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Original Article
The accuracy of widal test for typhoid fever diagnosis in Ethiopia: Systematic review and meta-analysis

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Abstract

Introduction: *Widal agglutination test is a serologic investigation that is used to diagnose typhoid fever. This is an easy, fairly inexpensive, and readily available test with questionable reliability. The test performance differs from setting to setting depending on the technique used and other factors. The accuracy of this test in Ethiopia is poorly understood. So, the aim of this scientific work was to analyze the accuracy of Widal agglutination in diagnosing typhoid fever in Ethiopia.*

Methods: *We performed a systematic review and meta-analysis. Two electronic databases (PubMed/Medline and Google scholar) were searched using preset search strategy to find relevant studies. The methodological quality of the studies included was evaluated with a QUADAS-2. We extracted important variables from the eligible articles. Statistical analysis was conducted using STATA version 14. The protocol of our systematic review and meta-analysis is registered in the International Prospective Register of Systematic Reviews (PROSPERO) with the record number CRD42020194252.*

Results: *The electronic quests yielded 42 papers of which 8 were eligible for analysis. The quality of these studies was rated to be moderate based on the QUADAS-2. The pooled sensitivity, specificity, and negative, and positive predictive values of the Widal test were 80.8%, 53.0%, 98.5%, and 2.1% respectively.*

Conclusion: *The widal agglutination test has average specificity, very good negative predictive value, and very poor positive predictive value for the diagnosis of typhoid fever. Depending on Widal to diagnose typhoid fever may lead to over-diagnosis of typhoid fever and related complications including inappropriate use of antibiotics. There is an urgent need for quick and dependable tests for diagnosing typhoid fever, particularly in settings like Ethiopia where doing timely culture is not feasible.*

Keywords*: Widal test, Accuracy, Typhoid fever, Systematic review, Meta-analysis, Ethiopia*
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Introduction

Typhoid fever is an important community health problem that affects nearly 21.6 million people and kills around 200,000 people each year globally (1).The illness is mainly acquired by ingesting of food or water contaminated by the stool and seldom by urine or vomitus of patients and carriers (2). Africa has an intermediate to high burden of the disease, 10-725 cases per 100 000 population per year (1,3).

It is common in low-resource countries including Ethiopia (4,5). It is commonly considered as one of the differential diagnoses in a patient presenting with short onset fever. However, as the symptoms are non-specific and similar with other acute febrile illnesses, clinical diagnosis is not straight forward (6). Microbiologic culture (bone marrow aspirate, blood, stool, and urine) that will enable one to isolate the etiologic agents (*S. Typhi and/or S. Paratyphi*) is the gold standard in the diagnosis of typhoid fever. Nevertheless, microbiologic culture is not readily available, takes 2-7 days for the result to be ready, and is more expensive. Getting a rapid, accurate, and affordable test(s) is of paramount importance.

Different rapid diagnostic methods are available to diagnose typhoid fever (7,8). Among the Rapid diagnostic methods, Widal, TUBEX, Typhidot, and TPTest are to be mentioned. The commonly available and utilized rapid diagnostic method in Ethiopia is Widal agglutination. Though, Widal agglutination test is commonly available and used to diagnose typhoid fever, it has questionable reliability.

Widal agglutination is a serologic diagnostic test that is used to diagnose typhoid fever. It detects the presence of agglutinin (antibody) in the serum of an infected person with *salmonella typhi*. The reaction between the agglutinin in the serum of the infected patient and the *salmonella typhi (S. Typhi)* antigens will result in agglutination. The antigens used to demonstrate agglutination are H (flagellar) and O (somatic) antigens of *S.typhi* (9). The Immunoglobulin M (IgM) somatic O antibody of S.typhi appears initially and represents the first serologic response in acute typhoid fever, while immunoglobulin G (IgG) flagellar H antibody usually develops more slowly and persists for longer (10,11).

After 100 years of its introduction as a serologic way of typhoid fever diagnosis, the Widal test still remains to be associated with controversies related to the quality of the antigens used and interpretation of the result, especially in endemic regions (9). Widal test is a simple, non-expensive and fairly non-invasive test with debatable reliability (12). Widal test has a reportedly high sensitivity and better specificity though variable. Similarly it has very good negative predictive value but has poor positive predictive value (13-15). Nevertheless; Widal agglutination test could remain reactive during convalescent time and has cross-reactivity with other microorganisms resulting in false positivity. These conditions will result in over-diagnosis of typhoid fever (16). Additionally, overreliance on this test combined with poor clinical judgment may result in over prescription of antibiotics and associated antibiotics resistance.

