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Original Research Article

ASSESSMENT OF THE DIAGNOSTIC ACCURACY OF WIDAL AND TYPHIDOT TESTS IN TYPHOID FEVER

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ABSTRACT

Typhoid fever continues to pose a major public health challenge in endemic regions such as South Asia, where inadequate sanitation, overlapping febrile illnesses, and limited diagnostic resources complicate timely detection. Although blood culture remains the gold standard, its sensitivity is compromised by prior antibiotic use and logistic constraints. The Widal test, widely used in resource-limited settings, has well-recognised limitations, whereas newer immunoassays such as Typhidot promise improved diagnostic performance. This study was undertaken to evaluate and compare the diagnostic accuracy of Widal and Typhidot tests against blood culture in suspected cases of enteric fever.

A prospective observational study was conducted at tertiary care hospitals between March 2018 and February 2019. A total of 300 patients with clinically suspected enteric fever were enrolled. Blood samples were collected for culture, Widal slide agglutination, and Typhidot testing. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated using blood culture as the gold standard. Statistical analysis was performed using SPSS version 25.0, with $p<0.05$ considered significant.

The mean age of participants was 23.79 years, with a predominance of cases in the 0–10-year age group (32%). Blood culture positivity was 22%, highest during 6–10 days of fever (71.2%). The Widal test showed a sensitivity of 72.7%, specificity of 76.5%, PPV of 46.6%, and NPV of 90.9%. In contrast, Typhidot demonstrated a markedly higher sensitivity of 98.5% and NPV of 99.2%, but lower specificity of 53.8% and PPV of 37.6%. Notably, all three tests were simultaneously positive in only 16% of cases, while 41.6% tested negative across all modalities.

Blood culture remains the gold standard but is limited by sensitivity and resource demands. The Widal test showed only moderate reliability, whereas Typhidot demonstrated excellent sensitivity and NPV, making it useful for rapid screening but limited by low specificity. No single test is sufficient; a multimodal approach combining rapid assays, culture, and clinical evaluation is the most effective strategy for accurate diagnosis in endemic, resource-limited settings.

Keywords: Typhoid fever; Widal test; Typhidot test; Sensitivity; Specificity.

INTRODUCTION

Typhoid fever is an infectious disease caused by Salmonella Typhi, which remains a major public health issue, especially in developing countries. These regions, often characterised by inadequate sanitation, poor water supply, and ineffective sewage systems, create ideal conditions for the faecal-oral transmission of the bacteria.[1,2] The disease presents with a wide range of clinical manifestations, from asymptomatic cases to systemic symptoms such as fever, malaise, and headache. Severe complications, including ileal

ulceration, perforation, and haemorrhage, can occur and may be life-threatening if not managed promptly.[1-3]

In 2017, an estimated 10.9 million cases of typhoid fever were reported globally. By 2021, enteric fever accounted for approximately 9.3 million cases (95% uncertainty interval: 7.3–11.9 million) and 107,500 deaths (95% uncertainty interval: 56,100–180,800). During this period, the global mortality rate declined from 1.87 per 100,000 person-years (95% UI: 0.95–3.18) in 2017 to 1.50 per 100,000 person-years (95% UI: 0.78–2.54) in 2021. Notably, there were significant geographical disparities, with South Asia contributing the highest burden of both cases and deaths.[4] However, accurately estimating the burden of enteric fever remains challenging due to the similarity of its clinical presentation to other febrile illnesses and the lack of highly sensitive and specific diagnostic tools in endemic areas.[1,5]

One of the major challenges in the effective control of typhoid fever is the difficulty of accurate diagnosis. In clinical settings, the diagnosis is often complicated by the non-specific nature of symptoms, which frequently overlap with those of other endemic infections such as malaria, dengue, and viral gastroenteritis.[1-3,5]

The diagnosis of typhoid fever includes a combination of clinical evaluation and laboratory testing, including serological assays, blood, bone marrow, and stool cultures, as well as antigen detection and DNA amplification techniques.[3] A definitive diagnosis is established by isolating Salmonella typhi, typically through blood or bone marrow cultures. Among these, blood culture is considered the gold standard, offering a diagnostic accuracy of approximately 70%–75% during the first week of illness.[6] Although blood, bone marrow, and stool cultures remain the most reliable diagnostic methods, their high cost and limited accessibility pose significant challenges, particularly in resource-limited settings.[2,3,6]

