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PREVALENCE OF TYPHOID FEVER AMONG PATIENTS ATTENDING MURTALA MUHAMMAD SPECIALIST HOSPITAL KANO

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ABSTRACT
Typhoid fever poses a serious public health problem in Nigeria and is largely diagnosed based on the Widal agglutination test which has been proven to be neither sensitive nor specific. This study determined the prevalence of typhoid fever using both Widal agglutination test and blood culture using automated microbiology systems (BD BACTEC). The study was carried out among 90 patients attending Murtala Muhammad Specialist Hospital in Kano. Out of the 90 cases recruited for the study, none of the cases (0, 0.0%) had S. Typhi isolated using blood culture. However, 18 cases (20%) had other bacteria which are not S. Typhi isolated using blood culture while 72 cases (80%) were negative. For the Widal test, 63 cases (70%) were positive for anti S. Typhi O antigen while 27 cases (30%) were negative. Similarly, 42 cases (46.7%) were positive for anti S. Typhi H antigen, while 48 cases (53.3%) were negative. Type of toilet system was found to be significantly associated with non S. Typhi bacteraemia (P=0.021). The study recommends the use of other diagnostic test such as molecular techniques to determine the sensitivity and specificity of both Widal and cultural methods.
Keywords: Widal, Blood culture, S. Typhi, Typhoid fever.

INTRODUCTION

Typhoid Fever (TF) is a systemic infection comprising of diseases mostly caused by *Salmonella enterica* (S. *enterica*) serovar Typhi and *S. enterica* serovar Paratyphi (A, B and C) which cause paratyphoid fever (Ajibola *et al.*, 2018). Salmonella is a Gram-negative rod-shaped facultative anaerobic bacterium of the family *Enterobacteriaceae* (Eng *et al.*, 2015). The clinical outcome of Salmonellosis depends various parameters including the serovar involved, the infected host species and the immunological status of the individual (Pham and McSorley, 2015). Gastroenteritis causing serovars of Salmonella are also able to cause systemic disease in individuals with a primary or acquired immune deficiency (Pham and McSorley, 2015). Certainly, Salmonella bacteremia is an emerging problem in Sub-Saharan Africa where it is associated with HIV, malaria or poor nutritional status (Pham and McSorley, 2015; Das *et al.*, 2022).

World Health Organization estimates the global typhoid fever disease burden at 11-20 million cases annually, resulting in about 128,000–161,000 deaths per year (W.H.O., 2018). Typhoid fever remains a major disease in Nigeria due to factors such as increased urbanization, insufficient supply of potable water, regional migration of large numbers of immigrant workers, insufficient human waste treatment facilities, overburdened health care delivery systems, and overuse of antibiotics that lead to the growth and spread of antibiotic-resistant *S.* Typhi (Akinyemi *et al.*, 2018).

Kano is one of the densely populated states in Nigeria where a study by Abdulkarim and Mohammed (2017) reported about 1,225 cases of typhoid fever within five years in a single hospital in Kano State. Nas *et al.* (2017) also reported an 18% prevalence of typhoid fever in Kumbotso local government area of Kano state.

Isolation of the causative bacteria in typhoid fever patients by culture remains the gold standard for diagnosis (Ajibola *et al.*, 2018). However, in most developing countries a serological test especially "Widal" test is most commonly applied (Ajibola *et al.*, 2018). In an effort to accurately highlight the burden of typhoid fever in Nigeria, positive serological tests must be accompanied with culture to avoid misappropriate prescription of antibiotics. This study thus, evaluates the prevalence of typhoid fever in Murtala Muhammad Specialist Hospital Kano using both Widal serology and blood culture.

MATERIALS AND METHODS

STUDY AREA

The study was conducted at Murtala Muhammad specialist hospital (MMSH), Kano Nigeria. Murtala Muhammad specialist hospital is one of the oldest government owned hospitals in Kano State located in the ancient city of Kano on Latitudes 11.9634° N, 8.5504° E.

Study Population

The study population comprised of patients with clinical signs and symptoms suggestive of typhoid fever with request of Widal test from Physicians at MMSH.