The test performance differs from setting to setting depending on the technique used and other factors. Single slide agglutination test is widely practiced point of care test in Ethiopia. The serial tube titration method is rarely used in clinical settings. There are multiple cross sectional studies done in Ethiopia to address the use of widal test in the diagnosis of typhoid fever. Most of the studies conducted revealed conflicting results on the accuracy of widal test in the diagnosis of typhoid fever in Ethiopia. There are studies in other settings that stated widal test is less accurate and no more recommended for the diagnosis of typhoid fever. In spite of this, widal test is widely used across Ethiopia. So, summarizing studies on the diagnostic accuracy of Widal test will help to inform decision makers to prepare diagnostic algorithms on the use of Widal tests, other rapid diagnostic tests and culture. Therefore, we set out to do systematic review and meta-analysis of the accuracy of Widal test in Ethiopia.

Methods of the Review

We conducted the study in accordance with the guideline of the PRISMA group (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) (17). We used the PRISMA check list (18) to safeguard the inclusion of all pertinent information.

Search strategy. The review focused on resources that described and explicate issues that are related to Widal test and its performance as compared to culture. Databases that were searched for resources included MEDLINE (Pub Med) and Google scholar, and reference lists of relevant papers. The search terms included "Widal" ,"Widal tests" , "typhoid"," typhoid fever", "Ethiopia" in all fields of the databases to establish previous literature on Widal tests for diagnosing typhoid fever. All relevant articles with no restriction on the time of publication were included in this study. The search terms used included but not limited to 'Widal' AND/OR 'Typhoid' AND 'Ethiopia', 'Widal tests' AND/OR 'typhoid fever' AND 'Ethiopia', and 'Widal tests' AND/OR 'typhoid' AND 'Ethiopia'.

Using the above mentioned search terms we searched for eligible articles. References for the applicable citations and reviews were manually searched for applicable citations and professionals in the field were consulted to enrich the search. The titles and abstracts of all recovered studies were independently evaluated by two authors (OA and SG). The same reviewers judged the full texts of the retained studies. At each step disagreements were resolved by discussion with the presence of a third reviewer (KS). The last search was conducted on September 11, 2020.

Inclusion and exclusion criteria

Inclusion criteria; All human studies that have compared Widal agglutination test with culture in Ethiopia.

Exclusion criteria; Studies with sample size less 50, studies that did not publish full articles or the full article not available, and studies done on asymptomatic participants were excluded.

Risk of bias in individual studies

Two reviewers (OA, SG) independently evaluated the risk of bias in the studies using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool. Disagreements were resolved by a third reviewer (KS). The results of the risk of bias assessment were described as part of the narrative synthesis.

Data Extraction

Each study was subjected to the following search: study author, year of publication, place of study, index test (Widal), study design, sample size, and the characteristics of the sample population. Comprehensive data about the Widal test was extracted from the studies that were included: the test brand, country and name of the manufacturer, the procedure of the Widal test. Similarly, we took the true positive (TP), false positive (FP), false negative (FN), and true negative (TN) of the Widal agglutination as compared to culture. Other important variables were also extracted.

Data synthesis process

We have used the two by two table variables; true positives, false negatives, false negatives and true negatives for producing pooled sensitivity, specificity, negative predictive value and positive predictive value. We extracted these important variables in Microsoft excel. The quantitative synthesis was performed using STATA version 14. For estimating the accuracy we used bivariate model to calculate sensitivity & specificity, positive & negative predictive vales along with 95 percent confidence intervals (CIs). These measures were pooled using the random effects model. Sensitivity (true positive rate) was defined as the probability that a test result will be positive when the disease exists and calculated as = TP/ (TP+FN). Similarly, specificity (true negative rate) was defined as the probability that a test result will be negative when the disease is not present and calculated as = TN/ (TN+FP). Heterogeneity (differences in reported estimates among studies) was evaluated by a Q test statistic (Chi square value with p values) and I^2values.

Quality Assessment

The studies quality assessment was conducted using Quality Assessment of Diagnostic Accuracy Studies) tool (QUADAS-2)(19). The QUADAS-2 tool was completed by following stepwise guidelines to judge risk of bias (four domains) and concerns about applicability (three domains) for each study.

