https://dx.doi.org/10.4314/dujopas.v10i2a.30
ISSN (Print): 2476-8316
ISSN (Online): 2635-3490
Dutse Journal of Pure and Applied Sciences (DUJOPAS), *Vol. 10 No. 2a June 2024*

Comparative Analysis of Widal Agglutination and Typhoid Humoral Response Assays in Febrile Patients at Kebbi State, Nigeria

Faruku N.[1]*, Sarkinfada F.,[2] Akande, A. O.[2] Taysir R. Hafiz, Khalid, I.M[2], Idris A. Kabuga[2], Musa S. [1], Umar K. [1], Faruk U. Magaji[4], Yusuf M. Mujahid N.S[2] and. Abbas M. A. [5],

[1]Department of Medical Laboratory Science,
Faculty of Allied Health Science,
Ahmadu Bello University,
Zaria.

[2]Department of Medical Microbiology and Parasitology,
Faculty of Clinical Sciences,
Bayero University,
Kano.

[3]Department of Medical Microbiology and Immunology,
Faculty of Medicine,
Al-Azhar University,
Cairo.

[4]Department of Biochemistry,
Faculty of Sciences,
Federal University,
Birnin Kebbi.

[5]Department of Community Medicine,
Faculty of Clinical Sciences,
Bayero University
Kano.

Email: maabbas.mph@buk.edu.ng

Abstract

Typhoid fever is a life-threatening diseases caused by Salmonella enterica serovar Typhi that poses high-level of public health concern especially in Sub-Saharan Africa. Essentially, inadequate or inappropriate diagnosis of the human salmonellosis could be a factor that facilitated its dissemination in Nigeria. To determine the sensitivity and specificity of serological widal and Typhoid IgG/IgM tests in comparison with blood culture as a reference for typhoid diagnosis among febrile patients attending three public hospitals in Kebbi State, Nigeria. A hospital based cross-sectional study was carried out among 406 febrile patients with suspected typhoid fever (mean age = 34 years). Blood culture for bacterial isolation, subjects widal and Salmonella enterica serovar typhi IgM/IgG tests were performed on all participants by the use of selective bacteriological media, slide agglutination, rapid Typhoid IgG/IgM lateral flow

Author for Correspondence

immunochromatographic assay, respectively. Positive Salmonella typhi culture was obtained in 6.4% of the participants, of which a higher rate was recorded among males 7.5% than in females 5.0% participants. Anti-typhoid IgM and IgG were detected 25.1% and 21.9% of the participants while the widal agglutination was positive in 52% of the participants.. The sensitivity, specificity, positive, and negative predictive value of typhoid IgM was found as 88.46%, 79.21%, 22.55%, and 99.01%, respectively. Whereas, 65.38%, 48.95%, 8.06%, and 95.39% were obtained for Widal agglutination test, respectively. Based on these findings, the typhoid IgM and IgG demonstrated better diagnostic performance for serological identification of typhoid in the first week of illness than widal test. Hence, so it is suggested that healthcare facilities to considering replacing the widal test by typhoid IgM/IgG tests in cases when urgent test results are necessary in lieu of culture that has long turnaround time.

Keywords: Typhoid fever, *Salmonella typhi,* Typhoid immunoassays, diagnostic performance, Widal test

INTRODUCTION

Typhoid fever is an enteric bacterial infection caused by the gram-negative rod bacterium *Salmonella* enterica serovar typhi (*S. typhi*), which is primarily transmitted by faecal-oral mode through the consumption of contaminated food or water (Crump, 2019). The disease is characterized by prolonged fever, fatigue, headache, nausea, abdominal pain, constipation or diarrhoea, and vomiting with severe cases leading to tissues and organ complications and even death (Mujahid *et al.,* 2022). 11 to 20 million typhoid cases were reported resulting in 128000 to 161000 deaths globally (WHO, 2022).

The disease is common in low and middle income countries including Nigeria due to the unavailability of accurate and reliable diagnosis, antimicrobial resistance (AMR), poor personal and environmental hygiene as well as poor safe drinking water and food hygiene. The true burden of the enteric fever is difficult to estimate due to the limited diagnostic resources and proper surveillance tools resulting in poor characterization of the burden of enteric fever (Bharmoria *et al.,* 2017; Eng *et al.,* 2015). Microbiological testing is usually required to confirm the diagnosis of *Salmonella* Typhi, with blood and bone marrow cultures currently considered the gold-standard tests (Moore *et al.,* 2014). However, blood culture tests are expensive, has a low sensitivity, and require infrastructure and skilled staff that are not always available in low- and middle-income countries (LMICs) and are not adequate for rapid patient management (Mather *et al.,* 2019).