The Widal test, developed over a century ago, remains the primary diagnostic tool in many developing countries despite its well-recognised limitations.[1] Interpretation of the Widal slide agglutination test is complicated by factors such as cross-reactivity with other infections, a time lag between infection and antibody production, and the persistence of antibodies long after treatment. Additionally, repeated exposure to Salmonella Typhi can lead to false-positive results. These issues significantly undermine the test's diagnostic accuracy and reliability for identifying active typhoid fever cases in endemic settings.[1,3,5] The tube dilution technique allows for quantification of specific antibodies, and changes in antibody titres can indicate active disease; however, this method is not widely accessible.[7]

An alternative diagnostic test, Typhidot,[8] which detects antibodies against the outer membrane antigens common to Salmonella Typhi and Salmonella paratyphi, has shown improved performance in many studies.[3,9-13] Nevertheless, variations in sensitivity and specificity have been reported between adults and children.[2,6,14,15] The Typhidot assay demonstrates good sensitivity (68–95%) and specificity (75–95%) during early infection stages. Importantly, its increased negative predictive value is particularly valuable in endemic areas for ruling out disease.[16]

The present study was therefore designed to accurately compare the sensitivity and specificity of the Widal and Typhidot tests for the diagnosis of enteric fever, using blood culture as the gold standard reference method.

MATERIALS AND METHODS

This study was conducted in the Department of Microbiology, Mysore Medical College and Research Institute, in collaboration with K.R. Hospital, Mysuru. The study population included both outpatients and inpatients presenting with febrile illness and clinically suspected of having enteric fever. Patients with acute febrile illness and those suspected of typhoid fever were included, while febrile patients with confirmed alternate diagnoses were excluded.

After obtaining written informed consent and recording relevant clinical details, blood samples were collected under aseptic precautions. Adults provided 15 mL and children 7 mL of venous blood. Of this, 10 mL (adults) or 5 mL (children) was inoculated into Brain Heart Infusion (BHI) broth for blood culture. The remaining sample was centrifuged at 1000 rpm for 3 minutes, and the separated serum was stored for Widal and Typhidot testing.

For blood culture, 5–10 mL of blood was inoculated into culture bottles and incubated at 37°C. Subcultures onto nutrient agar, blood agar, and MacConkey agar were performed after overnight incubation and repeated up to 7 days if necessary. Colony identification was based on morphology, Gram staining (showing Gram-negative bacilli) and biochemical tests. Salmonella Typhi colonies were non-lactose fermenting on MacConkey agar, grey-white, moist, and circular on nutrient and blood agar. They were oxidase-negative, catalase-positive, motile, MR-positive, VP-negative, and fermented glucose, mannitol, and maltose without gas production. They did not ferment lactose or sucrose and showed minimal H_2S production in KIA/TSI media. Other characteristics included a negative citrate test and no ornithine decarboxylation.

The Widal test was performed using both the slide screening and slide semi-quantitative methods. The slide screening test detected anti-O, H, and AH agglutinins, while the semi-quantitative method estimated titres ranging from 1:20 to 1:320. Agglutination indicated a positive result. This test is rapid and useful in resource-limited settings, though

it has limitations related to sensitivity and specificity.

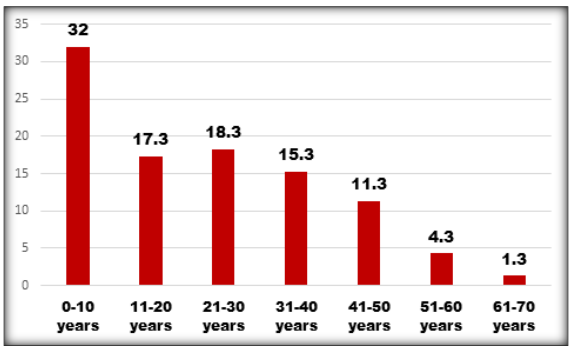
The Typhidot test, a rapid immunoassay, detects IgM and IgG antibodies against S.Typhi outer membrane proteins. Using a commercial kit, 3 µL of patient serum was mixed with sample diluent and incubated with pre-coated strips. Following a series of washes and addition of conjugate and colour developer, the strips were dried and interpreted by comparing test dots with control strips. IgM positivity alone indicated acute infection, IgM + IgG positivity suggested middle-stage infection, and IgG alone pointed to past infection or reinfection. Both negative results suggested an absence of typhoid. A known limitation of the test is that high IgG levels may inhibit IgM binding, resulting in false negatives, which may require confirmation using Typhidot-M.

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarize demographic and clinical characteristics. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the Widal and Typhidot tests were calculated using blood culture as the gold standard. Categorical variables were compared using the chi-square test or Fisher's exact test where appropriate. A p-value of less than 0.05 was considered statistically significant.