SAMPLE SIZE

The sample size used in this study was determined using the formula developed by Gberikon *et al.* (2019), based on previous prevalence of Salmonella Typhi infection in Kano State (4.5%) as reported by Akinyemi *et al.* (2018). The sample size was calculated to be 90.

Ethical Consideration

Ethical approval was sought and obtained from the ethical committee, Kano State Ministry of Health with reference number MOH/Off/797/T.I/1424.

Questionnaire Administration

A structured questionnaire was administered to obtain demographic characteristics and risk factors associated with *S.* Typhi infection among the study population.

Sample Collection and Transportation

Using a sterile syringe, exactly 10 ml of blood from each consented patient was aseptically collected; 7ml dispensed into the BACTEC aerobic plus culture vial and 3ml in a plain container which were both transported to the laboratory for further analysis (Andualem *et al.*, 2014).

Sample Processing

Widal Serology.

Widal slide agglutination test was done using *S.* Typhi, *S.* paratyphi A, *S.* paratyphi B and *S.* paratyphi C O and H antigens according to the instructions of the manufacturer (Chronolab systems). The antigen suspension commercially available in 5 ml volume from Chronolab systems, (Barcelona, Spain) was used. A direct qualitative slide agglutination technique was used in this study for determination of the agglutination ability of sera. The test was done by mixing one drop of serum with one drop each of O and H antigens separately on slide (Andualem *et al.,* 2014). After rocking the slide back and forth for 1 min, the mixture was observed for macroscopic agglutination. If there was agglutination within 1 min it was reported as reactive, otherwise, non- reactive (Deksissa and Gebremedhin, 2019).

Inoculation

Inoculation of Blood Samples for Culture

The blood samples were cultured using the BACTEC fluorescent series 9120 instruments (Becton Dickinson, USA) automated microbiology systems following the method described by Anvarinejad *et al.* (2016) with slight modifications. Exactly 7ml of blood was aseptically dispensed into the BD Bactec Plus aerobic/F culture vial containing soybean-casein digest broth and then loaded into the BACTEC machine within 30 minutes of sample collection. Whenever the machine gives an alert, the specific bottle was removed, Gram stained, and sub cultured on chocolate agar and MacConkey's agar. The isolates were identified as Salmonella based on Gram staining, the oxidase test, the catalase test, motility, triple-sugar iron (TSI) fermentation, and colony morphology. Negative were removed after the system confirms them as negative.

Data Analysis

Statistical association between anti- *S.* Typhi antibody and blood culture among the study subjects was determined at 95% confidence interval using Fisher's exact test with the aid of statistical package for social sciences (SPSS) version 22 where a p value of ≤ 0.05 was considered significant.

RESULTS

A total of 90 cases were recruited for the study. None of the cases (0, 0.0%) tested positive for *S.* Typhi blood culture. However, 18 cases (20%) were positive for non- *S* Typhi blood culture while 72 cases (80%) were negative. For the Widal test, 63 cases (70%) were positive for anti *S.* Typhi O antigen while 27 cases (30%) were negative. Similarly, 42 cases (46.7%) were positive for anti *S.* Typhi H antigen, while 48 cases (53.3%) were negative.

Table 1: Prevalence of Typhoid Fever based on Widal and Blood culture Methods

Parameters	No. Examined (%)	Culture Negative (%)	Non-Culture S. Typhi Positive (%)	*P-Value*	OR
Anti - Typhi O					
Positive	63 (70.0)	52 (57.8)	11 (12.2)	0.358	1.655
Negative	27 (30.0)	20 (22.2)	7 (7.8)		
Total	90 (100)	72 (80)	18 (20)		
Anti -Typhi H					
Positive	42 (46.7)	38 (42.2)	4 (4.4)	0.033*	3.912
Negative	48 (53.3)	34 (37.8)	14 (15.6)		
Total	90 (100)	72 (80)	18 (20)		

