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Comparative study of Widal test to stool culture for the diagnosis of suspected typhoid fever: A study in a primary health centre, Ghana

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ABSTRACT

Background: Misdiagnosis of typhoid fever is common since most primary healthcare relies on only the Widal test without further confirmation. This study compared stool culture to the performance of the Widal test at the Methodist Hospital, Wenchi, in the Bono Region of Ghana. **Methods:** This study recruited 178 persons suspected of typhoid fever. Venous blood and stool samples were collected to assess antibodies to the *Salmonella typhi* (*S. typhi*) H and O antigens using the Ozotex Widal test kit and isolate *S. typhi* using culture techniques. An antibody titre value $\geq 1:80$ was considered positive for the Widal test. Also, the performance of the Widal test was evaluated using the results from the stool culture as the standard reference. **Results:** The prevalence of typhoid fever, as confirmed by isolating *S. typhi* from the stool, was 6.2%. From the Widal test, the prevalence of typhoid fever was 71.3%, with 81.8% sensitivity, 29.3% specificity, 7.1% positive predictive value and 96.1% negative predictive value. Also, the Widal test has a lower agreement with the stool culture ($\kappa = 0.021$). **Conclusion:** The Widal test was not dependable for diagnosing typhoid fever; hence, microbial culture should always confirm results from the Widal test.

Introduction

Typhoid fever is a systemic disease caused by *Salmonella typhi* (*S. typhi*) and threatens global health, causing morbidity and mortality [1, 2]. Worldwide, about 21 million cases, with over 600 000 deaths, are reported annually. A higher typhoid fever burden in developing countries results from rapid population growth, inadequate safe drinking and limited health systems [3, 4]. With increasing drug resistance, it is recommended that febrile patients are diagnosed before commencing any

treatment to enhance the effective and efficient use of drugs, thereby limiting the misuse of drugs, which could contribute to drug resistance [5, 6].

The main setback of effectively controlling typhoid fever is associated with poor diagnosis. In clinical settings, typhoid fever diagnosis is complex due to overlapping symptoms with other common infections, including meningitis, malaria, and viral enteritis [7-10]. The gold standard for diagnosing typhoid fever is blood culture, but it is expensive for

patients, time-consuming, and in remote rural settings, culture facilities may be unavailable [11]. Stool culture could also be used where blood culture is inaccessible because studies have shown a strong agreement between blood and stool culture for diagnosing typhoid fever [2, 12].

The diagnostic methods currently used in Ghana for typhoid fever diagnosis include the Widal slide agglutination test, stool, and blood cultures. But in developing countries, equipment, supplies, and skilled personnel required, especially in primary healthcare, are limited. Hence, the most commonly used diagnostic tool in most remote rural healthcare settings is the Widal test, which is cheaper, easy to perform and does not require complicated expertise [13].

In the pathogenesis of *Salmonella* infection, the lipopolysaccharide in the bacteria cell plays an essential function. The lipopolysaccharide in the bacteria comprises mainly three components, with the O antigen (O-specific polysaccharide chain) being exposed on the bacteria cell surface [14]. The O antigen (also known as the somatic antigen) is an endotoxin and provides antigenic variations. This antigen is thermostable and resistant to complement-mediated serum killing of several Gram-negative bacterial [6, 16]. The most superficial of the somatic antigen is the capsular or envelope lipopolysaccharide (Vi antigen). The Vi antigen protects the *S. typhi* from antibody and complement-mediated lysis [17]. Moreover, the bacteria have an H antigen (the flagellar antigen). This flagellar antigen helps in bacterial motility and provides antigenic variation also in the bacteria. Hence, infected people with *S. typhi* produce three antibodies (anti-O, anti-H and anti-Vi) against these antigens [18].

Common Widal test assesses the presence of anti-O and anti-H antibodies in the sera of patients in the diagnosis of typhoid fever [4, 19]. This is because the anti-O is positive during the acute stage of the disease, whereas the anti-H rises late and disappears late. However, the anti-Vi is an indicator of the carrier stage [20]. Most studies have used blood culture as a gold standard in evaluating diagnostic tools for typhoid fever diagnosis [2, 4]. However, with the cost associated with blood culture, we compared the diagnosis of typhoid fever among suspected patients using stool culture and Widal test at Methodist Hospital, Wenchi, in the Bono Region of Ghana.

Material and Methods

Study design and study site

A cross-sectional hospital-based study was conducted from January to March 2022 among persons suspected of typhoid fever during their visit to the Methodist Hospital, Wenchi, in the Bono Region of Ghana. This hospital serves the indigenes of the Wenchi municipality. The people in this municipality derive their livelihood from farming and animal husbandry.