RESULTS

This prospective observational study enrolled 300 patients attending K. R. Hospital and Cheluvamba Hospital, Mysore, from March 2018 to February 2019, all of whom were clinically suspected with enteric fever. The demographic profile revealed a wide age distribution, with the mean age of participants being 23.79 years (±18.37). The age-wise distribution indicated that the most affected age group was 0-10 years, comprising 32% (n=96) of the cohort, followed by 11-20 years (17.3%, n=52) and 21-30 years (18.3%, n=55). A smaller proportion belonged to the 31-40 years (15.3%, n=46), 41-50 years (11.3%, n=34), and older age groups, with only 1.3% (n=4) above 60 years (Graph 1). These findings suggest that pediatric and young adult populations are predominantly affected in this setting.



Graph 1: Age distribution of the study participants

The gender distribution was nearly balanced, with 55.6% (n=167) males and 44.3% (n=133) females, resulting in a male-to-female ratio of approximately 1.26:1. This marginal male predominance aligns with prior epidemiological data indicating a slight male preponderance in enteric fever cases, possibly related to differential exposure or healthcare-seeking behaviours among genders.

The clinical characteristic of fever duration was categorized into three groups: 1-5 days, 6-10 days, and 11-15 days. The most common duration was 6-10 days, accounting for 67.6% (n=203) of patients, followed by 23.3% (n=70) with fever lasting 1-5 days, and 9% (n=27) with fever duration exceeding 10 days (Table 1). This distribution demonstrates that most patients present during the second week of illness, which is the typical window for optimal diagnostic testing.

Table 1: Duration of fever among the study participants. [n=300]

Duration of Fever	No. of patients	Percentages
1-5 days	70	23.3
6-10 days	203	67.6
11-15 days	27	9.0
Total	**300**	**100**

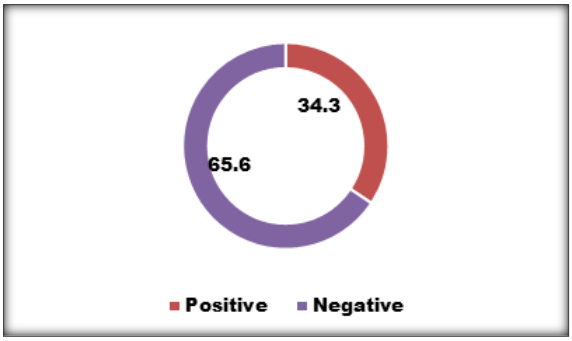
Regarding microbiological and serological investigations, blood culture positivity was observed in 22% (n=66) of patients, aligning with previous reports but indicating lower sensitivity potentially due to prior antibiotic use or timing of sample collection. Blood cultures yielded the highest positivity during the 6-10 days of fever (71.21%), reaffirming the importance of timely sample collection for pathogen detection.

Table 2: Percentage of positive results of blood culture, Widal test and Typhidot test according to the duration of fever

Fever duration (days)	Blood culture		Widal test		Typhidot test	
	No.	%	No.	%	No.	%
1 to 5	7	10.6	7	6.8	19	11
6 to 10	47	71.21	78	75.72	130	75.14
11 to 15	12	18.2	18	17.5	24	13.9
Total	66	100	103	100	173	100

The diagnostic yield of all three tests varied according to the duration of fever. Blood culture showed the highest positivity rate (71.21%) in patients with a fever duration of 6 to 10 days, while the positivity dropped significantly to 10.6% in those presenting within the first 1 to 5 days. Similarly, the Widal test showed the highest positivity (75.72%) during days 6 to 10 of illness, with much lower rates in the early phase (6.8% for 1 to 5 days) and moderate positivity (17.55%) between 11 and 15 days. The Typhidot test followed a comparable pattern, with positivity of 75.14% during days 6 to 10, 11% in the first 1 to 5 days, and 13.9% during days 11 to 15 (Table 2). These findings suggest that the diagnostic performance of all three tests improves notably after the first week of illness.

Serological testing showed variability in diagnostic performance. The Widal test identified 34.3% (n=103) of patients as positive (Graph 2), with a sensitivity of 72.72% and specificity of 76.49%. The positive predictive value (PPV) was 46.60%, and the negative predictive value (NPV) was 90.86%, indicating relatively high reliability in ruling out infection (Table 3). However, the test's moderate PPV suggests limitations in confirming active typhoid without corroborative testing.