***=significant statistical difference using fisher's exact test.**

Table 2: Culture status of studied subjects based on demographic factors

Parameters	No. Examined (%)	Culture Negative (%)	Non- S. Typhi Culture Positive (%)
Gender			
Male	32 (35.6)	25 (27.8)	7 (7.8)
Female	58 (64.4)	47 (52.2)	11 (12.2)
Total	**90 (100)**	**72 (80)**	**18 (20)**
Age (years)			
< 21	38 (42.2)	31 (34.4)	7 (7.8)
21-30	28 (31.1)	23 (25.6)	5 (5.6)
31-40	12 (13.3)	10 (11.1)	2 (2.2)
41-50	9 (10)	5 (5.6)	4 (4.4)
51-60	2 (2.2)	2 (2.2)	0 (0)
Total	**90 (100)**	**72 (80)**	**18 (20)**
Marital Status			
Single	40 (44.4)	30 (33.3)	10 (11.1)
Married	49 (54.4)	41 (4.6)	8 (8.9)
Divorced	1 (1.1)	1 (1.1)	0 (0)
Total	**90 (100)**	**72 (80)**	**18 (20)**
Educational Level			
Non-Formal	8 (8.9)	6 (6.7)	2 (2.2)
Primary	20 (22.2)	18 (20)	2 (2.2)
Secondary	39 (43.3)	32 (35.6)	7 (7.8)
Tertiary	23 (25.6)	16 (17.8)	7 (7.8)
Total	**90 (100)**	**72 (80)**	**18 (20)**
Occupation			
Self-employed	15 (16.7)	15 (16.7)	0 (0)
Civil Servant	15 (16.7)	12 (13.3)	3 (3.3)
Unemployed	4 (4.4)	3 (3.3)	1 (1.1)
Student	35 (38.9)	25 (27.8)	10 (11.1)
Full-time Housewife	21 (23.3)	17 (18.9)	4 (4.4)
Total	**90 (100)**	**72 (80)**	**18 (20)**

Table 3: Culture status of studied subjects based on some risk factors

Parameters	No. Examined (%)	Culture Negative (%)	Non-*S.* Typhi Culture Positive (%)	*P-Value*	OR
Suya Consumption					
Yes	48 (53.3)	41 (45.6)	7 (7.8)	0.170	0.481
No	42 (46.7)	31 (34.4)	11 (12.2)		
Total	**90 (100)**	**72 (80)**	**18 (20)**		
Family History of Typhoid Fever					
Yes	46 (51.1)	38 (42.2)	8 (8.9)	0.603	0.716
No	44 (48.9)	34 (37.8)	10 (11.1)		
Total	**90 (100)**	**72 (80)**	**18 (20)**		
Toilet System					
Pit Latrine	17 (18.9)	11 (12.2)	6 (6.7)	0.021*	-
Water Closet	72 (80)	61 (67.8)	11 (12.2)		
Open Defecation	1 (1.1)	0 (0)	1 (1.1)		
Total	**90 (100)**	**72 (80)**	**18 (20)**		
Drinking Water					
Bottled Water	7 (7.8)	6 (6.7)	1 (1.1)	0.671	-
Sachet Water	49 (54.4)	39 (43.3)	10 (11.1)		
Tap Water	11 (12.2)	8 (8.9)	3 (3.3)		
Well Water	10 (11.1)	7 (7.8)	3 (3.3)		
Borehole	13 (14.4)	12 (13.3)	1 (1.1)		
Total	**90 (100)**	**72 (80)**	**18 (20)**		

***=significant statistical association, - = Not computed, OR= Odds Ratio.**

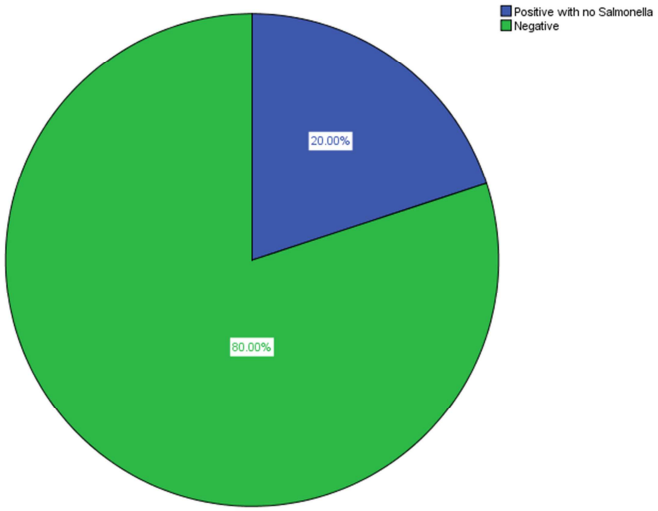

Figure 1: Blood Culture Result of Patients Investigated for Typhoid Fever.