Study population and sample size determination

All persons suspected of typhoid fever visiting the Methodist Hospital, Wenchi, in the Bono Region of Ghana, were considered the study population. The sample size was calculated using a 14.1% prevalence of typhoid fever [21] and the Fischer expression for cross-sectional studies [22]. At a 95% confidence level and a 5% margin of error, the sample size was 187, as illustrated below. However, 178 persons who consented were enrolled.

$$\begin{aligned} n &= \frac{z^2 \times p(1-p)}{m^2} \\ &= \frac{1.96^2 \times 0.141 \times (1-0.141)}{0.05^2} \\ &= 186.11 \approx 187 \end{aligned}$$

Where: n = sample size; z = z-score value (1.96) from a normal distribution at a 95% confidence level; m = margin of error of 5.0%; p = prevalence of typhoid fever: 14.1%.

Data collection and laboratory analysis

Questionnaires were administered to obtain demographic data, and 2 ml of venous blood and stool samples were obtained from the study participants after obtaining informed consent. The study participants on antibiotics therapy or finished antibiotics therapy within one month were excluded from the study. The stool samples were collected using sterile applicator sticks into screw-capped sterile containers. The stool samples were inoculated on *Salmonella Shigella* agar plates and incubated at 37 °C for 18 – 24 hours. The *Salmonella Shigella* agar was used in isolating *Salmonella typhi* because it is selective for only *Salmonella* spp and *Shigella* spp. The stool contains normal flora, which can outgrow *S. typhi* if the stool is culture an enriched media. Negative cultures were incubated for three days before being reported as negative. Bacteria growth on the agar plates was speciated using microbiological techniques, including Gram staining and biochemical tests

specific for *S. typhi*, such as oxidase, urea, indole, Simmon's citrate and triple iron sugar tests. The principles for these tests have been documented [23]. The characteristics of *S. typhi* are illustrated in **Table 1**.

The venous blood samples were dispensed into gel separator tubes, allowed to clot, and centrifuged at 3000 rpm for 5 minutes to obtain the sera to assess for the Widal test. The Widal test is based on assessing antibodies (agglutinins) in sera against the O (somatic) and H (flagella) antigens of *S. typhi* [24]. Following the manufacturer's instructions, the test was done using the Ozotex Widal test kit (CMC Medical Devices and Drugs, S. L., Málaga, Spain). The titre of anti-O and anti-H $\geq 1:80$ was considered positive.

Ethics statement

Ethical approval was sought from the Committee on Human Research, Publications and Ethics (CHPRE) of the School of Medicine and Dentistry, Kwame Nkrumah University of Science and Technology (KNUST), Ghana (approval number: CHPRE/AP/218/20). Also, informed consent was obtained from the study participants or guardians (for participants below 18 years) after explaining the purpose of the study in a language each participant or guardian understood.

Statistical analysis

Data were analysed using the Statistical Package for Social Sciences Statistical Software (version 20.0, IBM Corporation USA). The data were expressed in percentages and frequencies, and Fisher's exact test

was used to determine significant differences between the variables. The diagnostic performance of the Widal test was determined by calculating the sensitivity, specificity, predictive values, and kappa using stool culture as the standard [19]. The statistical significance was accepted in all comparisons at a *p*-value less than 0.05.

Table 1. Biochemical characteristics of *S. typhi*.

Biochemical test	Result
Oxidase	Negative
Indole	Negative
Urease	Negative
Simmon's citrate	Negative
TSI (lactose fermentation)	Negative
TSI (H ₂ S production)	Negative

Results

The study participants were 6 – 74 years, with most 20 years and below. However, females dominated the participants (**Table 2**). Overall, 6.2% of the participants tested positive for *S. typhi* by stool culture, dependent on the age group ($p = 0.025$) (**Table 2**). Also, according to the Widal test, the overall prevalence of typhoid fever was 71.3% (127/178). The Widal test had a higher sensitivity (81.8%) but lower specificity (29.3%) and agreement with the stool culture (kappa = 0.021) (**Table 3**).

Table 2. Prevalence of typhoid fever among the study participants by culture.

Variables	Negative [n (%)]	Positive [n (%)]	Total [N (%)]	<i>p</i> -value
Overall	167 (93.8)	11 (6.2)	178 (100.0)	
Age (years)				0.025
≤ 20	55 (32.9)	3 (27.3)	58 (32.6)	
21 – 40	40 (24.0)	7 (63.6)	47 (26.4)	
41 – 60	41 (24.6)	1 (9.1)	42 (23.6)	
> 60	31 (18.6)	0 (0.0)	21 (17.4)	
Gender				0.539
Males	64 (38.3)	3 (27.3)	67 (37.6)	
Females	103 (61.7)	8 (72.7)	111 (62.4)	

Statistical difference calculated by Fisher's exact test.

