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Comparative Evaluation of the Effectiveness of Widal Test and Typhidot Rapid Test in the Diagnosis of Typhoid Fever in Akwa Ibom State University Medical Centre, Mkpat Enin,Nigeria.

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Abstract

Typhoid fever is a major health problem in developing countries. An accurate diagnosis based on clinical signs and symptoms alone is difficult. In some areas where there is high incidence, bacterial isolation facilities for definitive diagnosis are often not available, so Widal test and rapid test (TyphiDot Combo) are used as the diagnostic tools. However, the efficiency and accuracy of these tests for diagnosis of typhoid fever have been debatable. The aim was to study the specificity and sensitivity pattern of Widal test and TyphiDot Combo rapid test in the diagnosis of typhoid fever. A total of 372 blood samples were collected from students attending the Medical Centre for one illness or the other. Using Widal test kits and TyphiDot Combo Rapid identification method for detection of the presence of Salmonella antibodies, IgG and IgM.Out of the 372 Students screened 120 (32.4%) with a titre value of 1:160 and above were significant and 72 (19.4%) of the students were positive to Typhidot IgG/IgM Salmonella typhi with IgG 44 (61%) positive and 28 (39.9%) to IgM. Widal tube agglutination test shows a high sensitivity but low specificity to Salmonella antibodies. TyphiDot IgG/IgM Combo is a new and reliable serodiagnostic test with significantly higher sensitivity and specificity. TyphiDot IgG/IgM is a practical alternative to Widal test in the diagnosis of typhoid fever on account of its increased sensitivity, early detection of cases and ease of procedure with minimal infrastructure and availability of results on the same day.

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Introduction

Typhoid Fever is a systemic disease caused by S*almonella typhi* and is the major cause of morbidity and mortality worldwide. The World Health Organization (WHO) estimates about 20 million cases with greater than six hundred thousand (600,000) deaths. These cases are more in India, south and central America, and Africa where they constitute serious source of morbidity and mortality due to rapid population growth, increased urbanization, limited safe portable water, infrastructure and other public health problems (Tam *et al.*, 2008).

Typhoid fever is a major health problem in developing countries and its diagnosis on clinical ground is difficult. Diagnosis in developing countries such as Sudan, Kenya, and Nigeria is mostly done by Widal test. However, the value of the test has been debatable. Hence evaluating the result of the test is necessary for correct interpretation. Accurate diagnosis of typhoid fever at an early stage is important not only for diagnosis of etiological agent, but also to identify individuals that must serve as a potential carrier. In many countries the Widal test is the most widely used test in typhoid fever diagnosis because it is relatively cheaper, easy to perform and require minimal training and equipment (Tam *et al.*, 2008).

TyphiDot-Combo was used for rapid serological analysis; this is a dot enzyme immunoassay for the detection of specific IgM and IgG to *S. typhi*. This test makes use of the 50 kD antigen to detect specific IgM and IgG antibodies to *S. typhi*. TyphiDot is a medical test consisting of a dot ELISA kit that detects IgM and IgG antibodies against the outer membrane protein (OMP) of the *S. typhi*. The typhiDot test becomes positive within 2–3 days of infection and

separately identifies IgM and IgG antibodies. The test is based on the presence of specific IgM and IgG antibodies to a specific 50Kd OMP antigen, which is impregnated on nitrocellulose strips. IgM shows recent infection whereas IgG signifies remote infection. It has undergone full-scale multinational clinical evaluation of its diagnostic value. This dot EIA test offers simplicity, speed, specificity (75%), economy, early diagnosis, sensitivity (95%) and high negative and positive predictive values (Reszon, 2013).

The objectives of the study were to compare the diagnostic performance of widal test and Typhidot Rapid test, as well as to evaluate the specificity and sensitivity of the widal test to Typhidot test.

Materials and Methods

Study area and population

The study was conducted in Akwa Ibom State University Medical centre, Mkpat Enin Local Government Area, Akwa Ibom State. Blood samples were collected from students both male and female visiting the clinic for medical attention. A total of 372 blood samples were collected from the students, one hundred and seventy-two (172) samples of blood were from male students and two hundred (200) samples from female students.

Preliminary investigation

The School medical centre was visited to obtain official permission to conduct the study and to discuss the feasibility of the study and to seek the cooperation of the Clinicians, Medical Laboratory Scientists and other staff.

Inclusion and Exclusion criteria

Subjects that were captured in the study were students of Akwa Ibom State University presented with sign and symptoms of Typhoid fever, staff were not inclusive. The study was conducted between November 2016 and June 2017 (8months period).

