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Original Research Article

Comparative Study of Widal test Against Stool Culture in Diagnosis of Typhoid Fever Suspected Cases in Kano, Northern Nigeria

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Abstract: The "gold standard" for diagnosis of typhoid fever is the isolation of *Salmonella typhi* from appropriate samples including blood, stool and urine. The study was aimed to compare Widal test against stool culture for diagnosis of typhoid fever cases in Kano, Northern Nigeria. A completely randomized design is used. A total of 125 subjects (male, n= 57 and female, n=68) presenting febrile conditions in 4 different health care centers within Kumbotso Local Government Kano State were used for the study. About 5ml of blood was obtained from each study participant for Widal test and freshly passed faeces were collected for stool culture. The result showed that 22 (17.6%) tested positive for *Salmonella typhi* by Widal test, whereas 17 (13.6%) tested positive by stool culture. From the result, patients within the age category 21 – 40 years has the highest incidence 9 (7.2%) and 7 (5.6%) for Widal test and stool culture respectively while male has the highest prevalence with 12 and 10 individuals (9.6% and 8%) while 10 and 7 individual positive samples were female accounted for 8% and 5.6% for widal test and stool microcopy respectively. In relation to stool culture, Widal test has high sensitivity (78.3%), specificity (93.6%), positive predictive value (68.2%) and negative predictive value (98.1%). There is no significant difference on the prevalence of the infection on the basis of gender, age category and diagnostic methods at $p<0.05$. It is concluded that Widal test can be used as a diagnostic method for detection of *Salmonella typhi*.

Keywords: Typhoid fever, *Salmonella*, stool culture, Widal test.

INTRODUCTION

Typhoid fever is a systemic disease caused by *Salmonella enterica* serotype typhi and is a major cause of morbidity and mortality worldwide [1]. It emerged as an important infectious disease in the early 19th century. Humans are the only natural host and reservoir for typhoid fever agent. Infection occurs in all age groups, and it is transmitted by ingestion of food or water contaminated with feces [2]. The highest incidence occurs where water supplies serving large populations are contaminated with feces [3].

The diagnosis of enteric fever poses several problems due to the non-specific and wide array of clinical features. The common symptoms and signs are fever, vomiting, cough, anorexia, diarrhea, abdominal pain, hepatomegaly, splenomegaly, and coated tongue. Enteric fever should be considered in the differential diagnosis of febrile patients with abdominal symptoms [4]. The common tropical infections such as dengue, enteric fever, leptospirosis, typhus fever, and malaria having similar early presentations can cause confusion in decision-making. Recognition of these diseases is important to diagnose them and treat them early, to avoid potentially fatal complications [5]. Laboratory diagnosis of enteric fever includes Blood culture, Stool Culture and Serological test. Widal test is a common agglutination test employed in the serological diagnosis of enteric fever. This test was developed by Georges Ferdinand Widal in 1896 and helps to detect presence of salmonella antibodies in a patient's serum [6].

In 1896, Widal developed a procedure for diagnosing typhoid fever based on the fact that antibodies in the blood of an infected individual cause the bacteria to bind together into clumps (the Widal reaction) [7]. The test was based on demonstrating the presence of agglutinin (antibody) in the serum of an infected individual, against the H (flagellar) and O (somatic) antigens of *Salmonella typhi* [8]. The "O" antigen is the somatic antigen of *S. typhi* and is shared by *S. paratyphi* A, *S. paratyphi* B, other Salmonella species and other members of the Enterobacteriaceae family [9]. Antibodies against the O antigen are predominantly IgM, rise early (appear on day 6 to 8) in the illness and disappear early [9]. The H antigens are flagellar antigens of *S. typhi*, *paratyphi* A and *paratyphi* B. Antibodies to H antigens are both IgM and IgG, rise late (on days 10 to 12) in the illness and persist for a longer time [9, 10]. Serological diagnosis relies classically on the demonstration of a rising titre of antibodies in paired samples at an interval of 10 to 14 days [11].