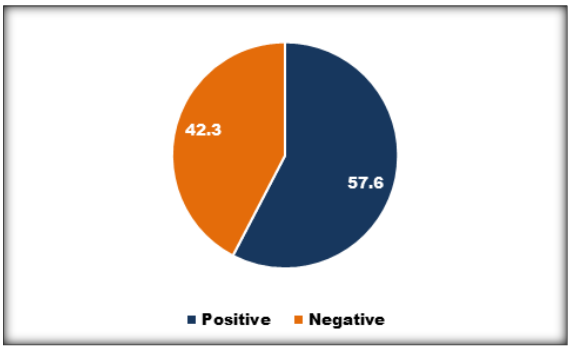


Graph 2: Widal test positivity among the study participants

Table 3: Overall diagnostic efficacy of Widal test in the diagnosis of Typhoid fever in contrast with Blood culture as the gold standard. [n= 300]

Statistics	Values (%)
Sensitivity	72.72
Specificity	76.49
Positive Predictive Value	46.60
Negative Predictive Value	90.86

The Typhidot test demonstrated a higher positivity rate of 57.6% (n=173) (Graph 3). Its diagnostic performance was notably high, with an impressive sensitivity of 98.48%, although specificity was moderate at 53.84%. The PPV was 37.57%, and NPV was 99.21%, indicating that negative Typhidot results strongly suggest absence of disease, making it a useful screening tool, especially in settings with limited blood culture facilities (Table 4). The high sensitivity supports its utility in early detection, while the lower specificity underscores the potential for false positives.



Graph 3: Typhidot Test positivity among the study participants

Table 4: Overall diagnostic efficacy of Typhidot test in the diagnosis of Typhoid fever in contrast with Blood culture as the gold standard. [n= 300]

Statistics	Values (%)
Sensitivity	**98.48**
Specificity	**53.84**
Positive Predictive Value	**37.57**
Negative Predictive Value	**99.21**

The combined analysis of diagnostic tests (blood culture, Widal, and Typhidot) revealed that all three tests were simultaneously positive in 16% (n=48) of patients. Isolated positivity of blood culture was rare, with only one case (0.33%) where blood culture was positive but Widal and Typhidot were negative. Conversely, 41.6% (n=125) of patients tested negative across all tests, highlighting challenges in diagnosing typhoid based solely on laboratory investigations and emphasizing the need for multiple diagnostic modalities.

DISCUSSION

The present study evaluated the diagnostic efficacy of the Widal test and Typhidot assay in comparison to blood culture, the gold standard, in 300 clinically suspected cases of enteric fever. The findings provide valuable insights into the continued reliance on traditional serological methods versus the adoption of rapid immunoassays in endemic, resource-limited settings.

Epidemiological Context

Our study population showed a predominance of paediatric and young adult cases, with the 0-10-year age group most affected (32%). This aligns with global estimates indicating a disproportionately high burden of enteric fever among children in South Asia and African countries.[3,17]

Ousenu K et al. (2021) also highlighted the greatest incidence, consistent with our observation that children and adolescents formed the bulk of the affected cohort.[9] Similarly, Mukhopadhyay B et al. (2019) reported that India continues to struggle with typhoid control, with children at greatest risk.[1] This suggests that age-targeted preventive strategies, including vaccination campaigns, remain crucial in endemic regions.

Blood Culture Findings

In our study, blood culture positivity was 22%. This is within the expected range (15–45%) reported but lower than optimal, possibly due to prior antibiotic exposure. Neupane et al. (2021) similarly emphasized that inappropriate antibiotic use before sample collection is a major contributor to false negatives in culture.[6] Our finding that yield peaked between days 6–10 of illness corroborates earlier work by Ajibola et al. (2018), who described culture sensitivity as highly dependent on the timing of collection.[5] Thus, while culture remains indispensable, its practical utility in endemic communities is limited by contextual factors.

Performance of the Widal Test

The Widal test in our study had a sensitivity of 72.7% and specificity of 76.5%. This is comparable to the range reported in prior studies from India and Africa.[3,8,13,16,17] Narayanappa et al. (2010) documented sensitivity of 70% and specificity of 75% in pediatric patients, which closely parallels our results.[10] Similarly, Singh & Singh (2021) noted moderate accuracy, reinforcing the notion that Widal cannot be relied upon in isolation.[11] Conversely, Yemeli Piankeu et al. (2024) found slightly lower sensitivity (65%) and specificity (70%) in Cameroon, suggesting regional variations likely due to baseline antibody titers in endemic populations.[12] The positive predictive value in our study (46.6%) was lower than many reported series, possibly reflecting background antibody persistence in a highly endemic setting, as described by Tegene et al,[2] Taken together, the evidence underscores Widal's utility as an adjunct rather than a standalone diagnostic tool.