Results

Search results

The electronic searches yielded 42 articles. Thirty-four of these papers were excluded from this review and meta-analysis (eleven of the studies were excluded because of duplication, 11 were excluded due to non-relevance after reviewing their titles, 9 articles excluded because they were done on asymptomatic participants, two of the articles were not accessed (these articles required fee and the authors could not afford), and one article was done on non-human participants). The details of the article inclusion and exclusion are shown in figure 1 below.

We included 8 cross-sectional studies in this systematic review and meta-analysis (20-27). All eight of the included studies were used in the qualitative synthesis while 6 of them were utilized for the quantitative synthesis (20-25). The studies used for quantitative synthesis had a total sample size of 1727 participants. The studies were conducted in six regions of the country, Addis Ababa (21,22), Oromia (25,26), South nations and nationalities (24), Tigray (20), Amhara (23), and Somali(27). No study was retrieved regarding the Widal study from the remaining regions of the country (Harar, Benishangl-Gumuz, Gambella, Afar, and Dire Dawa)(**Table 1).**

Table 1. Characteristics the included studies

Author, year	Region of the country	Sample/ participants	Widal test* positivity n (%)	Culture positivity n (%)	Remark
A.G. Wasihun et al, 2015	Tigray	502	343 (68.4)	8 (1.6)	
Andualem et al, 2014	Addis Ababa	270	88 (32.6)	11 (4.1)	
Legesse Garedew et al, 2018	Addis Ababa	288	148 (51.4)	1 (0.68)	66% of the Widal reactive patients were prescribed antibiotics
Meseret Birhanie et al, 2014	Amhara	200	38 (19)	1 (0.5)	
Deksissa and Gebremedhin , 2019	Oromia	372	209 (56.2)	10 (2.7)	
Ameya et al, 2017	SNNPR^^	95	65 (68.4)	19 (20)	
Dawit et al, 2019	Somali	203	N/A^	22 (11)	
Habte et al, 2018	Oromia	421	N/A^	21 (5)	

^ N/A- not available, ^^ SNNPR- Southern Nations, Nationalities and Peoples Region

*The numbers presented here regarding Widal test are for the Sliding agglutination technique.

