HSI Health Sciences Investigations Journal

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ISSN Online 2704-4890 | ISSN Print 2720-7609

Online first publication

Original Research Article

HSI Journal (2026) Volume 8 (Issue 2):1490-1497. https://doi.org/10.46829/hsijournal.2026.4.8.2.1490-1497

Open Access

Performance of Widal IgM/IgG rapid diagnostic tests, and stool culture in identifying asymptomatic typhoid infections

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Received December 2025; Revised February 2026; Accepted March 2026

Abstract

Background: Introduction: Non-febrile asymptomatic persons present a unique case definition for typhoid since the only means of diagnosis is the detection of typhoidal antigens or antibodies or the growth of Salmonella bacteria in cultures. However, in recent times, the diagnostic capacities of commonly used testing regimes have been called into question.

Objective: The study examined the test characteristics of three testing regimes, Widal, IgM/IgG Rapid Diagnostic test, and stool culture tests in the diagnosis of typhoid fever among non-febrile asymptomatic persons.

Methods: The cross-sectional study sampled healthy persons in Atebubu Municipality. Blood and stool samples were collected from each participant after they consented to the study and completed a sociodemographic questionnaire. Participants' sera were tested for antibodies against Salmonella "O" and "H" antigens, and the presence of IgM and IgG against Salmonella antigens. Stool samples were cultured on Salmonella-Shigella agar, and the characteristic colonies of Salmonella spp. identified. The degree of agreement between different tests and standard tests was analysed by calculating Cohen's kappa statistic.

Results: Eighty-three (83) study participants were studied. The diagnostic tests evaluated in this study vary significantly in their performance metrics. While immunological tests such as IgM and IgG show promise in detecting typhoid fever, they also present challenges in specificity and agreement with culture methods. The Widal test, despite its high specificity, has low sensitivity, limiting its usefulness for accurate diagnosis of typhoid fever. The IgM test demonstrated high sensitivity (89.3%) but low specificity (32.7%), suggesting its effectiveness in detecting true cases while exhibiting a considerable rate of false positives. Conversely, the IgG test showed lower sensitivity (50.0%) but higher specificity (65.5%), indicating better utility in ruling out non-cases. Notably, extended culture incubation (48hrs) significantly increased test sensitivity rates compared to 24hr cultures.

Conclusion: Complementary testing regimens should be adapted to increase diagnostic accuracy in typhoid fever, especially among non-febrile, asymptomatic patients.

Keywords: Widal IgM/IgG typhoid fever, enteric fever, *Salmonella typhi, Salmonella paratyphi*

Cite the publication as Yangnemenga E, Konlaan Y, Moses-Tetteh D, Adjomah VK. Performance of Widal, IgM/IgG rapid diagnostic tests, and stool culture in identifying asymptomatic typhoid infections. HSI Journal 8 (2):1490-1497. https://doi.org/10.46829/hsijournal.2026.4.8.2.1490-1497

INTRODUCTION

Typhoid fever, a grave systemic infection caused by *Salmonella enterica* serotype Typhi and Paratyphi, remains a formidable challenge to global public health [1]. Despite advances in medical science, typhoid fever continues to exert a significant toll, with an estimated 11 to 21 million new cases annually, resulting in approximately 128,000 to 161,000 deaths worldwide [2]. The clinical spectrum of typhoid fever is notoriously broad, encompassing everything from subtle febrile episodes to life-threatening systemic complications, which complicates

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timely diagnosis and effective treatment [3]. This variability in presentation not only challenges clinicians but also highlights the urgent need for enhanced diagnostic tools and public health interventions to mitigate the burden of this disease.

Accurate diagnosis of typhoid fever remains a significant challenge due to its nonspecific clinical manifestations, which often overlap with those of other febrile illnesses, such as malaria and dengue, particularly in regions where these diseases are endemic [4]. Clinical history, presentation and laboratory testing are often enough to diagnose typhoid fever in febrile patients. In non-febrile patients, the presentation is less helpful, as fever is a cardinal sign of typhoid fever. Thus, the importance of accurate diagnosis cannot be overemphasised, especially among non-febrile persons. Various testing regimes have been instituted, each with its peculiar strengths and weaknesses. Traditionally, the Widal test, which detects agglutinating antibodies against *Salmonella* O and H antigens, was the mainstay of clinical practice. Additionally, the test's sensitivity and specificity are highly variable, influenced by factors such as the local prevalence of typhoid fever, prior exposure to *Salmonella*, and vaccination history [5]. A meta-analysis by Wijedoru et al. highlights the test's limitations, revealing that its sensitivity and specificity can plummet to as low as 50-60%, contingent on the cutoff titers and the population being tested [6].

