, Tilahun K. Diagnostic value of widal test in the diagnosis of typhoid fever: A systematic review. J Med Microbiol Diagnosis. 2017;6(1):248.
5. Wasihun AG, Wlekidan LN, Gebremariam SA, Welderufael AL, Muthupandian S, Haile TD, et al. Diagnosis and treatment of typhoid fever and associated prevailing drug resistance in Northern Ethiopia. Int J Infect Dis. 2015;35:96–102.
6. Parry CM, Hien TT, Dougan G, White NJ, Farrar JJ. Typhoid fever. N Engl J Med. 2002;347(22):1770–82.
7. Lalremruata R, Chadha S, Bhalla P. Retrospective audit of the widal test for diagnosis of typhoid fever in pediatric patients in an endemic region. J Clin Diagn Res. 2014;8(5):DC22-5.
8. Siba V, Horwood PF, Vanuga K, Wapling J, Sehuko R, Siba PM, et al. Evaluation of serological diagnostic tests for typhoid fever in papua new guinea using a composite reference standard. Clin Vaccine Immunol. 2012;19(11):1833–7.
9. Mweu E, English M. Typhoid fever in children in Africa. Trop Med Int Health. 2008;13(4):532–40.
10. House D, Chinh NT, Diep TS, Parry CM, Wain J, Dougan G, et al. Use of paired serum samples for serodiagnosis of typhoid fever. J Clin Microbiol. 2005; 43(9):4889–90.
11. Fraser A, Goldberg E, Acosta CJ, Paul M, Leibovici L. Vaccines for preventing typhoid fever. Cochrane Database Syst Rev. 2007(3):CD001261. Available:http://www.ncbi.nlm.nih.gov/pubmed/17636661 (Accessed 19 Sep 2017)
12. Moorhead R. William budd and typhoid fever. J R Soc Med. 2002;95(11):561–4.
13. Franco-Paredes C, Khan MI, Gonzalez-Diaz E, Santos-Preciado JI, Rodriguez-Morales AJ, Gotuzzo E. Enteric fever: A slow response to an old plague. PLoS Negl Trop Dis. 2016;10(5):e0004597.
14. Husain M, Khan RN, Rehmani B, Haris H. Omental patch technique for the ileal perforation secondary to typhoid fever. Saudi J Gastroenterol. 2011;17(3):208–11.
15. Bhutta ZA. Current concepts in the diagnosis and treatment of typhoid fever. BMJ. 2006;333(7558):78–82.
16. World Heath Organisation. Typhoid vaccine: WHO position paper, Weekly epidemiological record. 2008:83. Available:http://www.who.int/wer/2008/wer8306.pdf?ua=1 (Accessed 24 Jan 2017)
17. Reller ME, Olsen SJ, Kressel AB, Moon TD, Kubota KA, Adcock MP, et al. Sexual transmission of typhoid fever: A multistate outbreak among men who have sex with men. Clin Infect Dis. 2003;37(1):141–4.
18. Nelson DB, Muscarella LF. Current issues in endoscope reprocessing and infection control during gastrointestinal endoscopy. World J Gastroenterol. 2006;12(25):3953–64.
19. Kovaleva J, Peters FTM, van der Mei HC, Degener JE. Transmission of infection by flexible gastrointestinal endoscopy and bronchoscopy. Clin Microbiol Rev. 2013; 26(2):231–54.
20. Danu MS, Urhekar AD, Goel N, Mane V, Yadav A, Ajit KG. Comparison of Widal test with immunochromatography and enzyme immuno assay for *Salmonella typhi* IgM and IgG antibodies. Res Rev J Microbiol Biotechnol. 2013;2(3):35–48.
21. Lavanya V, Shivakumar S Solabannavar SBS. Comparison of results obtained by semi-quantitative slide agglutination and tube widal tests in the diagnosis of