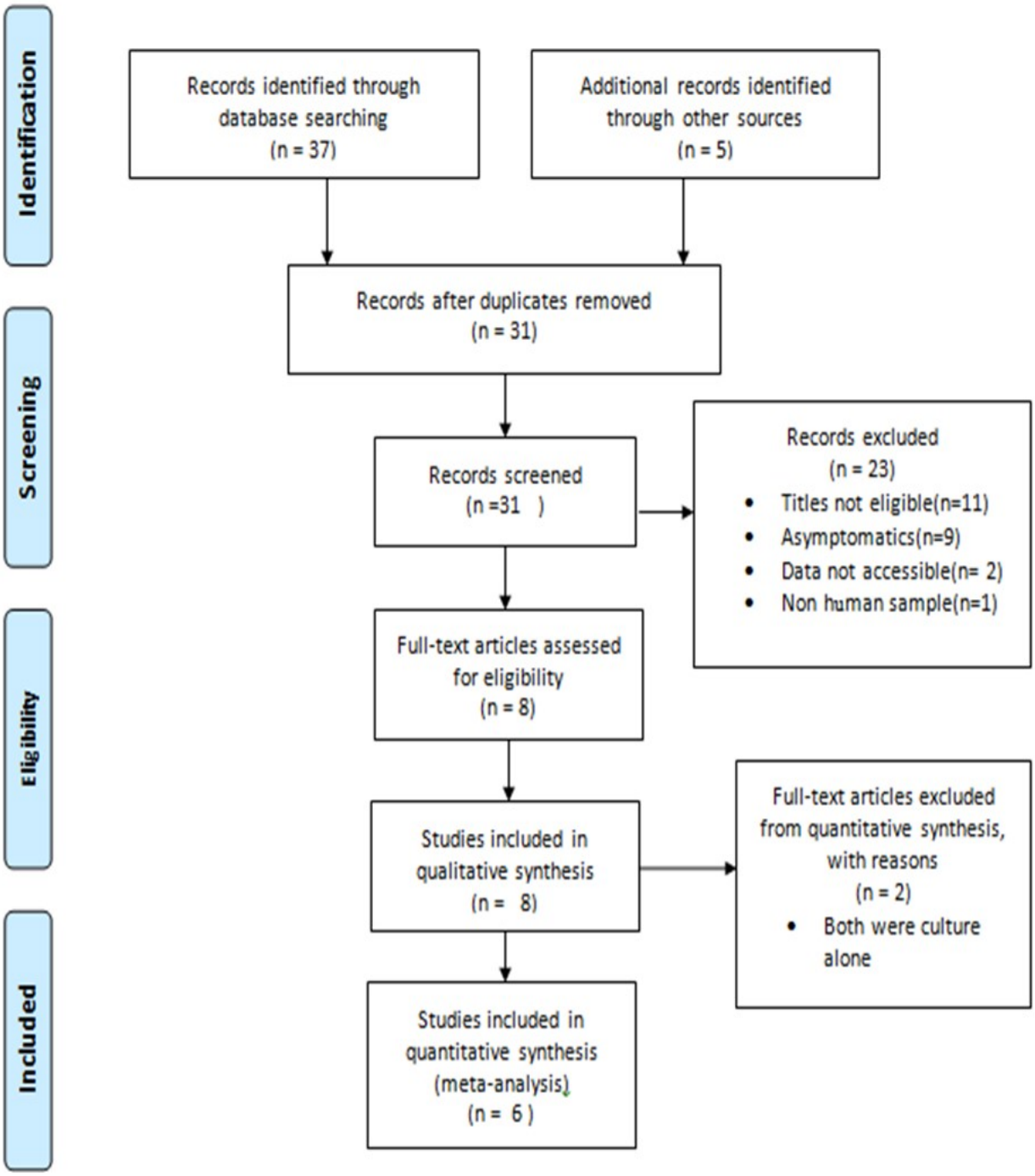

Figure 1 A flow diagram of the selection of eligible studies

Table 2 Summary of the quality assessment of the included studies

Study	Risk of bias				Applicability concerns		
	Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
A.G. Wasihun et al	Φ	Φ	Φ	Φ	Φ	Φ	Φ
Andualem et al	♣	♣	*	♣	♣	♣	♣
Legesse Garedew et al	Φ	Φ	Φ	Φ	Φ	Φ	Φ
Meseret Birhanie et al	Φ	Φ	*	Φ	Φ	Φ	*
Deksissa and Gebremedhin	*	♣	♣	♣	*	♣	♣
Ameya et al	*	♣	♣	*	*	♣	♣

♣ *Low risk;* Φ*High risk;* * *Unclear risk*

Qualitative synthesis

All the studies included in this review have analyzed febrile individuals with a clinical suspicion of typhoid fever. Among the 8 studies contained within in this review, 6 of them have compared the index Widal agglutination test to a reference blood (20-23) or stool (24,25) culture. On the other hand, two of the studies (26,27) just did blood culture alone. The sensitivity, specificity, negative predictive value, and positive predictive vales of the Widal test ranged from 71.2-100%, 0.68-68.4%, 90-100%, and 2.6-48.8%, respectively. The culture positivity rate ranged from 0.35%- 20%.

Quantitative synthesis

The main outcomes of concern were the sensitivity, specificity, negative predictive and positive values predictive values of Widal agglutination as compared to the reference test (culture in this case).

The pooled sensitivity of Widal agglutination test in the diagnosis 80.8% (95% CI, 68.6-92.9%). Similarly, the pooled specificity of Widal test was determined to be 53.0% (95% CI, 52.8-55.2%). The pooled negative and positive predictive values of the Widal test were 98.5% (95% CI, 97.2-99.7%) and 2.1% (95% CI, 1.1-3.0%) respectively. The pooled Widal and culture positivity in our study was 35.0% (95% CI, 33.2-36.6%) and 1.2% (95% CI, 0.8-1.7%). The forest plots of the pooled negative and positive predictive values are depicted below (Figure 2 and 3).