Rapid diagnostic tests (RDTs), unlike the Widal test, have gained traction in recent years due to their user-friendliness and swift turnaround times, offering a promising alternative to traditional diagnostic methods for typhoid fever [6]. Multiple studies have assessed the effectiveness of RDTs, yielding mixed outcomes [7]. A systematic review by Ochiai et al. reported that the sensitivity of RDTs for detecting typhoid fever varies widely, from 60% to 80%, while specificity tends to be higher, ranging from 75% to 90% [8]. However, the utility of these tests in detecting typhoid fever among non-febrile individuals remains poorly understood, particularly in areas with a high prevalence of asymptomatic carriers [9]. Asymptomatic carriers are pivotal in the transmission dynamics of typhoid fever, and their identification is crucial for effective disease control and prevention strategies [10]. This gap in understanding highlights the urgent need for further research to refine RDTs and enhance their capacity to detect both symptomatic and asymptomatic cases, thereby strengthening public health efforts to curb the spread of typhoid fever. Furthermore, the pronounced variability associated with the Widal test not only casts doubt on its diagnostic accuracy but also underscores the critical need for more reliable and standardised approaches to diagnosing typhoid fever, ensuring more consistent and accurate results across diverse settings.

Stool culture, although highly specific [11], has not enjoyed widespread use in Low- and Middle-Income Countries (LMICs) such as Ghana, unlike the previous two. Although several factors can affect the diagnostic yield of stool culture, such as prior antibiotic exposure, the volume of the specimen, and the presence of competing intestinal flora [12], stool culture continues to be a valuable diagnostic tool, particularly for the definitive confirmation of typhoid fever and for guiding targeted antimicrobial therapy [13]. In this study, we critically evaluate the diagnostic performance of the Widal test, rapid diagnostic immunoglobulin tests, and stool culture in detecting typhoid fever among non-febrile individuals in Atebubu Municipality. Anecdotal reports in this region indicate a high incidence of typhoid fever, where conventional diagnostic methods may inadequately capture the disease's epidemiological scope, especially among asymptomatic carriers.

MATERIALS AND METHODS

This cross-sectional study was conducted from January to August 2022 in Atebubu Municipality, Bono East Region, Ghana. The objective was to evaluate the diagnostic performance of Widal, IgM/IgG Rapid Diagnostic Tests (RDTs), and stool culture among asymptomatic non-febrile individuals. The study site was selected based on anecdotal evidence of a high burden of typhoid fever in the region.

The study population comprised apparently healthy, non-febrile individuals residing in Atebubu Municipality at the time of sampling. Non-febrile individuals were defined as those with a body temperature not exceeding 37.5°C and who exhibited no symptoms indicative of acute infection. Sample size was determined using the single population proportion formula with an assumed prevalence of 50%, a 95% confidence interval, and a 10% margin of error. This yielded a minimum of 96 participants; however, 83 individuals were ultimately enrolled based on available consenting participants within the sampling period. The recruitment of 83 participants from a target of 96 reduces statistical power, particularly for interpreting results from subgroup analyses. Participants were selected using a stratified random sampling technique to ensure representative coverage across different areas within the municipality. The community was stratified into various locales based on geographical and socio-economic factors, and participants were randomly selected from each stratum to mitigate selection bias. Inclusion criteria required participants to be non-febrile individuals aged 5 years or older, residing in Atebubu Municipality during the study period, and free of acute infection symptoms.

Children under 5 years were excluded due to challenges in obtaining adequate stool samples and the higher prevalence of non-typhoidal *Salmonella* infections in this age group. Exclusion was also based on ethical considerations for obtaining assent and consent from minors in this community-based setting. Also, individuals who had received antibiotic treatment within the previous three months, those with known immunocompromised

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conditions (such as HIV/AIDS, diabetes, or cancer), and individuals who were visibly ill or febrile at the time of sampling were excluded.

