COMPARATIVE ANALYSIS OF WIDAL TEST AND CULTURE FOR THE DIAGNOSIS OF SALMONELLOSIS AMONG PATIENTS IN ENUGU STATE UNIVERSITY TEACHING HOSPITAL.

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ABSTRACT

Salmonellosis, a disease caused by salmonella bacteria, causes significant morbidity and mortality globally especially in Sub Saharan Africa. The aim of this study is to compare the diagnostic accuracy of Widal test and stool culture for the diagnosis of salmonellosis at Enugu State University Teaching Hospital (ESUTH). This is a cross sectional comparative study carried out between July 22 to November 22 2024 at ESUTH Parklane involving 100 participants presenting with symptoms suggestive of salmonellosis. Ethical approval and informed consent was obtained. Paired blood and stool samples were collected following standard procedures and analyzed using the Widal slide agglutination test and stool culture on Salmonella-Shigella Agar (SSA). Laboratory analysis was done following standard procedures. Isolates were confirmed biochemically using Klinger Iron Agar (KIA), and antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method. Data was analyzed statistically using software SPSS version 25. Descriptive statistics and Chi-Square tests were employed to summarize demographic data and comparison between diagnostic methods. Widal test yielded 56(56%) positive results of which 32(57.14%) were males and 24(42.86%) were females, Stool culture yielded 42(42%) positive results of which 26(61.90%) were males and 16(38.10%) were females, Widal test exhibited a sensitivity of 82%, Specificity of 62%, PPV of 61.7% and NPV of 81.8%, while Stool culture exhibited 100% Sensitivity, specificity, PPV and NPV as the gold standard. Of the isolates, 85.7% was susceptible to Ofloxacin, 71.4% was susceptible to Ciprofloxacin making them the drug of choice, while no sensitivity was observed for Augmentin, and Ceftazidime. The results obtained indicate the limitations of the Widal test, which aligns with other studies conducted in Nigeria and Africa. Therefore, the Widal test should not be solely relied upon for salmonellosis diagnosis but used as a preliminary test, followed by confirmation with culture method and antimicrobial susceptibility testing to guide treatment decisions.

Keywords : Salmonellosis, Widal test, Stool Culture, Diagnostic accuracy, Laboratory analysis, Antimicrobial susceptibility testing.

1. INTRODUCTION

Salmonellosis is a significant global public health concern, particularly in developing countries where inadequate sanitation, poor hygiene, and substandard food safety measures prevail [1]. It is caused by Salmonella bacteria, with clinical manifestations ranging from self-limiting gastroenteritis to invasive diseases such as sepsis and enteric fever (typhoid and paratyphoid fever). Of these, enteric fever has drawn heightened concern due to its high morbidity and mortality rates, especially in regions with resource constraints [2]. The global burden of Salmonellosis is disproportionately borne by low- and middle-income countries (LMICs), where poor sanitation, inadequate water supply, and unsafe food practices are prevalent. According to the World Health

Organization (WHO), typhoid fever alone causes 11–20 million cases and 128,000–161,000 deaths annually. Additionally, paratyphoid fever accounts for 6 million cases and 54,000 deaths each year. Africa and South Asia carry the highest incidence rates, with Nigeria being one of the endemic regions [3] [4]. Non-typhoidal Salmonella infections, primarily foodborne, result in an estimated 93.8 million cases of gastroenteritis globally, with over 155,000 deaths annually [5]. In sub-Saharan Africa, NTS also contributes to invasive disease in vulnerable populations, including children and individuals with compromised immunity, such as HIV/AIDS patients [6].

Accurate and early diagnosis of Salmonellosis is critical for effective treatment and prevention, However, diagnostic challenges persist, particularly in resource-limited settings. In Nigeria, two primary methods for diagnosis are the Widal test and stool culture. The Widal test, a serological method, detects antibodies against *Salmonella Typhi* and *Salmonella Paratyphi* antigens. It is widely used in low-income regions due to its affordability and ease of use. Despite its popularity, the Widal test suffers from poor sensitivity and specificity, which can lead to diagnostic inaccuracies such as false positives due to cross-reactivity with non-Salmonella infections like malaria or prior vaccinations, and false negatives in the early stages of infection [2] [7].