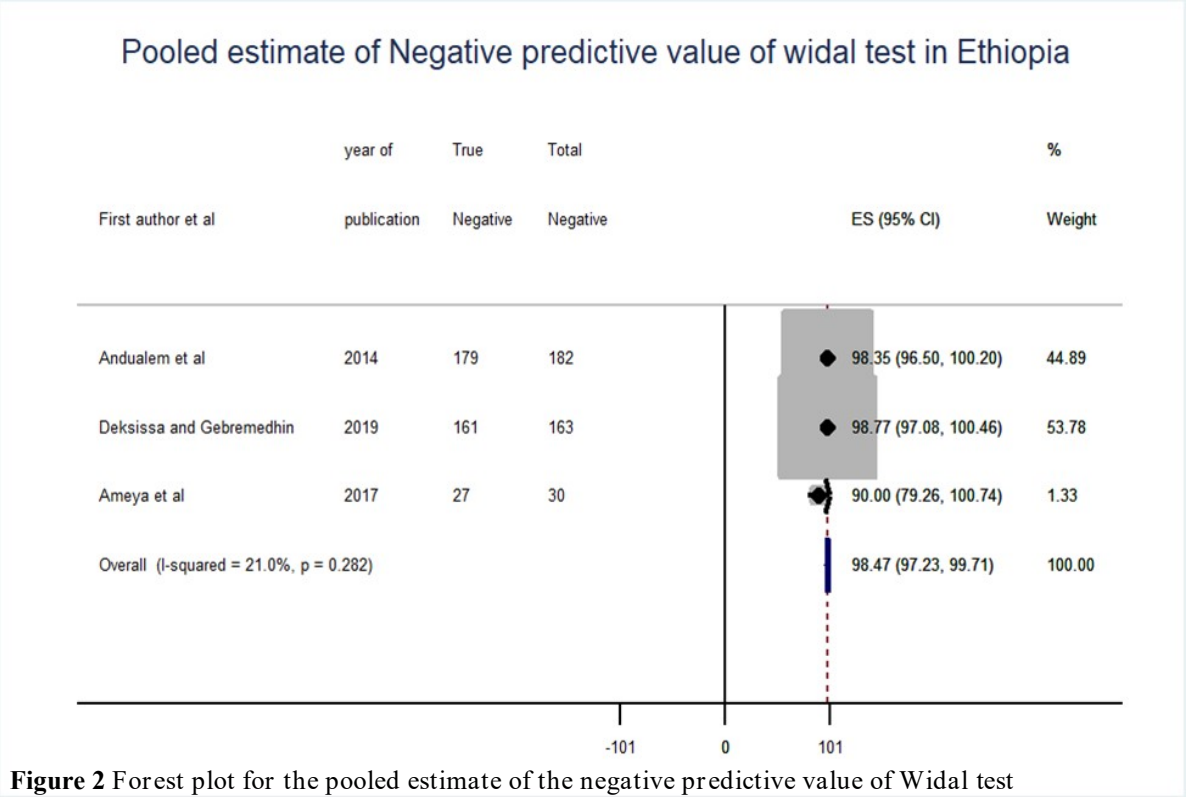

Figure 2 Forest plot for the pooled estimate of the negative predictive value of Widal test