A structured questionnaire was administered to gather demographic information, including age, sex, level of education, religion, marital status, workplace environment, residential location, history of exposure to typhoid fever, and pertinent medical history. Blood samples, approximately 5 mL in volume, were collected from each participant for the Widal test and rapid diagnostic immunoglobulin tests. Additionally, stool samples were obtained using sterile, wide-mouthed plastic containers. Participants were instructed to provide 2-3 grams of fresh stool, which was collected with sterile spatulas provided in the containers and processed within one hour of collection to preserve the viability of potential *Salmonella* organisms. Ethical approval was obtained from the Municipal Health Directorate of the Atebubu Municipal Assembly, consistent with the Declaration of Helsinki and Ghana Health Service ethical standards. Prior to participation, all individuals were adequately informed about the objectives, procedures, risks, and benefits of the study, including the potential for mild discomfort associated with venipuncture. Informed consent was obtained from each adult participant and from guardians of minors, with assent from children where applicable.

Laboratory analyses were performed at the Atebubu Municipal Hospital laboratory in accordance with standard operating procedures. The Widal test was conducted using the OZOTEX-WIDAL (OH) test kit (Medsource Ozone Biomedicals Pvt. Ltd.), a qualitative and semi-quantitative assay designed to detect somatic (O) and flagellum (H) antigens of *Salmonella Typhi* and *Salmonella Paratyphi*, following the manufacturer's guidelines. Serum was extracted from the collected blood samples and processed by dispensing 50 µL of the participant's serum onto each of the first paired circles on a slide, 50 µL of positive control serum onto the next paired circles, and 50 µL of normal saline onto the final paired circles. Antigen drops were added to each row of circles, and the mixture was gently agitated for 60 seconds. Agglutination patterns were examined to identify the presence of *S. Typhi* and/or *S. Paratyphi* antigens, with additional titration performed for samples showing positive reactions. Rapid diagnostic immunoglobulin tests were performed using the Juschek (Acrobiotech, California, USA) IgG/IgM test kit. This involved adding 25 µL of the participant's serum to the sample well, followed by two drops (approximately 50 µL) of reaction buffer, and allowing 15 minutes for result interpretation. The presence of IgM and/or IgG bands was assessed to determine different stages of infection, if present, as described by [14].

Stool samples were cultured on *Salmonella-Shigella* agar to identify the presence of *Salmonella spp*. Each stool sample was emulsified with normal saline, and a sterile wire loop was used to streak the suspension onto the agar plates. These plates were then incubated at 37°C for 24 hours. Following incubation, the plates were examined for the characteristic colony morphology indicative of *Salmonella* species. The plates were then incubated for an additional 24 hours and examined for possible *Salmonella* species. This was recorded as the 48-hour culture results. To ensure the accuracy and reliability of the results, quality control measures were implemented by including a known positive control with each batch of samples to assess agar quality and monitor environmental conditions. This study adhered to the 2015 Standards for Reporting Diagnostic Accuracy (STARD) guidelines to ensure completeness and transparency in reporting.

Data analysis

Data analysis was performed using SPSS version 26.0 (IBM Corp, Armonk, NY, USA). Descriptive statistics summarised the demographic characteristics of the participants. Diagnostic performance metrics, including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), were calculated using stool culture as the reference standard (high specificity for definitive confirmation of Salmonella shedding, particularly relevant for identifying asymptomatic carriers). Cohen's kappa statistic was employed to assess agreement between tests. Strength of agreement was interpreted using the established Landis and Koch (1977) scale: poor (< 0.20), fair (0.21–0.40), moderate (0.41–0.60), substantial (0.61–0.80), and almost perfect (>0.80) [15]. Chi-square tests were used to evaluate associations between demographic factors and typhoid positivity across the different diagnostic modalities. Potential sources of diagnostic disagreement, such as prior antibiotic exposure, chronic carrier state, and cross-reactivity, were considered and discussed. The statistical significance threshold was set at $p < 0.05$, and all estimates were reported with 95% confidence intervals.