In contrast, stool culture is considered the gold standard for diagnosing Salmonellosis because it allows direct isolation and identification of the pathogen. This method is highly specific but is constrained by its resource-intensive nature, prolonged turnaround times, and dependence on optimal sample handling and storage conditions. In many cases, patients' prior use of antibiotics further reduces the sensitivity of culture-based diagnostics, compounding the challenge [4].

An additional layer of complexity arises from the growing problem of antimicrobial resistance (AMR). Multidrug-resistant (MDR) strains of *Salmonella*, including those resistant to fluoroquinolones and third-generation cephalosporins, are increasingly reported, particularly in sub-Saharan Africa and South Asia [6]. These resistant strains limit therapeutic options and highlight the critical need for accurate diagnostics to guide treatment protocols and prevent resistance propagation. [8]. The aim of this study is to compare the diagnostic accuracy of Widal test and stool culture in the diagnosis of salmonellosis in other to evaluate and adopt a more effective diagnostic techniques for the benefit of the patients, Furthermore, it will evaluate the antimicrobial susceptibility patterns of isolated Salmonella strains, providing insights into the prevailing resistance trends in this setting.

2. MATERIALS AND METHODS

2.1. Study Area

The study was conducted at the Enugu State University Teaching Hospital (ESUTH) Parklane, Enugu, Nigeria. ESUT-TH Parklane is a tertiary healthcare facility that provides specialized medical services to Enugu State and surrounding regions. The hospital's catchment area includes urban, peri-urban, and rural populations, making it a suitable location for studying a broad spectrum of patients from different demographic and socioeconomic backgrounds. The laboratory at ESUT-TH is equipped for microbiological testing, including culture methods and serological assays.

2.2 Study Design and Period of Study

This is a cross-sectional comparative study designed to evaluate the diagnostic efficacy of the Widal test versus stool culture in diagnosing Salmonellosis among patients at ESUT-TH Parklane. The study also included antimicrobial susceptibility testing (AST) to assess resistance patterns of Salmonella isolates from the samples. The study was conducted between 22nd of July to 22nd of November 2024.

2.3 Study Population

The study included patients presenting with clinical symptoms suggestive of Salmonellosis, such as prolonged fever, abdominal pain, diarrhea, and general malaise. A total of 100 participants were enrolled in this study

Only consented patients who presented with symptoms suggestive of salmonellosis at the Enugu State University Teaching Hospital were included in this study. Patients who didn't provide consent, patients who has taken antibiotics in the last two weeks, Vaccinated individuals and those who didn't present with symptoms suggestive of salmonellosis was excluded from the study.

2.4. Sample collection

A total of two hundred samples (200) (100 blood samples and 100 stool samples)

About 3-4ml of blood sample were collected aseptically from each participant and transferred into sterile plain tubes allowed to clot and further centrifuged to make ready for Widal test. Fresh stool samples were also collected from same patients in sterile universal (plastic) disposable bottles with screw cap and immediately transferred to the laboratory for analysis.

2.5 Laboratory Analysis

2.5.1 Widal Agglutination Test Method

The Widal test was performed using a standard slide agglutination method. Widal agglutination test was performed using Widal agglutination kit (Pro-med Widal kit) and was carried out according to manufacturers instruction. The reagents contained Salmonella O and H antigens and Salmonella Paratyphi AO, AH, BO, BH, CO, CH antigens. Positive and negative controls were included and a titre of greater than 1/80 indicates the presence of Salmonella infection. The reagents and samples were brought to room temperature and the antigens was shaken properly for proper mixing before dispensing. A drop of patient's serum to be tested was placed into each of the required circles on the glass slide, and one drop of Widal antigen suspension was added to the reaction circles containing patient's serum using a capillary pipette. Using different applicator sticks provided, the contents of each circle was mixed properly, the tile was rocked and observed for agglutination macroscopically for one minute.

2.5.2 Stool Culture

A portion of the stool was quickly collected from the universal bottle using a heat fixing wire loop and streaked on Salmonella Shigella Agar (SSA). SSA is used for isolation, cultivation and differentiation of gram negative enteric microorganisms from both clinical and non clinical specimens. The plates were incubated at 37°C for 24-48 hours. SSA enhance the growth of Salmonella and the presence of Salmonella is indicated by pink-red colonies with black centres due to production of Hydrogen Sulphide (H2S), if there was no growth the culture was considered negative, presence of growth with morphological characteristics of Salmonella colonies was followed up with a biochemical test using Klinger Iron Agar (KIA), to read acid production of the slant, gas and H_2S production. The presence of Red slant, yellow butt, black precipitate in the butt (H_2S production), gas or no gas production depending on Salmonella species is indicated a positive KIA reaction for Salmonella.