With the emergence of multi-drug resistance for *Salmonella* Typhi, treatment has become difficult and has further complicated the situation. Due to the nonspecific symptoms presented by typhoid patients with other diseases, there is an urgent need for specific, rapid and reliable laboratory tests for the diagnosis of typhoid fever.

Availability of rapid diagnostic report could facilitate the early commencement of treatment with suitable empirical antimicrobial agents before culture and antimicrobial susceptibility test results become available. Widal test is the most common rapid serological test for detecting both O and H antibodies over three decades, however has limited sensitivity and specificity. On the contrary molecular test detecting the nucleic acid of the bacilli have a higher sensitivity and specificity but high costs which limits its routine use in low and middle income countries (LMICs) (Hosoglu *et al.,* 2008). The role of the Widal test is also under scrutiny due to its variable sensitivity, specificity, and predictive values that vary between geographical areas (Agarwal *et al.,* 2004). Typhoid IgM/IgG is a new rapid serological test that is based on

the principle of lateral flow immunochromatographic. It detects the presence of specific IgM and IgG antibodies to *Salmonella* Typhi lipopolysaccharide. It becomes positive as early as in the first week of the fever, the results interpreted visually and be available within 15 minutes. Hence, it could serve as an alternative for rapid and early diagnosis of typhoid fever. In cognisance of these, the present study aimed to determine the sensitivity and specificity of serological widal and Typhoid IgG/IgM tests in comparison with blood culture as a reference for typhoid fever diagnosis among febrile patients attending three public hospitals in Kebbi State, Nigeria.

MATERIALS AND METHODS

Study Area

Kebbi is a state in the north-western Nigeria, with its capital city at Birnin Kebbi. The state was created out of a part of Sokoto State in 1991. Kebbi State is bordered by Sokoto State, Niger State, Zamfara State, the Dosso region of the Republic of Niger and the Benin Republic. As at 2022, the state has a total population of 5,563,900 people and an area of 37,201 km².

Ethical Clearance

This study was conducted based on the principles expressed in the Declaration of Helsinki/Belmont Report. The ethical clearance was obtained from the Research and Ethics Committee of the Department of Research and Statistics of Kebbi State Ministry of Health, Birnin Kebbi with reference number MOH/KSREC/VOL.1/56 and KSHREC Reg. No. 105: 6/2020.

Study Participants and Collection of Blood

A total of 406 febrile study participants were recruited for this study, who were clinically suspected of having enteric fever.

Inclusion and Exclusion Criteria

Enrolment criteria for being a suspected enteric fever case included being 1–70 years of age, and having fever of >39 °C for 2 days.. We collected 10 mL and 20 mL of venous blood for children and adults for microbiologic culturing and serological tests at the time of clinical presentation, respectively.

Blood Culture Method

We performed microbiological culturing of venous blood using a BacT/ALERT 3D automated detection system, sub-culturing positive bottles on Salmonella-Shigella agar (SSA), MacConkey agar, blood agar, and chocolate agar plates, and identifying colonies using colony morphology, standard biochemical identification tests.

Widal Agglutination Test

The Widal test was conducted to assess for the presence of antibodies reacting to the *Salmonella* flagellar (H) and/or lipopolysaccharide (O) antigens; we used a commercially available kit (Omega Diagnostics, Scotland, UK). We performed the Widal test using slide agglutination test, results were considered positive with a titre ≥1:160 antibody agglutination titre as significant.

Typhoid IgG/IgM Test

Typhoid IgG/IgM rapid test cassette (BioPanda, UK) is a qualitative, membrane-based immunoassay device for the detection of IgG and IgM antibodies to *Salmonella* Typhi in

human whole blood, serum or plasma. The diagnostic test consists of two components, IgG component and IgM component separately. The test was performed following manufacturer's instructions.

Statistical Analysis
We calculated the sensitivity and specificity of these tests with a 95% confidence interval of the diagnostic methods using GraphPad Prism version 8.