In typhoid fever, stool cultures are usually positive from the second week of the infection. Stool is usually plated on agar and also inoculated into fluid enrichment media such as selenite broth. Suspicious colonies from culture plates are tested directly for the presence of salmonella O antigens by slide agglutination and sub-cultured to peptone water for determination of H antigen structure and for further biochemical analysis [12]. The objective of this study was to evaluate the performance of Widal test and stool microscopy and to compare the diagnostic accuracy of these tests as tools for diagnosis of enteric fever.

MATERIALS AND METHOD

Study Area

This research was conducted in Kumbotso Local Government Area of Kano State, Nigeria. The Local Government area was created in 1976 [13]. The Local Government area has eleven (11) wards. It has an area of 158 km² and total population of two hundred and ninety four thousand, three hundred and ninety one (294,391) residents with population density of 2,197.47 person/ km^2 [13]. According to National population commission, the populations are expected to reach to 374,200 by the year 2011. It is located at an elevation of 450 meters above sea level. Its coordinates on a map are $11^0$53'N latitudinally and $8^0$30'E longitudinally [14]. Major towns in the local government are Kumbotso town, Chiranci, Sheka, Dan-bare, Challawa, Panshekara etc. Farming remains a major occupation in the area. However many educated indigenes in the area are employed in the formal sector while others engaged in various trading activities.

Study Population

A completely randomized design is used. A total of 125 subjects (male, n= 57 and female, n=68) presenting febrile conditions suggestive of malaria, dengue virus fever or typhoid fever in 4 different health care centers which are the most populated area in the Local Government (Chiranchi n= 43, Sheka n= 42, Dan-bare n=38 and Panshekara n=37) within Kumbotso Local Government Kano State were used for the study. It included individuals of all ages and sexes.

Sample Collection

Specimens were collected from the febrile patient, who presented themselves at the health centers within the study site from a period of April 2015 to October, 2015. Specimens for this study were blood and stool for Widal and stool culture respectively. 5ml of blood was obtained from each study participant upon routine venepuncture for Widal test. Blood samples in plain tubes were allowed to clot and the clot removed by centrifuge and the supernatant obtained was serum. Freshly passed faeces were collected in a sterile wide mouthed container. Each sample container was labeled with the patient's code number, date and time.

Questionnaire Design, Distribution and Retrieval

A total of a hundred (125) questionnaires were designed using open ended questions to provide information about the socio-demographic factors of participants and predisposing factors to both infections. Informed consents were obtained from all participants before inclusion. Guardians gave consent for minors.

Widal Agglutination Test for *Salmonella* Antibodies

Widal agglutination test was performed on each blood sample using the Widal agglutination kit (Biotech Lab, United States) according to manufacturer's instruction. The reagents contained *Salmonella typhi* O and H antigens and *Salmonella paratyphi* A, B and C antigens. Positive and negative controls were included and a titre greater than or equal to 1/80 indicates salmonella infection. The reagents and samples were brought to room temperature and the antigens were shaken properly to mix well before dispensing. A drop of patient's serum to be tested was placed onto each of the required number of circles on the tile, and then one drop of Widal antigen suspension was added to the reaction circles containing patient serum. Using different mixing applicator sticks provided, the tile was rocked gently back and forth and observed for agglutination macroscopically for one minute.

Stool Culture

Feces were inoculated onto the surface of Nutrient, MacConkey and *Salmonella Shigella* Agar (SSA) (Life save Biotech, USA) for isolation, cultivation and differentiation of *Salmonella typhi* according to method describe by Prescott

et al., [15]. During the process, a sterile wire loop was deep into the fecal sample of the patients and streaked onto the surface of the agars. The procedure was repeated for all the samples and the plates were incubated 37^0 C for 24 hours. The presumptive colony of *Salmonella typhi* from each plate was further sub-cultured to obtained pure culture. The pure isolates of *Salmonella typhi* were preserved in peptone water for further use. The preserved isolates were confirmed as *S. typhi* using conventional microbiological methods which include Gram staining, lactose fermentation and motility test as well as biochemical (Indole, methyl orange, Voges Proskauer, nitrate reduction and citrate utilization) tests according to the methods described by Cheesbrough [16] and Holt *et al*., [17].