RESULTS

The demographic profile of the eighty-three (83) study participants reveals a diverse distribution across several characteristics, with a mean age of 32.4. Most (55%) of the participants identified as Christians. Other demographics are shown in Table 1. Table 2 summarises the prevalence of typhoid fever based on various diagnostic tests. The immunological tests revealed that the IgM test identified 62 positive cases (74.7%) compared to 21 negative cases (25.3%), while the IgG test showed a lower positive rate of 33 cases (39.8%) against 50 negative cases (60.2%). The Widal test results indicated a lesser positivity in anti-TO titre (> 1:80) (9.6%) than the anti-TH titre (> 1:80) (14.5%). A similar trend was observed in the 24-hour culture incubation (33.7%) compared to the 48-hour incubation (72.3%). Table 3 displays the prevalence of typhoid fever based on diagnostic tests across two different locations: Zongo and Residential areas. For the immunological tests, IgM test results showed a higher prevalence in the Residential area (82.6%) than in Zongo (64.9%), although

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this difference did not reach statistical significance (p = 0.065). Testing positive for IgG was more strongly associated with living in a Residential area than with living in a Zongo area (p-value of 0.034). Similarly, testing positive for anti-TH titre (>1:80) was significantly more prevalent in the Residential area (21.7%) than in Zongo (5.4%), with a p-value of 0.035.

Table 4 presents a bivariate analysis of demographic factors associated with typhoid fever prevalence, as measured by culture results at 24 and 48 hours. Positivity rates using either test culture incubation time were similar across demographics, except for workplace location, where 24-hour incubation showed a significant difference in positivity across the demographic variable. Table 5

Table 1. Sociodemographic characteristics of study participants

Variable	Categories	n	%	Mean ± SD
Age				32.4 ± 10.2
Level of education	No formal education	39	47	
	Primary school	21	25.3	
	High school and above	23	27.7	
Religion	Christian	55	66.3	
	Muslim	14	16.9	
	Other	14	16.9	
Marital status	Single	39	47	
	Married	35	42.2	
	Separated/divorced	9	10.8	
Location of workplace	Market area	21	25.3	
	Along a road	19	22.9	
	School premise	12	14.5	
	Lorry park/station	4	4.8	
	Church/mosque premises	6	7.2	
	Non-response	21	25.3	
Place of residence	Zongo	37	44.6	
	Residential	46	55.4	

Table 2. Prevalence of typhoid by different test regimes

Diagnostic test	Negative		Positive	
	n	%	n	%
Immunological test				
IgM	21	25.3	62	74.7
IgG	50	60.2	33	39.8
Positive for both (IgM+ IgG)	21	25.3	62	74.7
Widal test				
anti-TO titre (>1:80)	75	90.4	8	9.6
anti-TH titre (>1:80)	71	85.5	12	14.5
Positive for both (TO+TH)	69	83.1	14	16.9
Culture				
24 hours	55	66.3	28	33.7
48 hours	23	27.7	60	72.3

Table 3. Prevalence of typhoid based on location

	Zongo (n=37)	Residential (n=46)	P- value
Immunological test			
IgM	24 (64.9)	38 (82.6)	0.065
IgG	10 (27.0)	23 (50.0)	0.034
Positive for both (IgM+ IgG)	24 (64.9)	38 (82.6)	0.065
Widal test			
anti-TO titre (>1:80)	4 (10.8)	4 (8.7)	0.746
anti-TH titre (>1:80)	2 (5.4)	10 (21.7)	0.035
Positive for both (TO+TH)	5 (13.5)	9 (19.6)	0.464
Culture			
24 hours	11 (29.7)	17 (37.0)	0.489
48 hours	29 (78.4)	31 (67.4)	0.266

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Table 4. Bivariate analysis of demographic factors associated with typhoid