2.6. Data Management and Statistical Analysis

Raw data were entered into Microsoft Excel, statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 25. Descriptive statistics, including mean, median, and standard deviation, were used to summarize the demographic characteristics of the participants. Inferential statistics were used to compare the diagnostic accuracy of Widal test and stool culture. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the Widal test were calculated using stool culture as the gold standard. Chi-square tests were used to compare categorical variables between diagnostic methods.

Logistic regression analysis was used to assess the impact of demographic factors and resistance

patterns on test outcomes. A p-value of <0.05 was considered statistically significant.

2.6. Ethical Considerations

The study was conducted in accordance with the ethical standards outlined in the Declaration of Helsinki. Ethical approval was gotten from the Enugu State University Teaching Hospital Health Research Committee. Informed consent was obtained from all participants before inclusion in the study. Participants was informed about the study's objectives, procedures, potential risks, and benefits. Confidentiality of patient information was maintained, and participation was entirely voluntary, with the option to withdraw at any point without any negative consequences.

3. RESULTS

3.1 Socio- Demographic Characteristics of Respondents

A total of 100 participants presenting with symptoms suggestive of Salmonellosis at Enugu State University Teaching Hospital (ESUTH) Parklane were enrolled in this study, Majority of the participants were males accounting for 58% while 42% we're females. With respect to age distribution, the mean age of participants was 31.2±12.8 years. The highest number of participants were aged 20–29 years (32%), followed by 30–39 years (22%), while the lowest representation was in the age group above 60 years (6%).

Characteristic	Frequency (n)	Percentage (%)	Mean ± SD
Gender			
Male	58	58.0	
Female	42	42.0	
Age Group (Years)			31.2 ± 12.8
0–9	5	5.0	
10–19	13	13.0	
20–29	32	32.0	
30–39	22	22.0	
40–49	12	12.0	
50–59	10	10.0	
60 and above	6	6.0	
Total	100	100.0	

3.2 Diagnostic outcomes of Widal and stool culture test

Out of the 100 participants, the Widal test recorded 56(56%) positive cases, while stool culture identified 42(42%) positive cases. Stool culture, being the gold standard, suggests that some of the positive results from the Widal test may represent false positives. The Widal test detected more positives among males 32(57.14%) than females 24(42.86%) while stool culture detected 26(61.90%) positive cases in males and

16(38.10%) in females. Chi-Square analysis showed no statistically significant association between gender and the performance of either diagnostic method ($p>0.05$). The Widal test detected more positive cases in the 20–29 age group 18(18%), while stool culture identified 12(12%) cases in the same group. Chi-Square analysis revealed no significant association between age groups and diagnostic performance for either method ($p>0.05$).

Table 2 Comparison of Widal Test and Stool Culture

Diagnostic Test	Positive Cases (n, %)	Negative Cases (n,%)	Total (n, %)
Widal Test	56 (56%)	44(44%)	100 (100%)
Stool Culture	42(42%)	58(58%)	100(100%)

Table 3: Gender Distribution and Diagnostic Results

Gender	Widal Positive (n,%)	Widal Negative (n,%)	$\chi2$	p-value	Stool Positive (n,%)	Stool Negative (n,%)	$\chi2$	p-value
Male	32(57.14%)	26(59.09%)	1.023	0.312	26(61.90%)	32(55.17%)	0.976	0.323
Female	24(42.86%)	18(40.91.%)			16(38.10%)	26(44.83%)		
Total	56(100%)	44(100%)			42(100%)	58(100%)		

Table 4: Age Distribution and Diagnostic Results

Age Group (Years)	Widal Positive (n)	Widal Negative (n)	$\chi2$	p-value	Stool Positive (n)	Stool Negative (n)	$\chi2$	p-value
0–9	3	2	0.576	0.448	2	3	1.224	0.269
10–19	9	4			6	7		
20–29	18	14			12	20		
30–39	12	10			10	12		
40–49	8	4			6	6		
50–59	4	6			4	6		
60+	2	4			2	4		
Total	56	44			42	58	-	-

3.3 Sensitivity, Specificity, PPV, and NPV of Widal Test when compared to Stool Culture Test for the Diagnosis of Salmonellosis.