Statistical Analysis

Statistical analysis package for social sciences (SPSS) version 10.0 was used for statistical analysis of the data generated. Chi square was used to compare between two or more variables. Statistical significance was considered at p-value <0.05

Ethical Consideration

The study was conducted following ethical approval obtained from the Health Services Management Board, Kano State based on the consent of Ethical Committee of Health Department of Kumbotso Local Government Area of Kano State.

RESULTS

Socio-demographic Factors of the Participants

A total of 57 (46%) males and 68 (54%) females took part in this study with ages ranging from less than 20 to over 40 years. Majority of the participants were 20 to 40 years age bracket. Participant from rural area accounted for 58% (72 subjects) while 42% (53 subjects) are from urban area. Most female participants are house wives males participant are mostly students, civil servant and farmers.

Table-1: Socio-demographic Factors of the Participants with Percentage Frequency

Parameter	Male (n)	Female (n)	Total (n)
Age (Years)			
0 – 20	14 (12%)	18 (14%)	32 (26%)
21 – 40	25 (20%)	22 (17%)	47(37%)
41 – 60	12 (10%)	18 (14%)	30 (24%)
61 – Above	06 (05%)	10 (08%)	16 (13%)
Settlement			
Rural	33 (27%)	39 (31%)	72 (58%)
Urban	24 (19%)	29 (23%)	53 (42%)
Occupation			
Student	17 (13%)	12 (10%)	29 (23%)
Civil servant	12 (10%)	01 (01%)	13 (11%)
Farming	14 (11%)	05 (04%)	19 (15%)
Trading	08 (06%)	02 (02%)	10 (08%)
House wives	00 (00%)	48 (38%)	48 (38%)
Others	06 (05%)	00 (00%)	06 (05%)

Prevalence of Typhoid fever according to Diagnostic Methods

The prevalence of typhoid fever based on the diagnostic method used in this study is presented in Table-2. The result showed that out of the 125 patients involved in the study, 22 (17.6%) tested positive for *Salmonella typhi* by Widal test, whereas 17 (13.6%) tested positive by stool culture.

Table-2: Prevalence of typhoid fever based on the diagnostic methods used

Methods	No. of samples	No. of positive	No. of negative	P value
Widal test	125	22 (17.6%)	103 (82.4%)	.383482*
Stool culture	125	17 (13.6%)	108 (86.4%)	

Key: * Result not significant (p<0.05)

Prevalence of Typhoid fever based on Sex and Age

Table-3 represents the prevalence of typhoid fever based on gender and age category of the patients. The result showed that patients within the age category 21 – 40 years has the highest incidence of typhoid fever 9 (7.2%) and 7 (5.6%) for Widal test and stool culture respectively. On the basis of gender, despite they have less number of febrile

patients, male has the highest prevalence of typhoid fever with 12 and 10 individuals (9.6% and 8%) while 10 and 7 individual positive samples were female accounted for 8% and 5.6% for widal test and stool culture respectively.

Table-3: Prevalence of typhoid fever based on Sex and Age of the respondents

	Widal test			Stool culture		
Parameter	**+**	**-**	**P value**	**+**	**-**	**P value**
Age (years)						
0 – 20 (n=32)	05	27	.903279*	03	29	.847159*
21 – 40 (n=47)	09	38		07	40	
41 – 60 (n=30)	06	24		05	25	
Above 61 (n=16)	02	14		02	14	
Sex						
Male (n=57)	12	45	.3533385*	10	47	.238918*
Female (n=68)	10	58		07	61	

Key: * Result not significant (p<0.05)

Sensitivity and Specificity of Widal test against Stool culture

The sensitivity, specificity, positive and negative predictive values of Widal test against stool culture is presented in Table 4. This means that since stool culture was considered the ideal to which Widal would be compared to, its sensitivity and specificity when cultured for *S. typhi* using the MacConkey and *Salmonella Shigella* agar were 100% each. Sensitivity is the probability that a truly infected individual will test positive, while specificity is the probability that a truly uninfected individual will test negative. Among 22 positive Widal tests, only 15 were positive for stool culture, and the two more positive stool cultures were negative with Widal test. Widal sensitivity was 78.9% while its specificity was 93.6% as compared to the ideal 100% sensitivity and specificity of stool culture. The Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of Widal were 68.2% and 98.1% respectively