			Culture for 24h			Culture for 48h		
Variable	categories	N	Negative	Positive	p-value	Negative	Positive	p-value
Age groups	</=25	25	19 (34.5)	6 (21.4)	0.438	10 (43.5)	15 (25.0)	0.307
	26-36	30	18 (32.7)	12 (42.9)		6 (26.1)	24 (40.0)	
	37-47	16	9 (16.4)	7 (25.0)		3 (13.0)	13 (21.7)	
	48+	12	9 (16.4)	3 (10.7)		4 (17.4)	8 (13.3)	
Level of education	No formal education	39	28 (50.9)	11 (39.3)	0.293	12 (52.2)	27 (45.0)	0.59
	Primary school	21	11 (20.0)	10 (35.7)		4 (17.4)	17 (28.3)	
	High school and above	23	16 (29.1)	7 (25.0)		7 (30.4)	16 (26.7)	
Religion	Christian	55	36 (65.5)	19 (67.9)	0.476	14 (60.9)	41 (68.3)	0.744
	Muslim	14	8 (14.5)	6 (21.4)		5 (21.7)	9 (15.0)	
	Other	14	11 (20.0)	3 (10.7)		4 (17.4)	10 (16.7)	
Marital status	Single	39	28 (50.9)	11 (39.3)	0.089	11 (47.8)	28 (46.7)	0.433
	Married	35	19 (34.5)	16 (57.1)		8 (34.8)	27 (45.0)	
	Separated/divorced	9	8 (14.5)	1 (3.6)		4 (17.4)	5 (8.3)	
Location of workplace	Market area	33	16 (29.1)	17 (60.7)	0.003	8 (34.9)	25 (41.7)	0.734
	Along a road	26	17 (30.9)	9 (32.2)		9 (39.1)	15 (25.0)	
	School premise	16	14 (25.5)	2 (7.1)		5 (21.7)	12 (20.0)	
	Church/mosque premises	8	8 (14.5)	0 (0.0)		1 (4.3)	8 (13.3)	
Place of residence	Zongo	37	26 (47.3)	11 (39.3)	0.489	8 (34.8)	29 (48.3)	0.266
	Residential	46	29 (52.7)	17 (60.7)		15 (65.2)	31 (51.7)	

Table 5. Comparison of the diagnostic performance of the diagnostic tools using 24h culture as standard

Diagnostic test	% Sensitivity (95%CI)	%Specificity (95%CI)	%PPV (95%CI)	%NPV (95%CI)	Kappa Index (95%CI)
IgM	89.3 (82.6-95.9)	32.7 (22.6-42.8)	40.3 (29.8-50.9)	**85.7** (78.2-3.2)	0.170 (0.031-0.308)
IgG	50.0 (39.2-60.8)	65.5 (55.2-75.7)	42.4 (31.8-53.1)	72.0 (62.3-1.7)	0.148 (-0.067-0.363)
Positive for both (IgM+ IgG)	89.3 (82.6-95.9)	32.7 (22.6-42.8)	40.3 (29.8-50.9)	85.7 (78.2-3.2)	0.170 (0.031-0.308)
anti-TO titre (>1:80)	14.3 (6.8-21.8)	92.7 (87.1-98.3)	50.0 (39.2-60.8)	68.0 (58.0-8.0)	0.085 (-0.092-0.262)
anti-TH titre (>1:80)	17.9 (9.62-26.1)	87.3 (80.1-94.4)	41.7 (31.1-52.3)	67.6 (57.5-7.7)	0.060 (-0.134-0.253)
Positive for both (TO+TH)	21.4 (12.6-30.3)	85.5 (77.9-93.0)	42.9 (32.2-53.5)	68.1(58.1-78.1)	0.078 (-0.124-0.281)
48 h culture	100.0	41.8 (31.2-52.4)	46.7 (35.9-57.4)	100.0	0.327 (0.191-0.462)

Table 6. Comparison of the diagnostic performance of the diagnostic tools using 48h culture as standard

Diagnostic test	% Sensitivity (95% CI)	%Specificity (95% CI)	% PPV (95% CI)	% NPV (95% CI)	Kappa Index (95% CI)
IgM	73.3 (63.8-82.9)	21.7 (12.9-30.6)	71.0 (61.2-80.7)	23.8 (14.7-33.0)	-0.051 (-0.258 - 0.157)
IgG	36.7 (26.3-47.0)	52.2 (41.4-62.9)	66.7 (56.5-76.8)	24.0 (14.8-33.1)	-0.181 (-0.369 - 0.007)
Positive for both (IgM+ IgG)	73.3 (63.8-82.9)	21.7 (12.8-30.6)	71.0 (61.2-80.7)	23.8 (14.7-33.0)	-0.051 (-0.258 - 0.157)
anti-TO titre (>1:80)	13.3 (6.0-20.7)	100	100	30.7 (20.8-40.6)	0.079 (0.019-0.138)
anti-TH titre (>1:80)	16.7 (8.65-24.7)	91.3 (85.2-97.4)	83.3 (75.3-91.4)	29.6 (19.8-39.4)	0.049 (-0.044-0.141)
Positive for both (TO+TH)	20.0 (11.4-28.6)	91.3 (85.2-97.4)	85.7 (78.2-93.2)	30.4 (20.5-40.3)	0.070 (-0.028-0.168)
24 h culture	46.7 (35.9-57.4)	100	100	41.8 (31.2-52.4)	0.327 (0.191-0.462)