The Widal test demonstrated a sensitivity of 82%, specificity of 62%, PPV of 61.7% and NPV of 81.8% indicating it's moderate ability to detect positive results and exclude negative result

Table 5: Sensitivity and Specificity of the Widal Test

Parameter	Result (%)
Sensitivity	82
Specificity	62
PPV	61.7
NPV	81.8

3.4 Antimicrobial susceptibility testing of salmonella isolates to gram negative antibiotics disc.

The isolates showed the highest sensitivity to ofloxacin (85.7%), followed by ciprofloxacin (71.4%) and ceftriaxone (59.5%). Resistance was highest against Augmentin and ceftazidime, with no isolates showing sensitivity.

Table 6: Antimicrobial Susceptibility of *Salmonella Typhi* Isolates

Antibiotic	Number of Sensitive Isolates (n)	Percentage (%)
Streptomycin	18	42.9
Ofloxacin	36	85.7
Ciprofloxacin	30	71.4
Gentamycin	20	47.6
Ceftriaxone	25	59.5
Ceporex	12	28.6
Peflacine	10	23.8
Cefuroxime	4	9.5
Augmentin	0	0.0
Ceftazidime	0	0.0

4.1 DISCUSSION

The routine laboratory diagnosis of Salmonellosis is dependent on either isolation of salmonella from stool culture or detection of raised titre of agglutinating serum against the Lipopolysaccharide somatic (O) or flagella (H) antigens (the Widal test). This study explored the comparative diagnostic efficacy of the Widal test and stool culture in the diagnosis of Salmonellosis at Enugu State University Teaching Hospital, with 100 paired samples (blood and stool) analyzed.

The findings in this study as presented in table 2 suggest a high prevalence of 56% in patients using the Widal test method and a prevalence of 42% using the stool culture method. This high prevalence is in agreement with several other studies such as studies conducted in 2020, 2019 and 2013 by Olorode et al (7), Wam et al (4), Ramyil et al (2) respectively which also reported high incidence of Salmonellosis, this high prevalence from this study could be due to cross-reactivity with non-Salmonella infections and antibodies from prior exposures or vaccinations, overcrowding with poor access to clean water and lack of proper sanitation.

The findings from this study as presented in table 3 and 4 also showed that individuals of all ages are susceptible to infection by Salmonella species. The age group more susceptible in this study was those between 20-29 age group with 32.1% positive results. This is in agreement with studies carried out by Olorode et al, Wam et al and Ramyil et al (2), they found 21-30, 10-29 and 24-29 years respectively were more susceptible to Salmonella infection respectively. Wan et al then stated that this might be due to improper sanitation and hygiene. Both children and adults can get infected with Salmonellosis through ingestion of contaminated food and water. Overcrowding and poor access to safe drinking water, improper sanitation and hygiene are risk factors for one getting infected by Salmonellosis. The results showed that there's more prevalence of Salmonellosis in males (57.14%) than females (42.86%) using Widal test and Males (61.7%) than females (38.3%) using Stool culture. This findings also agree with Olorode et al (7) and Ramyil et al (2) which reported that males were more positive to both Widal test and Stool culture than females. Udeze et al (9) stated that the reason why male are more susceptible is because females are not usually exposed to the activities that are normally associated with disease like sanitation, poor sewage disposal, swimming, fishing or resting in a dirty environment as their male counterparts (9).

However, this findings doesn't agree with the findings in the study by Wam et al who reported higher prevalence of Salmonella infections in females than males in both Widal test and Stool culture, In their findings they found out females dominated with 73.44% while msles came with 26.56% using Widal test, in stool culture females also had higher prevalence with 54.55% when compared to males with 45.45%.