RESULTS
Of the 406 febrile subjects recruited in this study, 55.9% were male and females were 179 (44.1%). A total of 25.2% of the participants represented the age group 31- 40 years while age group of 1-10 years constituted the lowest number with only 21 (5.2%) participants. Participants with primary level of education had the highest frequency of 107 (26.4%) while 23.4% had secondary form of education. Farmers among the participants based on their occupation were the highest recruited with 27.6% and only 9.4%students were recruited. Out of the 406 participants, 55.4% were urban resident and 44.6% were from rural areas (Table1).

Standard widal slide agglutination antibody titre for *Salmonella* O and H antigens.
The total of 406 blood specimens collected from febrile study subjects were tested by widal slide agglutination test as shown in Table2. Of the 406 specimens, 52% participants had positive/significant widal agglutination titres (≥1:160) for the O antigen and 48% participants were negative/insignificant (< 1:160), while 50.0% had positive widal agglutination titres (titre ≥1:160) for the H antigen and 50.0% were negative (titre <1:160). The positive widal slide agglutination titres results were interpreted and reported based on this agglutination titre scale of ≥ 1/160 as significant/positive.

Sero-prevalence of typhoid fever antibodies among study subjects attending three selected hospitals
Out of the 406 study subjects tested by widal and IgG/IgM tests, the overall seroprevalence of typhoid fever base on the widal slide agglutination test was 52 % for O antigens and for H antigens was 50.0% respectively, while base on the typhoid IgG/IgM test were 25.1% and 21.9% for IgG and IgM respectively as shown in Table 3.

Detection frequency of culture and biochemical reaction methods among study subjects
Of the total 406 collected blood specimens of study subjects, 31.8% samples were BacT/ALERT 3D culture positive. 129 BacT/ALERT 3D positive bottles were sub-cultured on brilliant green agar, MacConkey agar, blood agar and chocolate agar, out of which 26 isolates were biochemically identified as *Salmonella* Typhi (Table 4).

Table 1: Socio-demographic characteristics of febrile study subjects.

Variable	Frequency (n=406)	Percentage (Confidence Interval)
Sex		
Male	227	55.9 (51.0 – 60.7)
Female	179	44.1 (39.3 – 49.0)
Age Group (years)		
1 – 10	21	5.2 (3.4 – 7.8)
11 – 20	27	6.7 (4.6 – 9.5)
21 – 30	86	21.2 (17.5 – 25.4)
31 – 40	102	25.2 (21.2 – 29.6)
41- 50	100	24.6 (20.7 – 29.0)
>50	70	17.2 (13.9 – 21.2)
Level of Education		
Primary	107	26.4 (22.3 – 30.8)
Secondary	95	23.4 (19.5 – 27.8)
Tertiary	99	24.4 (20.5 – 28.8)
Informal	105	21.8 (21.8 – 30.3)
Occupation		
Civil Servant	77	19.0 (15.5 – 23.1)
Merchant	72	17.7 (14.3 – 21.7)
Farmer	112	27. 6 (23.5 – 32.1)
Daily labourer	54	13.3 (10.3 – 17.0)
House wife	53	13.1 (10.1 – 16.7)
Student	38	9.4 (6.9 – 12.6)
Residence		
Urban	225	55.4 (50.6 – 60.2)
Rural	181	44.6 (39.8 – 49.4)

Table 2: Standard widal slide agglutination titres among febrile study subjects

Reaction titre	**O-Antigen** Frequency (%) (n=406)	**H-Antigen** Frequency (%) (n=406)
<1:20	28 (6.9)	28 (6.9)
1:20	48 (11.8)	49 (12.1)
1:40	60 (14.8)	66 (16.3)
1:80	59 (14.5)	60 (14.8)
1:160	149 (36.7)	151 (37.2)
1:320	60 (14.8)	52 (12.8)
1:640	2 (0.5)	0 (0)
Total	406 (100)	406 (100)

Key; n=Number of subjects

Table 3. Overall seroprevalence of *Salmonella* Typhi antibodies among study subjects.