Sampling methods

A total of 372 blood samples were collected from the students in the AKSU main campus, medical centre who presented with or without clinical symptoms of enteric fever. Aseptically, 5mls of venous blood were taken with sterile vacutainer for serological analysis; the samples were labelled with date and time of collections boldly. The samples were transported to the laboratory with minimum delay to avoid death of enteric pathogens (Cheesbrough, 2002; Tam *et al.*, 2008).

Serological analysis of blood samples using Widal test

The blood specimen was analysed using Widal test kits (standard tube test method) and Typhidot Combo Rapid identification method for detection of the presence of *Salmonella* antibodies, among the students (Reszon, 2013).

Procedures for Widal Identification test

Ten test tubes were placed in a rack; 4 ml of saline was added to tube 1 and 1ml to tube 2-10.

One ml of serum was added to tube 1 and gently mixed to dilute the serum 1 in 5. That is 1ml of serum plus 4ml of saline, giving 5ml volume. There after One ml of serum-saline solution was transferred to tube 2. This dilutes the serum 1 in 10. The procedures were repeated until tube 10. To obtain serum dilutions of 1 in 5, 1 in 10, 1 in 20, 1 in 40, 1 in 80, 1 in 160, 1 in 360, 1 in

640,1 in 1280, and 1 in 2560. Using a fresh pipette, and starting from the highest dilution, 0.5ml were transferred from each test-tube into a corresponding agglutination tube rack.

Point five ml of *Salmonella* antigen was added to each tube; the addition of an equal quantity of antigen dilutes the serum again, producing the final serum dilution being 1 in 10 in the first tube, 1 in 20 in the second tube, etc. To another agglutination tube, 0.5ml of saline and 0.5ml of antigen was added. This tube serves as a control to show if the antigen is self- agglutinable.

The agglutination racks were placed in the water-bath and water level adjusted until it covers one third of the tube. The tubes were examined for agglutination after two hours of incubation.

The Widal test was positive when TO antigen titre was more than or equal to 1:160 in an active infection, or when TH antigen titre was more than or equal to1:160 in past infection or in immunized persons (Perilla, 2003; Tam *et al.*, 2008)

Typhidot Rapid serological test.

Test cassettes and chase buffer were brought to room temperature (if precipitates are noted in the chase buffer reagent, shake the bottle vigorously and allow to warm up further). The test cassettes were gently removed from the pouch and label with the sample name. The following steps were taken: Thirty five micro litres (35 µl) of serum/ plasma was added to each sample well; making sure that there is no air bubbles. Serum/ plasma will start wicking up the membrane. The cassette may be tapped gently on the table to facilitate the sample to flow up the membrane, to observe that, the wet sample front of the serum/plasma reaches area marked "C". A drop of buffer was added to each sample well and the results were

read within 15-20 minutes. In the rare event that the sample front stops wicking up after a minute, an additional drop of buffer was added.

Quality control

Control experiments (positive and negative) were set-up to monitor the efficiency of the media, reagents and different biochemical and serological test performed.

Results

Table 1: Detection of *Salmonella* antibodies in widal tube agglutination test.

Groups	No. of Specimens	Titres						Total (%)
		1:20	1:40	1:80	**1:160**	**1:320**	**1:640**	
Female	200	49	44	42	**42**	**32**	**00**	74(19.4)
Male	172	50	36	31	**28**	**18**	**00**	46(12.4)
Total	372	99	80	73	**70**	**50**	00	**120 (32.3)**

Significant Titre: 1:160 and above. No; number of specimens

Table 2: Distribution of *Salmonella* Antibodies among students attendingAkwa Ibom State University medical Centre for one illness or the other,using Widal Tube agglutination method.

Groups	No. of samples	Widal test		Total positive	% positive
		1:160	1:320		
Female	200	42	32	74	19.9
Male	172	28	18	46	12.4
Total	**372**	**70**	**50**	**120**	**32.3**

Key: %- percent.

Table 3: Distribution of *Salmonella* Antibodies among students attending Akwa Ibom State University Medical Centre for one illness or the other, using TyphiDot combo diagnostic test

Groups	No. of sample	Typhidot test IgG	IgM	Total positive	% positive
Female	200	28	17	45	12.1
Male	172	16	11	27	07.3
Total	**372**	**44**	**28**	**72**	**19.4**

Key: %- percent.

Table 4: Comparative distribution of *Salmonella* Antibodies using Typhidot Combo and Widal diagnostic kits among Subjects attending Akwa Ibom State University Medical Centre.