evaluates the diagnostic performance of various typhoid fever tests using 24-hour culture as the reference standard. Compared to culture (24-hour incubation) results, IgM showed the highest sensitivity (89.3%), whilst anti-TO titre showed the highest specificity (92.7%). Furthermore, the positive predictive value for all tests was below 50%, whilst the negative predictive value was above 60%. All tests also showed poor agreement with culture (24-hour incubation) results (kappa index > 0.2). Table 6 presents a comparative analysis of the diagnostic performance of various tests for typhoid fever, with the 48-hour culture as the standard. Compared to culture (48hr incubation) results, IgM showed the highest sensitivity (73.3%) whilst anti-TO titre showed the highest specificity (100%). Furthermore, anti-TO titre showed the highest positive predictive value of 100% and the highest negative predictive value of 30.7%.

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DISCUSSION

This study provides important insights into the comparative diagnostic utility of the Widal test, IgM/IgG rapid serologic assays, and stool culture in a community-based sample of asymptomatic individuals in a typhoid-endemic setting. The immunological tests show a striking prevalence of positive IgM (74.7%) and IgG (39.8%) antibodies. However, the IgM assay demonstrated high sensitivity (89.3%) but markedly low specificity (32.7%), indicating its utility as a screening tool but a high potential for false-positive results. In contrast, the Widal test showed high specificity (up to 92.7% for anti-TO titres) but limited sensitivity, consistent with previous studies that caution against its use in isolation due to its poor diagnostic accuracy in endemic areas [16,17]. These findings emphasise the classical diagnostic trade-off between sensitivity and specificity: while high sensitivity improves the likelihood of detecting true cases, it also increases false positives if specificity is low; conversely, highly specific tests reduce false positives but may miss true infections. The Widal test results reveal that a significant majority of participants tested negative for anti-TO and anti-TH titers (>1:80), with figures of 90.4% and 85.5%, respectively. These findings underscore the importance of interpreting Widal test results with caution, particularly in endemic regions where cross-reactivity with other infections may lead to false positives [18]. This disconnect between clinical suspicion and laboratory confirmation of enteric fever raises critical questions about the reliability of traditional diagnostic methods and highlights the need for improved diagnostic accuracy in these settings.

A comparative analysis based on residence reveals that individuals living in Residential areas exhibit a higher positivity rate for IgG antibodies (50.0%) than those in Zongo areas (27.0%). Interestingly, individuals residing in residential (higher-income) areas had higher IgG and Widal positivity than those in Zongo communities, which are traditionally associated with poor sanitation. This counterintuitive finding may reflect differential healthcare access, prior antibiotic exposure, or health-seeking behaviour. In a community where people are constantly moving from Zongo areas to residential areas, it is not surprising that this finding could reflect the number of people who previously lived in Zongo areas. This observation raises concerns about asymptomatic carriage in less suspected populations and underscores the need for targeted health education and screening programs. The diagnostic performance metrics further emphasise the utility of these tests in clinical settings. The IgM test demonstrates high sensitivity (89.3%) but relatively low specificity (32.7%), indicating its potential as a screening tool while highlighting the risk of false positives. Conversely, the Widal test maintains high specificity, suggesting its utility for confirming enteric infections; however, variability in results across populations necessitates careful interpretation of findings.