Test	**Frequency (%)**	
	Typhi O antibody	**Typhi H antibody**
Widal test	211 (52.0)	203 (50.0)
IgG/IgM	89 (21.9)	89 (21.9)
IgG	102 (25.1)	102 (25.1)
IgM	89 (21.9)	89 (21.9)

Key: IgG; Immunoglobulin G, IgM; Immunoglobulin M

Table 4. Detection frequency of culture and identification methods among study subjects

Parameters	Positive	Total	Percentage (%)
BacT/ALERT 3D	129	406	31.8
Biochemically identified *Salmonella* Typhi	26	406	6.4

Comparison of widal slide agglutination test against culture biochemically identified *Salmonella* Typhi isolates.

Among 211 widal positive, 17 specimens were both culture and widal positive for *Salmonella* Typhi isolates, 9 were culture positive for *Salmonella* Typhi but negative by widal slide agglutination test. The sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) were calculated and determined using WHO standard method. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were recorded as 65.38%, 48.95%, 8.06% and 95.38% respectively (Table 5).

Comparison of IgG/IgM test against culture biochemically identified *Salmonella* Typhi isolates.

Of the 102 IgG/IgM test positive, 23 were both blood culture and IgG/IgM test positive for *Salmonella* Typhi isolates, however, 3 isolates were culture positive for *Salmonella* Typhi but were negative by IgG/IgM test. The sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) were calculated and determined using WHO standard method. The sensitivity, specificity, positive predictive value and negative predictive value were recorded as 88.46%, 79.21%, 22.55% and 99.01% respectively (Table 4.6).

Table 5. Comparison of Widal slide agglutination and IgG/IgM test against culture positive.

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Widal test	68.4	48.8	6.2	96.9	62.8
Typhoid IgG/IgM test	89.5	78.0	16.7	99.3	78.6

Key: PPV; Positive predictive value, NPV; Negative predictive value

DISCUSSION

The most widely used serological test in typhoid fever diagnosis is to detect antibody against O and H antigen of *Salmonella* Typhi by Widal test. In the present study, Widal test was 52% positive for TO and 50% positive for TH, this was found to be similar in a study done by Shukala *et al.*, (1997) who reported that 44.2% had TO titre of >160 in the early phase of illness from patients suspected to have typhoid in an endemic area of central India. These findings were most probably attributable to a hyper-immune or immunologically sensitized population which is continually exposed to *Salmonella* Typhi and other Salmonellae (Rani *et al.*, 2021). The results obtained are also of relevance to the concept that specimens which are taken in the first week of illness are of little use in the sero-diagnosis of typhoid fever. Although widal test only becomes positive from the second week of infection, its usually negative during early infection and as well became false positive due to cross-reacting antibodies from viral infection, malaria and others gram negative bacteria, this has led to its non-reliability. The seroprevalence of 52.0% widal test was lower than the result of a similar study conducted in Kano by Mujahid *et al.,* (2022) with 70% seroprevalence, Sokoto by Gambo *et al.* (2017) with 60% seroprevalence, by Azhar and Hadeel in 2019 who recorded 68% seroprevalence, Abioye *et al.,* (2021) in Karu and a study by Ali *et al.,* (2019) with 64%

seroprevalence. Also, a study conducted in Kaduna by Igiri *et al.* (2018) who reported 80.5% seroprevalence. This was comparatively higher than the study conducted in Zaria by Igiri *et al.* (2018) who seroprevalence of 33.3%.

IgG/IgM test is a new serological-based assay that detect antibodies against lipopolysaccharide and flagella of *Salmonella* Typhi impregnated in a cassette and which is rapid and highly conversant and user-friendly.

In contrast to our findings, similar studies by Sharanya *et al.* (2016), Bhutta and Mansurali (1999) and Sherwal *et al.* (2004). They reported rapid test positive 70% and 79%, Widal positive 54% and 57%, respectively, among clinically suspected typhoid fever.