Table 3. Diagnostic performance of the Widal test compared to stool culture.

Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	kappa
81.8 (48.2 - 97.7)	29.3 (22.6 - 36.8)	7.1 (3.3 - 13.0)	96.1 (86.5 - 99.5)	0.021

CI: confidence interval, PPV: positive predictive value, NPV: negative predictive value

Discussion

Early and accurate typhoid fever diagnosis prevents life-threatening complications such as intestinal perforations. The prevalence of *S. typhi* isolated from the stool was 6.2%. This low prevalence could be attributed to the irregular bacteria shedding in stool [25]. Our finding was comparable to a study conducted at Jimma Health Centre in Ethiopia, where a stool culture prevalence of 6.2% was reported [26]. However, the prevalence of *S. typhi* isolated from stool reported in this current study was higher than a study conducted among febrile patients at the Ambo Hospital in Ethiopia, where a stool culture prevalence of 0.8% was recorded [25]. The difference in the prevalence could be due to ecological variations among the study sites, cultural differences and hygienic practices, and the sample collection time [25].

This current study recorded a high Widal test seroprevalence (71.3%). Poor personal and food hygiene and lack of potable water have been mainly attributed as the major risk factors associated with high enteric fever seropositivity [27, 28]. Further, people living in enteric countries, including Ghana, are known to have background antibodies due to repeated or long-standing infections with pathogens of enteric fever, which could be cross-reactive with the Widal test [29, 30]. Considering these factors, the high seropositivity recorded in this current study is understandable. However, the results from the Widal test suggest that a single Widal test has limited diagnostic applicability in endemic areas since it could lead to overdiagnosis [31, 32].

The sensitivity of the Widal test was 81.8%, comparable to a study in Ethiopia with a higher sensitivity (84.2%) of a Widal test compared to stool culture [13]. However, the sensitivity recorded in this current study was lower than in a study in Cameroon, where a sensitivity of 40.9% was reported [19]. These variations in sensitivities observed may be due to differences in immune response among study participants, geographical location, and sample size. In this current study, the

ability of the Widal test to detect true negative results compared to the stool culture was 29.3%. This specificity was lower than the specificity (98%) recorded in Tanzania [33] but comparable to a study conducted in Ethiopia, where a specificity of 33.5% was reported [13]. The positive predictive value of the Widal test was 7.1% which was very low. However, the negative predictive value of the test kit was 96.1%, which indicates that a negative test result has good predictive power for the absence of typhoid fever.

Moreover, the false-positive results from the Widal test could be due to infection with other enteric bacteria possessing cross-reactive epitopes or cross-reaction with antigens of malaria parasites [25]. Also, the Widal test detects IgG antibodies of *S. typhi* antigens which could persist in circulation for 2 – 6 months post-antibiotic treatment [34]. Further, the false-negative test results from the Widal test may arise due to testing of patients at the early stage of the disease before full antibody response or persons ingesting bacterial loads that are inadequate to induce antibody production [25, 35]. Such negative test result makes the exclusion of enteric fever difficult in patients with a history and symptoms matching enteric fever. Such negative Widal test results make excluding enteric fever problematic in patients with signs pointing to enteric fever. Therefore, the limited specificity of the Widal tests, together with treating patients with a single Widal test result, could lead to needless treatment, unfavourable treatment outcomes or missing the appropriate treatment opportunities [31, 32, 35].

Conclusion

The overall prevalence of typhoid fever using stool culture was 6.2%. The Widal test had a high sensitivity, low specificity, low positive predictive value, and high negative predictive value compared to the stool culture; hence, culture is always required to confirm typhoid fever. We recommend the Widal test be continually assessed in-house for effective typhoid fever diagnosis in primary healthcare settings.

Limitations of the study

Blood culture has been the gold standard for evaluating the Widal test. However, we could not perform this culture due to limited resources. Also, previous antibiotic treatments may contribute to negative stool culture tests, which were not assessed in this study.

Conflicts of interest

The authors declare that they have no competing interests.

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Data availability

All the data obtained and analysed are included in this manuscript.

Authors' contributions

SAD conceived and supervised the work, and AM and LS conducted the experiments. SAD analysed the data and wrote the manuscript. All authors read and approved the final manuscript.

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