Notably, the PPV of all tests remained below 50%, indicating a significant proportion of false positive results. However, the high NPVs (>60%) suggest that these tools may be more useful for ruling out infection than for confirming it. These findings align with practical clinical reasoning, in which tests with high NPV are valuable for screening asymptomatic individuals in population-level surveillance. Studies conducted in Dar es Salaam, Tanzania [19], Nigeria [20], and Ethiopia [21] have emphasised that the Widal test is more useful for excluding typhoid fever than for confirming its diagnosis. The calculated Kappa (κ) values for all tests against the 24-hour culture reference standard were low (ranging from 0.060 to 0.170), indicating poor agreement. For clinical and public health decision-making, the low kappa values underscore the substantial risk of misclassifying individuals—either missing true carriers (low sensitivity/poor agreement) or unnecessarily treating non-carriers (low specificity/poor agreement)—which can lead to inefficient resource allocation and improper antimicrobial stewardship. Extending the incubation time from 24 to 48 hours increased the Salmonella positivity rate (prevalence of 72.3%). These findings may be consistent with the afebrile state of the participants and the relatively high IgG positivity rate (39.8%) and TH titres (14.5%) – metrics that are generally indicative of previous or chronic infections. These findings, from this methodological novelty of doubling the incubation time, suggest that longer incubation periods may be important for culturing Salmonella spp. in afebrile and chronic infections, and support its routine inclusion when evaluating asymptomatic individuals, whose bacterial shedding may be intermittent or low-density.

This study's strengths include its population-based design, inclusion of asymptomatic individuals, and methodological innovation of prolonged culture incubation. Limitations include the exclusion of children under five years due to ethical constraints and the inability to perform blood cultures, which remains the definitive diagnostic method. The use of stool culture alone as the reference standard, while suitable for identifying chronic carriage in a resource-limited setting, may misclassify acute or non-shedding infections. Clinically, our findings advocate for a composite testing strategy-using highly sensitive tools (e.g., IgM) for screening and specific tests (e.g., Widal) for confirmation. However, given the limited scope and sample size of the current study (n = 83), these strategies should be cautiously interpreted and require validation in larger cohort studies. Identifying asymptomatic carriers is paramount for breaking the chain of typhoid transmission. The challenges highlighted by the study, such as the low specificity of RDTs and the low sensitivity of the Widal test, underscore the difficulty in this endeavour. Public health interventions must address asymptomatic transmission, particularly in high-prevalence, resource-limited settings.

Conclusion

This study highlights critical insights into the diagnostic performance of various tests for typhoid fever, using both

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24-hour and 48-hour cultures as reference standards. The findings indicate that while immunological tests, such as IgM and IgG, exhibit some utility in detecting typhoid fever, they are hampered by low specificity and limited agreement with culture methods. These results underscore the challenges of achieving a reliable and accurate diagnosis of typhoid fever using current methods. The varying performance of these diagnostic tools suggests that no single test provides a comprehensive solution for all diagnostic needs. There is a need for further research to refine these diagnostic methods and explore complementary strategies to improve the overall accuracy of typhoid fever detection, ultimately contributing to better public health outcomes and more effective disease management.

DECLARATIONS

Ethical consideration
The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki and was approved by the Municipal Health Directorate of the Atebubu Municipal Assembly of the Bono East Region, Ghana. Informed consent was obtained from all participants, and data confidentiality was strictly maintained. Participants testing positive for typhoid fever were referred for appropriate clinical management according to Ghana Health Service guidelines. Participants were enrolled after obtaining informed consent from adults and assent from minors, with consent from a parent or guardian, where applicable.

Consent to publish
All authors agreed on the content of the final paper.

Funding
None

Competing Interest
The authors declare no conflict of interest

Author contribution
All authors contributed to the conception and design of the study. EY and VKA were responsible for participant recruitment, data collection, and analysis. EY, YK, DMT, and VKA contributed to drafting the manuscript. EY, YK, and DMT provided analytical and statistical support. All authors reviewed, revised, and approved the final version of the manuscript.

Acknowledgement
We extend our sincere appreciation to the Director of the Atebubu Municipal Health Directorate and the management of the Atebubu Municipal Hospital for their institutional support throughout this study. We also acknowledge the invaluable assistance of the laboratory staff of the Atebubu Municipal Hospital, Ghana. Finally, we express our profound gratitude to all individuals who voluntarily consented to participate in this research.

Availability of data
Data is available upon request to the corresponding author

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