The present study revealed that, of the 406 specimens subjected to BacT/ALERT 3D machine, 129 were BacT/ALERT 3D positive, of which 26 (6.4%) were culture positive for *Salmonella* Typhi isolates. and this constituted the bacteriologically proven enteric fever base on biochemical test reactions. The result was similar with previous studies in Nigeria by Omotola *et al.,* (2020), Ethiopia by Habte *et al.,* (2018) and Egypt by Srikantiah *et al.,* (2006). A study by Sharanya *et al*., 2016 in India recorded higher culture positive for *Salmonella* Typhi (16%). Similarly, a lower proportion of culture positive was reported by previous studies done in Asian (2%) by Gupta *et al.* (2013), and Laos (1.5%) by Robert *et al.* (2020). Variations in *Salmonella* Typhi prevalence can occur due to differences in place, time and even in consecutive years at the same geographical location as suggested by Dewan *et al.* (2013) and/or seasonal variability as demonstrated by Marchello *et al.* (2019). Furthermore, differences in the laboratory detection methods used might also contribute to the difference observed. In contrast to our findings, previous studies in Nigeria by Ohanu *et al.* (2019), Jigjiga, Ethiopia by Admassu *et al.* (2019), India by John *et al.* (2016), and Sudan by Ali *et al.* (2019) reported a higher culture-confirmed typhoid fever cases among febrile patients ranging from 11 to 14.1%. This might be due to the varied incidence of typhoid fever in different study areas and periods (Mogasale *et al.,* 2016). Moreover, the geographically heterogeneous nature of typhoid fever burden might also contribute to the difference observed (Marchello *et al.,* 2019). The present study reported the sensitivity, specificity, and positive and negative predictive value of Widal slide agglutination test results when compared with the results of the culture and biochemically identified *Salmonella* Typhi isolates as 68.4%, 48.8%, 6.2% and 96.9% respectively. Based on this findings, we suggest that the positive widal titre detected in the blood of the study participants by the widal test was not enough to demonstrate typhoid fever illness, these findings are supported to the studies conducted by Park *et al.* (2009), Ahmad *et al.* (2010), Andualem *et al.* (2014), Ali *et al.* (2019) Wahdah and Neni (2020) and Shahapur *et al.* (2021). Other similar findings were revealed to have higher Widal test specificity in contrast to our findings by Mathew and Jobin (2013), Sharanya *et al.,* (2016), Islam *et al.,* (2016) and Jyotshna *et al*. (2022). Decreased Widal test sensitivity is due to the long latent period after which the test may become positive, negative result in early infection or due to prior antibiotic therapy, while decreased specificity might be attributed to cross reactivity with other Gram negative bacteria and non-typhoidal salmonella (Ahmad and Naseem, 1999). Diagnosis of typhoid fever based on culture method which is World Health Organisation (WHO) gold standard has proven the unreliability of Widal agglutination test, which is mostly used as a diagnostic tool for typhoid fever in Kebbi, most part of Nigeria and most under developed and developing countries. The unreliability and uncertainty of the Widal agglutination test due to its limited sensitivity and specificity has been reported in some literatures. The Widal test is plagued with many controversies involving the quality of the antigen used and

interpretation of the result. Hence, the suitability of blood culture alongside serological tests in diagnosing active infection (Adeleke *et al.,* 2006). Similarly, Okonko *et al.* (2010) reported that the reliability of Widal test in solely diagnosing typhoid fever has suffered doubt. It has been reported to remain positive month after effective therapy of the infection, such that a positive may not necessarily indicate active infection, making the test relevant in diagnosing post infection complications. Ibekwe *et al.* (2008) also suggested that only bacteriological isolation of the enteric bacteria from the patient's blood, bone marrow, stool and urine constitute unequivocal infection. According to the World Health organisation (WHO) 2020, Widal test can lead to false positive results because *Salmonella* Typhi shares O and H antigen with other *Salmonella* serotypes, and has cross reacting epitopes with other enterobacteriaceae. In addition, such results can occur in other clinical conditions such as malaria, typhus, bacteremia caused by other organisms and cirrhosis. Blood culture which is the gold standard for diagnosis of typhoid fever is not routinely requested by most physicians possibly due to the limitation of the laboratory media, volume of the blood cultured, presence of antibiotics and final result can only be obtained at the earliest of three days after specimen collection (WHO,2020).

In this current study, it was found that typhoid IgG/IgM test had sensitivity of 89.5%. This results were slightly lower than the findings in India by Wahdah and Neni, (2020), Tarupiwa *et al*. (2015), Sherwal *et al.* (2004) from India, who reported 92% sensitivity and Choo *et al.* (1994), who reported 90.3% sensitivity. Several studies have reported much higher sensitivity for rapid tests in diagnosing typhoid fever cases. Jesudasson *et al.* (2006) reported 100% sensitivity to rapid tests in detecting enteric fever. This difference might be in those studies unlike ours, have included all typhoid patients irrespective of severe cases of the fever and the result of the repeated cases.