Table-4: Sensitivity and Specificity of Widal test against Stool Culture

Results	**Widal test**
Sensitivity (%)	78.9
Specificity (%)	93.6
PPV (%)	68.2
NPV (%)	98.1

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

DISCUSSION

In developing countries like Nigeria, Widal test is the common serological test used to diagnose typhoid fever. The findings in the present study suggest a low prevalence of 17.6% and 13.6% among febrile patients using Widal test and stool microscopy respectively. This prevalence rate is lower than that of Wam *et al*., [18] in Cameroun who a prevalence of 39.3% in patients using the stool culture method and a prevalence of 57.1% using the Widal test method. The result is lower than that of Gemechu *et al.*, [19], which showed a high prevalence of typhoid fever (20%) using stool culture and 68.4% using Widal test method in Ethiopia. On the other hand, this result is lower than that of Verma *et al.*, [20] in Northern India who found prevalence rate of 10.8% and 3.3% for Widal test and stool culture respectively. Low prevalence rate of typhoid fever among febrile patients in this study is attributed to high malaria prevalence in the area.

There is high prevalence of typhoid fever from Widal test when compared to stool culture according to the present study. This finding was in conformity with the findings of Wam *et al*., [18]; Gemechu *et al.*, [19] and Verma *et al*., [20]. The high Widal prevalence in the present study could be associated with cross-reacting antibodies from febrile patients other than typhoid fever [18]. According to the result of the present study, individuals of all age are susceptible to infection by *S. typhi*. The age group more susceptible to the present study was those between 21- 40 years old (However, the result is not significant p<0.05). This is in agreement with the studies conducted Wam *et al*., [18] and Ramyil *et al*., [21] who both found 21 – 30 and 24 – 29 years were more susceptible to typhoid fever respectively. According to Wam *et al*., [18], this could be due to improper sanitation and hygiene. Both children and adults can get typhoid fever through ingestion of contaminated food and water. Travelling to high-risk destinations presents a high risk of contracting typhoid fever [22].

With respect to the gender of the febrile patients, males has the highest prevalence of typhoid fever with 12 and 10 individuals (9.6% and 8%) while 10 and 7 individual positive samples were female accounted for 8% and 5.6% for widal test and stool microcopy respectively. This finding also agrees with that of Ramyil *et al*., [21] which showed that the males were more positive to Widal test and stool culture than females. However, the result contradicts those of Wam

et al., [18] and Gemechu *et al*., [19] who both found higher incidence of typhoid fever in female than male. High prevalence of typhoid fever among male in the study area is attributed to high involvement in outdoor activities by male than female in the study area, hence, they are more exposed to the infection.

The result of the present study demonstrated high sensitivity and specificity of Widal test as 78.9% and 93.6% respectively. This finding was in conformity with the finding of Gemechu *et al.*, [19] high sensitivity of Widal test (84.2%). This result contradicts that of Wam *et al.*, [18] who recorded low sensitivity and specificity of Widal test when compared to stool culture as 40.9% and 32.4% respectively. The positive predictive value (PPV) and negative predictive value (NPV) of Widal test were high according to the present study, that is, 68.2% and 98.1% respectively. This means that most of the proportion of patients with positive test results that are correctly diagnosed. On the other hand, the NPV value of Widal test was 98.1%. This indicates that a negative Widal test result has a good predictive value for the absence of the disease.

CONCLUSION

In the present study, there is low prevalence of typhoid fever among febrile patients in the study area. There is high prevalence of typhoid fever from Widal test (17.6%) when compared to stool culture (13.6%). According to the result, more male were infected than female. Based on the findings of this study, Widal test has high sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). Therefore, Widal test can be used as a diagnostic method for detection of *Salmonella typhi*. It is recommended that Widal test can still be used for the diagnosis of enteric fever

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