Performance of Typhidot

The Typhidot assay outperformed Widal in sensitivity (98.5% vs. 72.7%) and NPV (99.2% vs. 90.9%). These findings mirror those of Narayanappa et al. (2010), who showed Typhidot-M sensitivity of 95% compared to 70% for Widal.[10] Ousenu et al. (2021) in Cameroon also reported Typhidot's higher sensitivity (92%) than Widal (68%).[9] Our results are therefore consistent with the broad consensus that Typhidot is superior for early detection. However, our study found Typhidot specificity to be only 53.8%. This was lower than the 75–95% reported by Wijedoru et al. (2017) in a Cochrane review and by Najib et al. (2021) in their meta-analysis.[8,16] Possible reasons include high background antibody levels in endemic India, cross-reactivity with S. paratyphi, and IgG interference, as highlighted by Olsen et al. (2004).[14] Interestingly, Goenka et al. (2025) in Delhi reported a specificity closer to 90%, suggesting intra-country variation possibly linked to patient selection and kit versions.[13] Thus, while Typhidot offers excellent utility for ruling out disease due to its high sensitivity and NPV, its moderate specificity requires cautious interpretation to avoid overtreatment.

Comparative Assessment of Tests

Our findings align with those of Yemeli Piankeu et al. (2024) and Ousenu et al. (2021), who also concluded that Typhidot is more reliable than Widal in early illness.[9,12] However, like previous authors, we caution that no single test is definitive. Sapkota et al. (2023) stressed that diagnostic algorithms in endemic countries should integrate rapid serological assays with culture and clinical evaluation.[15] This approach balances sensitivity with specificity and helps optimize antimicrobial stewardship.

Clinical and Public Health Implications

The clinical implication of our study is clear: in endemic areas where culture facilities are scarce, Typhidot may serve as a frontline test, particularly valuable for rapid screening of febrile children. However, positive results should ideally be confirmed by culture or at least interpreted alongside Widal and clinical features. At the public health level, improved diagnostics are essential for accurate disease burden estimates and guiding vaccination strategies, as underscored by Mukhopadhyay et al. (2019).[1]

Strengths and Limitations

The strengths of our study include its large sample size (n=300), comprehensive comparison of three modalities, and focus on a high-burden region. Limitations include possible underestimation of culture positivity due to prior antibiotics, exclusion of bone marrow culture, and use of a single Typhidot kit variant rather than Typhidot-M, which might improve accuracy. The single-centre design may also limit generalizability.

Future Directions

Emerging molecular platforms, such as multiplex PCR and next-generation sequencing, show promise but remain cost-prohibitive in resource-limited settings.[6,15] Future research should prioritise affordable point-of-care antigen detection kits with improved specificity, while strengthening laboratory infrastructure to sustain blood culture capacity. Combining diagnostics with antimicrobial resistance monitoring will be essential for both clinical care and public health surveillance.

CONCLUSION

The present study highlights the ongoing challenges in diagnosing enteric fever in endemic regions. While blood culture remains the gold standard, its limited sensitivity and dependence on infrastructure restrict its real-world applicability. The Widal test, though still widely used, demonstrated only moderate accuracy, reaffirming its inadequacy as a sole diagnostic tool. In contrast, the Typhidot assay showed exceptional sensitivity and negative predictive value, making it a valuable frontline test for rapid screening, particularly in resource-limited settings. However, its reduced specificity raises concern for overdiagnosis and inappropriate antibiotic use, which could further fuel antimicrobial resistance. These findings strongly suggest that no single diagnostic test can be relied upon in isolation. A pragmatic multimodal approach, combining rapid assays with confirmatory culture and clinical evaluation, appears to be the most effective strategy.

Declaration

Ethics approval and consent to participate

The study was approved by The Institutional Ethics Committee (IEC). All procedures were followed in compliance with applicable rules and regulations. Everyone who participated, including their legal guardian(s), gave their informed consent.

Consent for publication

Everyone who participated, including their legal guardian(s), gave their informed consent.

Availability of supporting data - None

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Authors' contributions

(1), (3) & (4) designed the study and prepared the first draft of the paper.

(1), (2) & (3) contributions to the acquisition and interpretation of data, they are guarantor.

(1), (3) & (5) contributed to the design of the work and the interpretation of data.

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