In our findings, typhoid IgG/IgM was revealed to have a high specificity of 90%. This is in agreement with findings of Das *et al.* (2013) with 85%, Sapkota *et al*. (2022) with 82.8%, Sharanya *et al,* (2016) with 90%, Bhutta and Mansurali (1999) found 89% specificity, Sherwal *et al.* (2004) with 87.5%, and Gopalakrishnan *et al.* (2002) with 100% specificity of rapid tests in the diagnosis of typhoid fever. In disagreement with this study, a study by Shahapur *et al*. (2021) reported a much lower specificity of 64% by IgG/IgM test.

This study shown lower sensitivity and specificity by the Widal test compared to the IgG/IgM test. A research study by Soha, (2015) revealed that the Widal test was positive among patients giving a sensitivity of 85%, and a specificity of 88%, thus, typhoid IgG/IgM test shown 95% sensitivity and 90.4% specificity. The study by Setiana, (2016), reported that the Widal test has sensitivity of 44 -77% and specificity of up to 50-92%, the IgG/IgM test had sensitivity of 65-88% and specificity of 63-89%.

CONCLUSION

The present study results demonstrated effectiveness for its high sensitivity, specificity, PPVs, and NPVs of the Typhoid IgG/IgM test over the Widal test in the diagnosis of typhoid fever. However, the typhoid rapid IgG/IgM test should be encouraged for routine use in place of Widal test. Likewise, both tests cannot be considered gold standards for typhoid fever diagnosis owing to their low PPVs. Nevertheless, the high NPVs of both diagnostic tests indicate that the negative test will most certainly rule out infection and avoid unnecessary antimicrobial treatment.

REFERENCES

Crump JA. (2019). Progress in typhoid fever epidemiology. Clin Infect Dis 68:S4–S9. https://doi.org/10.1093/cid/ciy846.

Amicizia D, Micale RT, Pennati BM, Zangrillo F, Iovine M, Lecini E, Marchini F, Lai PL, Panatto D. (2019). Burden of typhoid fever and cholera: similarities and differences: prevention strategies for European travelers to endemic/ epidemic areas. *J Prev Med Hyg* 60:E271–E285.

Mather RG, Hopkins H, Parry CM, Dittrich S. (2019). Redefining typhoid diagnosis: what would an improved test need to look like? *BMJ Glob Health* 4:e001831. https://doi.org/10.1136/bmjgh-2019-001831.

Moore CE, Pan-Ngum W, Wijedoru LPM, Sona S, Nga TVT, Duy PT, Vinh PV, Chheng K, Kumar V, Emary K, Carter M, White L, Baker S, Day NPJ, Parry CM. (2014). Evaluation of the diagnostic accuracy of a typhoid IgM flow assay for the diagnosis of typhoid fever in Cambodian children using aBayesian latent class model assuming an imperfect gold standard. *Am J Trop Med Hyg* 90:114–120. https://doi.org/10.4269/ajtmh.13-0384.

Eng, S. K., Pusparajah, P., Ab Mutalib, N. S., Ser, H. L., Chan, K. G. and Lee, L. H. (2015). Salmonella: A review on pathogenesis, epidemiology antimicrobial resistance *Journal Frontiers in Life Science*. Volume 8(3):Pages 284–293.

Bharmoria, A., Shukla, A. and Sharma, K. (2017). Typhoid Fever as a Challenge for Developing Countries and Elusive Diagnostic Approaches Available for the Enteric Fever. *Int J Vaccine Res.*; 2 (2):1–16.

Senewiratne B. and Senewiratne K. (1977). Reassessment of the Widal test in the diagnosis of typhoid. *Gastroenterology*;73 (2):233-6.

Mujahid, N.S., Yusuf, I., Abbas, M.A., Yusuf, M., Sani, N.M., 1Shehu, A.A., Akande, A.O. Yakubu, A.B. and Sani, B.I. (2022). Prevalence of Typhoid Fever Among Patients Attending Murtala Muhammad Specialist Hospital Kano. *Bayero Journal of Pure and Applied Sciences*, 15(1): 57 – 63.