Comparing Widal test method and stool culture generally, it was observed from findings in this study that Widal test recorded more positive cases with 56% compared to stool culture with 42% positivity. The discrepancy in positive cases between the Widal test (56%) and stool culture (42%) suggests significant false-positive rates with the Widal test. This findings in this study is in agreement with the study conducted by Wam et al at North West region of Cameron which also found out that there's more positive cases with Widal test (57.1%) than stool culture (42.9%). The high prevalence with Widal test results align with the literature, which underscores the limitations of the Widal test in endemic areas due to cross-reactivity with non-Salmonella infections and antibodies from prior exposures or vaccinations, (). These findings is not in agreement with the study carried out by Olorode et al in Niger Delta region of Nigeria which recorded higher positive cases with stool culture (62%) than Widal test (54%).

The findings in this present study also recorded that Widal test has a high sensitivity of 82% and a low specificity of 62% when compared to stool culture which is the gold standard. This findings correlates with study conducted by Gemechu et al

(10) at southern Ethiopia which showed that Widal test has high sensitivity of 84.2% and a low specificity of 35.5%. The findings contradicts that of Wam et al which recorded low sensitivity of 40.9% and low specificity of 32.4%. The findings in this study also showed that Widal test has a PPV of 61.7% and NPV of 81.8%. This correlates with the study carried out by Gemechu et al (10) which showed a PPV of 24.6% and NPV of 90.0% but contradicts the study carried out by Wam et al (4) which showed PPV of 28.13 and NPV of 6.44%. So from the result, Sensitivity: 82%, the Widal test is moderately sensitive, meaning it can detect about 82% of individuals who actually have the disease (typhoid fever). However, this also means that about 18% of individuals with the disease may test negative (false negatives), Specificity of 62%, The Widal test has relatively low specificity, indicating that about 38% of individuals without the disease may test positive (false positives). This can lead to unnecessary treatment and resource waste. PPV: 61.7%, The Positive Predictive Value (PPV) is moderate, suggesting that about 61.7% of individuals who test positive actually have the disease. However, this also means that about 38.3% of positive results may be false positives. NPV: 81.8%, The Negative Predictive Value (NPV) is relatively high, indicating that about 81.8% of individuals who test negative are truly disease-free.

The stool culture in the study was considered the gold standard to which Widal was compared, it's sensitivity, specificity, PPV and NPV when cultured for Salmonella species using SSA were 100%. Salmonella is an enteric microorganism which primarily inhabit the intestine of humans, so it is highly detectable in stool. The 42 salmonella isolates obtained was further confirmed biochemically using KIA agar confirming the accuracy of stool culture in the diagnosis of salmonella infections.

In table 6, isolates of Salmonella species from stool culture were found to be more susceptible to Ofloxacin (85.7%), followed by ciprofloxacin (71.4%) and Ceftriaxone (59.5%), making them the drug of choice for the treatment of Salmonellosis. On the other hand they were more resistant to Augmentin (0%) and Ceftazidime (0%) and Cefuroxime (9.5%) showing the growing antimicrobial resistance of salmonella species.

4.2 CONCLUSION

From the study, Widal test method recorded a prevalence of 56% and stool culture method recorded 42% positive cases. The study showed that Widal test has moderately high sensitivity (82%), moderately low specificity (62%), PPV of 61.7% and NPV of 81.8%. This shows that Widal test has limitations when compared to stool culture and should not be totally relied on for the diagnosis of Salmonellosis, rather it should be used as a preliminary test as it has moderately high ability to identify negative results and confirm individuals to Salmonellosis free, therefore stool culture on the other hand should be used to confirm Widal positive results, isolates obtained used for antimicrobial susceptibility testing to guide disease diagnosis and treatment outcome.

4.3 RECOMMENDATIONS

1. Integrated Diagnostics:

Combining the Widal test with stool culture can enhance diagnostic accurancy. Policymakers and healthcare facilities should implement standard protocols to integrate these methods, where feasible.

2. Capacity Building and infrastructure development:

Training laboratory personnel in culture techniques and ensuring the availability of essential diagnostic infrastructure are critical steps in strengthening the diagnostic framework for Salmonellosis.

3. Antimicrobial Stewardship:

Regular antimicrobial susceptibility testing should be mandated to inform treatment guidelines and prevent the escalation of multidrug resistance in Salmonella spp.

4. Public Health Strategies:

Public awareness campaigns and improved sanitation practices can reduce the incidence of Salmonellosis, decreasing the diagnostic burden on healthcare systems.

5. Further Research:

Additional studies should explore the cost-effectiveness of integrating diagnostic methods and investigate novel diagnostic technologies, such as molecular assays, in endemic settings.

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