suspected typhoid fever cases. Int J Biol Med Res. 2013;4(1):3001–3.
22. Gaikwad UN, Rajurkar M. Diagnostic efficacy of Widal slide agglutination test against Widal tube agglutination test in enteric fever. Int J Med Public Heal. 2014;4(3):227–30.
23. Verma D, Kishore S, Siddique ME. Comparative evaluation of various tests for diagnosis of concurrent malaria and typhoid Fever in a tertiary care hospital of northern India. J Clin Diagn Res. 2014; 8(5):DC41-4.
24. WARISO KT. Re-appraising Widal test as a diagnostic tool in Nigeria. Niger Heal J. 2017;16(4):249.
25. Gopalakrishnan V, Sekhar WY, Soo EH, Vinsent RA, Devi S. Typhoid fever in Kuala Lumpur and a comparative evaluation of two commercial diagnostic kits for the detection of antibodies to *Salmonella typhi.* Singapore Med J. 2002;43(7):354–8.
26. Andualem G, Abebe T, Kebede N, Gebre-Selassie S, Mihret A, Alemayehu H. A comparative study of Widal test with blood culture in the diagnosis of typhoid fever in febrile patients. BMC Res Notes. 2014;7:653.
27. Okonko IO, Soleye FA, Eyarefe OD, Amusan TA, Abubakar MJ, Adeyi AO, et al. Prevalence of *Salmonella typhi* among Patients in Abeokuta, South-Western Nigeria. Br J Pharmacol Toxicol. 2010;1(1):6–14.
28. Ohanu ME, Mbah AU, Okonkwo PO, Nwagbo FS. Interference by malaria in the diagnosis of typhoid using Widal test alone. West Afr J Med. 2003;22(3):250–2.
29. Bakr WMK, El Attar LA, Ashour MS, El Toukhy AM. The dilemma of widal test - which brand to use? a study of four different widal brands: a cross sectional comparative study. Ann Clin Microbiol Antimicrob. 2011;10:7.
30. Enabulele O, Awunor SN. Typhoid fever in a Tertiary Hospital in Nigeria: Another look at the Widal agglutination test as a preferred option for diagnosis. Niger Med J. 2016;57(3):145–9.
31. Frimpong EH, Feglo P, Essel-Ahun M, Addy PA. Determination of diagnostic Widal titres in Kumasi, Ghana. West Afr J Med. 2000;19(1):34–8.
32. Ley B, Mtove G, Thriemer K, Amos B, von Seidlein L, Hendriksen I, et al. Evaluation of the widal tube agglutination test for the diagnosis of typhoid fever among children admitted to a rural hdospital in Tanzania and a comparison with previous studies. BMC Infect Dis. 2010;10:180.
33. Taiwo SS, Fadiora SO, Oparinde DP, Olowe OA. Widal agglutination titres in the diagnosis of typhoid fever. West Afr J Med. 2007;26(2):97–101.
34. Zailani SB, Oyelese AO, Aboderin AO. Determination of baseline antibody titre to *S. typhi/paratyphi* in Ile-Ife, Nigeria. Afr J Med Med Sci. 2003;32(3):307–10.
35. Adhikari A, Rauniyar R, Raut PP, Manandhar K Das, Gupta BP. Evaluation of sensitivity and specificity of ELISA against Widal test for typhoid diagnosis in endemic population of Kathmandu. BMC Infect Dis. 2015;15:523.
36. Willke A, Ergonul O, Bayar B. Widal test in diagnosis of typhoid fever in Turkey. Clin Diagn Lab Immunol. 2002;9(4):938–41.
37. Eleazar C, Iroegbu C, Udoh I. Comparison of widal test with cultural methods for evaluation of *Salmnella typhi/paratyphi* Infection. Br Microbiol Res J. 2015; 8(5):577–84.
38. Omuse G, Kohli R, Revathi G. Diagnostic utility of a single widal test in the diagnosis of typhoid fever at Aga Khan University Hospital (AKUH), Nairobi, Kenya. Trop Doct. 2010;40(1):43–4.
39. Sattar AA, Yusuf MA, Islam MB, Jahan WA. Different diagnostic procedure of typhoid fever: A review update. J Curr Adv Med Res. 2014;1(2):35–41.
40. Zorgani A, Ziglam H. Typhoid fever: misuse of widal test in Libya. J Infect Dev Ctries. 2014;8(6):680–7.
41. Thriemer K, Ley B, Menten J, Jacobs J, van den Ende J, Crump J, et al. A systematic review and meta-analysis of the performance of two point of care typhoid fever tests, tubex TF and typhidot, in endemic countries. Way SS, editor. PLoS One. 2013;8(12):e81263.
42. Ley B, Thriemer K, Ame SM, Mtove GM, von Seidlein L, Amos B, et al. Assessment and comparative analysis of a rapid diagnostic test (Tubex®) for the diagnosis of typhoid fever among hospitalized children in rural Tanzania. BMC Infect Dis. 2011;11(1):147.
43. Olsen SJ, Pruckler J, Bibb W, Nguyen TMT, Tran MT, Nguyen TM, et al. Evaluation of rapid diagnostic tests for typhoid fever. J Clin Microbiol. 2004; 42(5):1885–9.

44. Keddy K, Sooka A, Letsoalo M, Hoyland G, Chaignat CL, Morrissey A, et al. Sensitivity and specificity of typhoid fever rapid antibody tests for laboratory diagnosis at two sub-Saharan African sites. Bull World Health Organ. 2011; 89(9):640–7.
45. Bakr WM, El Attar LA, Ashour MS, El Tokhy AM. TUBEX test versus widal test in the diagnosis of typhoid fever In Kafr El -Shekh, Egypt. J Egypt Public Health Assoc. 2010;85(5–6):285–96.
46. Wijedoru L, Mallett S, Parry CM. Rapid diagnostic tests for typhoid and paratyphoid (enteric) fever. Cochrane Database Syst Rev. 2017;5:CD008892. Available:http://www.ncbi.nlm.nih.gov/pubmed/28545155 (Accessed 19 Sep 2017)
47. Khanam F, Sheikh A, Sayeed MA, Bhuiyan MS, Choudhury FK, Salma U, et al. Evaluation of a typhoid/paratyphoid diagnostic assay (TPTest) detecting anti-salmonella IgA in secretions of peripheral blood lymphocytes in patients in Dhaka, Bangladesh. Baker S, editor. PLoS Negl Trop Dis. 2013;7(7):e2316.
48. Islam K, Sayeed MA, Hossen E, Khanam F, Charles RC, Andrews J, et al. Comparison of the performance of the TPTest, tubex, typhidot and widal immunodiagnostic assays and blood cultures in detecting patients with typhoid fever in Bangladesh, Including Using a Bayesian Latent Class Modeling Approach. PLoS Negl Trop Dis. 2016;10(4): e0004558.
49. Wain J, Bay PVB, Vinh H, Duong NM, Diep TS, Walsh AL, et al. Quantitation of bacteria in bone marrow from patients with typhoid fever: Relationship between counts and clinical features. J Clin Microbiol. 2001;39(4):1571–6.
50. Tanyigna KB, Bello CS, Okeke N, Onwukeme KE. Comparison of blood, bone marrow aspirate, stool and urine cultures in the diagnosis of enteric fever. Niger J Med. 2001;10(1):21–4.
51. Ambati SR, Nath G, Das BK. Diagnosis of typhoid fever by polymerase chain reaction. Indian J Pediatr. 2007;74(10): 909–13.
52. Ngwu BA, Agbo JA. Typhoid fever: Clinical diagnosis versus laboratory confirmation. Niger J Med. 2003;12(4):187–92.

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