Ali, M. B., Hiba, M. A., Nahla, Y. A., Hala, H. A and Sara, M. A. (2019). A Comparison Study of Serological Test, Culturing Technique and Molecular Detection of Salmonella Typhi among Febrile Patients in Khartoum State, Sudan. *Advance Biotechnology & Microbiology*; 13(4): 555866. DOI: 10.19080/AIBM.2019.13.555866.

Abioye Joshua, Adiuku Beverly, and Adogo Lillian (2021). The prevalence of typhoid fever in Bingham University GSC *Biological and Pharmaceutical Sciences*, 01(03), 037–043.

Igiri, B. E., Inyang-Etoh, P. C., Ejezie, G. C., Jimoh, O., (2018). Diagnostic Investigations and Prevalence of Enteric fever in Ahmadu Bello University Teaching Hospital, Zaria, Kaduna State, Nigeria. *Clinical Microbiology and Infectious Diseases*. 3(1): 1-5. DOI:10.15761/CMID.1000131.

Gambo, S., Mohammad, U-K., Baki A.S., Bello, A. and Gambo, A. (2017). Incidence of *Salmonella* species isolated from presumptive Typhoid fever patients attending Specialist Hospital Sokoto. S*cholarly Journal of Biological Science.* 6(2), pp. 23-27.

Azhar and Hadeel, A. (2019). Burden of typhoid and paratyphoid fever in a densely populated urban community, Dhaka, Bangladesh. *IJID*. **14S**, e93–e99.

Jyotshna S., Rumina H., Robert O., Sonia A., Sam K., Sadia S., Farah Q., Sheillah M., Frida N., Rael T., Elizabeth N., Jason R. A. and Sabine D., (2022). Comparative Analysis of Commercially Available Typhoid Pointof- Care Tests: Results of a Prospective and Hybrid Retrospective Multicentre Diagnostic Accuracy Study in Kenya and Pakistan. *Journal of Clinical Microbiology*; 60:12; 10.1128/01000-22.

Omotola, J., Ogbonna, I., and Iheukwumere, C. H. (2020). Prevalence of Typhoidal *Salmonella* Infections and Associated Risk factors in Kaduna Metropolis, Nigeria. *Journal of Medical Microbiology and Infectious Diseases.* 8 (3).

Sherwal B. L., Dhamija R. K., Randhawa V. S., Jais M., Kaintura A. and Kumar M. A.(2004). comparative study of Typhidot and Widal test in patients of typhoid fever. *JIACM*; 5(3):244-6.

Wahdah Norsiah and Neni Oktiyani, (2020). Evaluation of the Diagnosis of Typhoid Fever Using the Widal Test and the Anti *Salmonella* Typhi IgM Test. *Medical Laboratory Technology Journal;* 6 (2), 2020, 128-134.

Bhutta ZA, Mansurali N. 1999. Rapid serologic diagnosis of pediatric typhoid fever in an endemic area: a prospective comparative evaluation of two dot-enzyme immunoassays and the Widal test. Am J Trop Med Hyg 61:654–657. https://doi.org/10.4269/ajtmh.1999.61.654.

Gupta, V., Singla, N., Bansal, N., Kaistha, N. and Chander, J. (2013). Trends in the antibiotic resistance patterns of enteric fever isolates-a three year report from a tertiary care centre. *Malaysia Journal of Medical Science*; **20**(4), 71–75.

Habte, L., Tadesse, E., Ferede, G. & Amsalu, A. (2018). Typhoid fever: Clinical presentation and associated factors in febrile patients visiting Shashemene Referral Hospital, southern Ethiopia. *BMC Res Notes.* **11**, 605. https:// doi. org/ 10. 1186/ s13104- 018-3713.

Mathew R, Jobin SR. (2013). A comparative study on methods for diagnosis of enteric fever. Int J Curr Res Rev ;5(14):88-95.

Roberts, T. *et al.* (2020). Typhoid in Laos: An 18-year perspective. *Am. J. Trop. Med. Hyg*. **102**(4), 749–757.

Srikantiah, P. *et al.* (2006). Population-based surveillance of typhoid fever in Egypt. *Am. J. Trop. Med. Hyg*. **74**(1), 114–119.