DISCUSSION

Enteric fever is globally recognised as one of the serious public health problems being a potentially fatal and multi systemic infectious disease (Mehta *et al.*, 2017). Socio-Demographic factors have been found to influence the prevalence of enteric fevers like typhoid where it mainly affect children and young adults whereas in high-income countries it is primarily a disease of returning travellers (Deksissa and Gebremedhin, 2019).

From this study, females were found to report to hospitals with symptoms suggestive of typhoid fever than males with frequencies of 58 (64.4%) and 32 (35.6%) respectively.

This is similar to the findings of Deksissa and Gebremedhin, (2019) where they conducted a study to estimate the prevalence of enteric fever among febrile patients visiting Ambo hospital, Ethiopia and found the majority of patients to be females (59.1%). A similar study by Mawazo *et al.* (2019) also found the percentage of females to be higher than males with a percentage of 61.1%. The higher percentage of female patients might be due to the fact that females constitute a larger number of the Kano state population which statistically means there is a higher probability of females falling sick than males .

In this study, the mean age of patients was 25.7 ± 1.35 years with participants less than 21 years of age constituting a larger proportion of the study population (38, 42.2%). This is similar to the findings of Wam *et al.* (2019) where they also recorded 41.1% of participants with complains of typhoid fever within the age range of 10-29 years. This might be due to the fact that people of this age group tend to be influenced by peer group and not mindful of what they eat or drink because contaminated food and water are major sources of febrile illnesses (Wam *et al.*, 2019).

This study found the preponderance of married patients (49, 54.4%) over single (40, 44.4%) and divorced (1, 1.1%) respectively which is in good agreement with the findings of Deksissa and Gebradhin, (2019) where they recorded higher number of married (71%) participants than unmarried (29%). This preponderance is not too far from the fact that most of the participants are females and within their child-bearing age which implies that most of them might be having pregnancy related problems which necessitate their regular hospital visits. Also, by nature of their responsibility as mothers, they are solely concerned with taking children to the hospital when sick. This makes them prone to nosocomial infections.

With regards to educational status, those with secondary level of education recorded higher frequency (39, 43.3%) as opposed to those with tertiary level of education (23, 25.6%), primary (20, 22.2%) and non-formal (8, 8.9%). This is in line with the findings of Mawazo *et al.* (2019) were they also recorded higher number of participants with secondary level of education. This trend observed might be due to the fact that most of the patients are married females less than 21 years of age. Female children in northern Nigeria often get married at young age where they hardly get the opportunity to continue with their studies as such, end up having secondary education as their highest qualification.

Enteric Fever has been considered mainly, a disease of afflicting persons with low economic status where individuals with little daily earnings are at greater risk than their higher income earning counterparts. This study confirms the above statement as patients with student status recorded higher frequency 35 (38.9%) followed by full-time housewives 21 (23.3%). Conversely, patients who are civil servants or self-employed had the same proportion 15 (16.7%).

The main factors contributing towards the prevalence of typhoid fever in developing countries are rapid population growth, increasing urbanisation, contaminated water supply, poor hygiene and lack of sanitation. These illnesses are not easily distinguishable especially in the initial stage and hence pose a great challenge in clinical diagnosis. Undiagnosed and untreated cases may result in serious complications such as typhoid perforation (Mehta *et al.*, 2017).

This study, found out that those who consumed meat from "Suya joints" had a lower percentage of non – *S.* Typhi blood culture positivity (7, 7.8%) than those who don't (11, 12.2%) as indicated in table 3. Fisher's exact test also shows that there exist no significant difference between "suya" consumption and blood culture status (p=0.170 OR=0.481). These findings are in contrast with that of Deksissa and Gebremedhin, (2019) where they found higher prevalence of typhoid fever in those who consumed raw meat (60.87%) than those who don't (46.22%). The difference in findings might be due to the fact that this study used grilled meat while as the risk factor while Deksissa and Gebremedhin, (2019) used raw meat consumption as the risk factor.