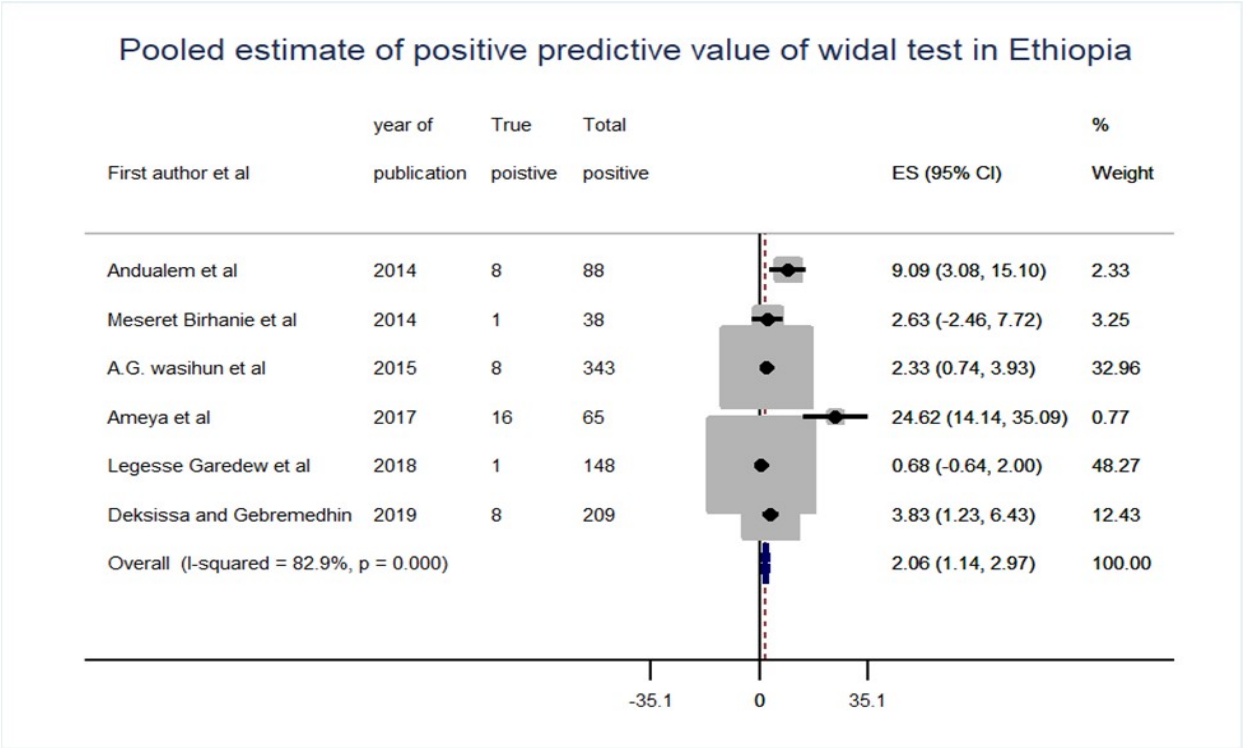

Figure 3 Forest plot for the pooled estimate of positive predictive value of the Widal test

Discussion

In our review, we have pooled the sensitivity, specificity, negative and positive predictive value of sliding Widal agglutination test using culture as a reference test. The pooled estimate from this review has revealed that the sliding Widal agglutination test has good sensitivity and excellent negative predictive value. However, it has a low specificity (53.0%) and positive predictive value (2.1%).

The pooled sensitivity of Sliding Widal agglutination test in this study was 80.8% (95%CI, 68.6-92.9%) and the negative predictive value was 98.5 %(95% CI, 97.2-99.7%). The sensitivity was comparable to a review conducted by Mengist and Tilahun (SN=73.5%) (28) and R. Bundalian et al (SN=32-95%) (29) while the negative predictive value of our study (98.6%) was much higher as compared to that reported by Mengist and Tilahun (NPV=60%) (28) and Begum et al (NPV=62.9%) (30). The NPV of our study was comparable to that reported by Taiwo et al (98.3%) (31) and Ley et al(100%) (13).

The pooled specificity and positive predictive value of Widal test in this study were 53.0% and 2.1% respectively. This positive predictive value is extremely low as compared to different other studies (15,30,32-34). One of the reasons may be the lower cuts –off for the O (1:80) and H(1:160) antibodies used in our study in contrary to that by Willke et al (1:200) (15). The other reason might be the lower culture positivity rate of our study.

The serologic (sliding agglutination Widal) diagnosis of typhoid fever occurred in up to a third of the study participants (35.0%) in this study. On the other hand, the culture confirmed typhoid fever was observed in 1.2% of the participants in our study. There is similar report of serologic diagnosis rate in Nigeria (35) and a higher rate (81%) in Tanzania (36). The culture proven diagnosis rate of typhoid fever was comparable to that of Congo (2.4%) (37) but lower as compared to that in Nigeria (22.1%) and Tanzania (11%) (35,36).

As mentioned earlier this study has shown a very low PPV (2.1%). Positive predictive value is the probability that subjects with a positive screening test truly have the disease. Thus, sliding Widal agglutination test is poorly reliable in identifying those truly diseased by typhoid fever. Nevertheless, this test has high NPV (98.5%) that means a subject with a negative Sliding Widal agglutination test is highly likely not to have Typhoid fever.

The strengths of our study include the relatively larger total sample size as compared to previous studies done in Ethiopia and the assessment of an important research question. Among the limitations of this study are our use of a reference test (blood and stool) that has lower sensitivity and the studies retrieved covered only some portion of Ethiopia.

Conclusion and recommendations

Our study is the first systematic review and meta-analysis to evaluate the accuracy of Widal test in Ethiopia. Widal agglutination test has average specificity, very good negative predictive value and very poor positive predictive value for the diagnosis of typhoid fever. Relying on Widal for the diagnosis of typhoid fever may lead to over-diagnosis of typhoid fever and related complications including inappropriate use of antibiotics. There is an urgent need of rapid and reliable tests for the diagnosis of typhoid fever particularly in settings like Ethiopia where doing timely culture is not feasible.

Declarations

Ethics consideration

This study is a systematic review and meta-analysis and obtaining an ethical approval was not necessary.

Availability of data and materials

All data generated or analyzed during this study are included in the manuscript. However, the raw data are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

OAM and SG carried out this study, drafted the manuscript and coordinated the study. KS participated in the design of the study and performed the statistical analysis and edited critically the manuscript. OAM and SG suggested the title of the research. All the authors read and approved the final manuscript for submission to EMJ.

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