Admassu, D., Egata, G. & Teklemariam, Z. Prevalence and antimicrobial susceptibility pattern of *Salmonella* enterica *serovar* Typhi and *Salmonella* enterica *serovar* Paratyphi among febrile patients at Karamara Hospital, Jigjiga, eastern Ethiopia. *SAGE Open Med.* **7**, 1–7 (2019).

Marchello, C. S., Hong, C. Y. & Crump, J. A. (2019). Global typhoid fever incidence: A systematic review and meta-analysis. *Clin. Infect. Dis.* **68**(S2), S105–S116.

John, J., Van Aart, C. J. C. & Grassly, N. C. (2016). The burden of typhoid and paratyphoid in India: Systematic review and meta-analysis. *PLoSNegl. Trop. Dis.* **10**(4), e0004616.

Dewan, A. M., Corner, R., Hashizume, M. & Ongee, E. T. (2013). Typhoid fever and its association with environmental factors in the dhaka metropolitan area of Bangladesh: A spatial and time-series approach. *PLoSNegl. Trop. Dis.* **7**(1), e1998.

Mogasale, V. V., Ramani, E., Mogasale, V., Park, J. Y., & Wierzba, T. F. (2018). Estimating typhoid fever risk associated with lack of access to safe water: a systematic literature review. *Journal of environmental and public health*; 2(10):570–80.

Park SH, Kim HJ, Cho WH, Kim JH, and Oh MH (2009). Identification of Salmonella enterica subspecies I, Salmonella enterica serovars Typhimurium, Enteritidis and Typhi using multiplex PCR. FEMS microbiology letters 301(1): 137-146.

Shahapur P R, Shahapur R, Nimbal A., Kumar T. S., Silver R. G. D. and Kandi V. (2021). Traditional Widal Agglutination Test Versus Rapid Immunochromatographic Test in the Diagnosis of Enteric Fever: A Prospective Study From South India. Cureus 13(10): e18474. DOI 10.7759/cureus.18474.

Sharanya K., Vinod K. and Lakshmi K. (2016). Comparison of Widal and Typhoid immunoglobulin M and immunoglobulin G in Rapid and early Diagnosis of Enteric Fever. *Asian Journal of Pharmaceutical and Clinical Research*; l 9, 3, 243-246

Andualem, G., Abebe, T., Kebede, N., Gebre-Selassie, S., Mihret, A., Alemayehu, H. A. (2014). A comparative study of Widal test with blood culture in the diagnosis of typhoid fever in febrile patients. *BMC Research Notes*. 7: 653.

Ahmed, B. Z. and Mansurali, N. (1999) Rapid serologic diagnosis of pediatric typhoid fever in an endemic area: a prospective comparative evaluation of two dot-enzyme immunoassays and the Widal test. *American Journal of Tropical Medicine and Hygiene*; 61(4): 654-657.

Ibekwe, A. C., Okonko, I. O., Onunkwo, A. U., Donbraye, E., Babalola, E. T. and Onoja, B. A. (2008). Baseline *Salmonella* agglutinin titres in apparently healthy freshmen in Awka, South Eastern, Nigeria. *Sci. Res. Essay,* **3**(9): 225-230.

Okonko, I.O., Soleye, F.A., Eyarefe, O.D., Amusan, T.A., Abubakar, M.J., Adeyi, A.O., Ojezele, M.O. and Fadeyi, A. (2010). Prevalence of *Salmonella Typhi* among Patients in Abeokuta, South-Western Nigeria. *British Journal of Pharmacology. and Toxicology.* **1**(1)**:** 6-14, 2010**.**

Islam K., Sayeed M. A., Hossen E., Khanam F., Charles R. C., Andrews J., Ryan E. T. and Qadri F. (2016). Comparison of the performance of the TPTest, Tubex, Typhidot and Widal immunodiagnostic assays and blood cultures in detecting patients with typhoid fever in Bangladesh, including using a Bayesian latent class modelling approach. *Neglected Tropical Diseases*; 10:e0004558. https://doi.org/ 10.1371/journal.pntd.0004558.

Adeleke, O. E., Adepoju, T. J. and Ojo, D. A. (2006). Prevalence of typhoid fever and antibiotic susceptibility pattern of its causative agent *Salmonella Typhi*. *Nigeria Journal of Microbiology.* **20**(3): 1191-1197.