Non *S.* Typhi blood culture positivity was found to be higher in those who had no family history of typhoid within the last three months (10, 11.1%) than those whose family members suffered typhoid (8, 8.9%). The former might have immunologic memory to *S.* Typhi antigens thereby conferring immunity against infections with bacteria having similar antigenic properties. However, chi-square test showed no statistical relationship between family history of typhoid and blood culture status (P=0.603 OR=0.716) as indicated in table 3.

Slum areas where sanitary facilities and provision of clean water are not fulfilled coupled with poor municipal waste management, might predispose people to the use of faecal contaminated stream water for food preparation and drinking purposes (Deksissa and Gebremedhin, 2019).

Type of toilet system was found to significantly affect blood culture status among the patients used in this study. Patients who use Water closet were found to constitute a larger proportion of those with non – *S.* Typhi blood culture positivity (11, 12.2%) than those using pit-latrine (6, 6.7%). This might be due to the fact that water closet systems require proper maintenance and hygiene which most people in developing countries do not pay attention to. These unhygienic water closet systems serve as potential reservoirs for transmission of *Salmonella* and other enteric bacteria. Fisher's exact test showed as statistical association between the type of toilet system used and blood culture status among the study population (P=0.021).

Source of drinking water was also found to differ significantly with the proportion of those having non- *S*. Typhi blood culture positivity. Patients who use sachet water (10, 11.1%) have non- *S.* Typhi positivity more than those who use tap (3, 3.3%) and well water (3, 3.3%) respectively. This might be due to the increase in number of sachet water producers where most of them do not treat the water properly and people tend to use it more than other sources of drinking water as seen in table 3. Those who use bottled water or borehole as their sources of drinking had the least frequency of blood culture positivity (1, 1.1%) respectively. This justifies the previous statement as borehole is usually contamination free while bottled water is relatively pure due to regulations by government agencies. However, there was no statistical significance between the type of drinking water and blood culture status (p=0.671).

From the present study, the prevalence of typhoid fever based on widal slide agglutination was found to be 63 (70%) based on reaction to *S.* Typhi O antigen, and 42 (46.7%) based on reaction to *S.* Typhi H antigen. This is in good agreement with the result of Deksissa and Gebremedhin, (2019) where they recorded a Seroprevalence of 49.5% for both *S.* Typhi O and H antigens. However, no single *S.* Typhi isolate was recovered from patient's blood using blood culture which is gold standard (table 1). The findings of this research are in good agreement with that of Igiri *et al*. (2018) where they determined the prevalence of enteric fever in Ahmadu Bello university teaching hospital Zaria and found 70% prevalence using Widal but 0% using standard blood culture. Fisher's exact test showed a statistical difference between widal and non- *S.* Typhi blood culture (p=0.035) as reported in table 1. This disagreement between blood culture and widal result might be due to the fact that Widal test is based on the reaction of *S.* Typhi antigens (O and H) to specific antibodies in patient's serum where cross reactivity with similar antigens is possible. Cross reactivity of widal has been reported in patients with dengue fever by Bhatti *et al*. (2015) where they recruited patients who had clinical and serological evidence of dengue virus infection and subjected them to widal screening test. They found about 33% of the patients with dengue fever to be widal positive with no single *Salmonella* Typhi bacterium isolated from their blood. A similar trend was also recorded in this research where other pathogenic bacteria were isolated from the blood of (18, 20%) of the patients tested. High seropositivity of widal might also be due to cross reactivity of the antigens to antibodies against malaria due to its endemic nature in Nigeria, leading to misdiagnosis and treatment of the wrong disease by physicians (Igiri *et al*., 2018). The results obtained in this study might give an insight to the reason for rapid increase in the emergence of drug resistant bacterial infections.

CONCLUSION

The prevalence of typhoid using Widal test was relatively high but blood culture confirmed that none of the study participants truly has active *Salmonella* infection. This suggests that most patients presenting with symptoms of febrile illnesses may not actually be suffering from typhoid fever but other bacterial infections and may therefore be mistreated for typhoid fever.

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