Methods	No. of samples	Gender Male	Female	Total positive	% positive
Typhidot	372	27	45	72	19.4
Widal	372	46	74	120	32.3
Total	**372**	**73**	**119**	**192**	**51.7**

Key: %-percent.

Discussion

Typhoid fever continues to pose significant public health challenges both in developed and developing countries, though improvement in environmental sanitation has reduced the incidence of typhoid fever in developed nations. Typhoid fever remains endemic in most developing countries with large scale transmission through contaminated food and drinking water.

Widal test has been used for over a century in developing countries but its diagnostic utility has been limited due to low sensitivity, specificity and positive predictive value (Sherwal*et al.*, 2004). Decreased sensitivity is due to the long latent period after which the

test may become positive. Decreased specificity is due to prior infection, vaccination with TAB vaccine, cross reaction with other gram negative infections. In the present study, the prevalence rate of typhoid fever was 51.7% and Widal test was positive in 32.3% (120/372) while Typhidot Combo rapid test was 19.4% (72/372) of the patients. The finding of the present study indicates a low specificity for Widal test. Similar results have been reported in other studies from endemic areas, where there may be high levels of specific and cross reacting antibodies (Sherwal *et al.*, 2004).

TyphiDot test is based on detection of antibodies which appear in detectable titres as early as the second day of illness. It showed sensitivity of 20.8% and specificity of 45%. We do not believe that our data support the use of either the Widal or TyphiDot test as a substitute for cultures in typhoid fever. It must be emphasized that although cultures are associated with a lag period of at least 48 hr for preliminary confirmation of infection, with the recent emergence of drug resistance among *S. typhi*, they remain an essential investigation. In many circumstances, especially among partially treated cases presenting to health facilities, combining Widal and TyphiDot test may reduce the diagnostic difficulty in typhoid fever. The TyphiDot offers an additional advantage among serologic diagnostic tests for typhoid fever in that the test strips do not require an ELISA reader for evaluation. Also, only minimal operator training is required. Nevertheless, the higher cost of the test in comparison with the Widal test, as well as cold-storage requirements for test reagents, are additional impediments in using this test in developing countries.

In the present study we conclude that TyphiDot is a practical

alternative to Widal test in the diagnosis of Typhoid fever on account of its increased sensitivity, early detection of cases, and ease of procedure with minimal infrastructure and availability of results on the same day. In studies by Andualem*et al., 2014,* Widal test had relatively good NPV 98.9% but very low PPV (5.7%) positive predictive value is more important than other measures of clinical diagnostic methods.The semi-quantitative agglutination (TyphiDot test) had shorter turnaround time than the Widal tube test. However, the results of all the tests were available the same day the specimen was received in the laboratory (Crump *et al.*, 2011).

Similarly, human immunodeficiency virus (HIV) infection is highly prevalent in Africa, and Nigeria, the prevalence of HIV infection among participants in the study on febrile illness can influence the sensitivity of the test. It is possible that HIV-associated immune dysregulation affects the production of antibodies specific to *S. typhi* outer membrane proteins, present in both the TyphiDot and the older Widal tests. Findings on the Widal test and the newer typhoid rapid antibody tests (TyphiDot Combo) are similar to those from studies conducted in Asia and Egypt (Macdonald, 2008),none of the rapid tests performed nearly as well as blood culture for the diagnosis of typhoid fever. Some reports suggest that the TyphiDottest may be more useful in Asia (Jesudason *et al.*, 2000). On the other hand TyphiDot Combo is a new, inexpensive and reliable serodiagnostic test recently available commercially. It has been studied in many countries and they found significantly higher sensitivity and specificity. Keeping the described in mind we studied TyphiDot combo (IgM) and (IgG) test

for its usefulness in diagnosis of typhoid fever in Nigeria.In our study, maximum titre rise of 4 fold was taken as the level of significant. This findings correlate with Tupasi*et al.*, (2000) who stated that clinically four-fold rising titre is commonly demonstrated as significant for enteric fever. This may be due to effect of antimicrobial therapy which inhibits further antibody rise.In the current study, the TyphiDot Combo for IgM/IgG and Widal test was done on 372 subjects. Positive TyphiDot Combo and Widal results were read in comparison with the results of control sera. 72 (19.4%) were positive for TyphiDot combo. Our results are consistent with the finding of Bhutta and Mansurali (2001) in Pakistan who found 63 (15.8%) out of 400 typhoid fever cases and Sherwal*et al.*, (2004) in India they found 98 (21.8%) out of 450 cases were TyphiDot-M positive. Closely similar findings were also reported by Choo *et al.*, (2002) who found that 44 (11.3%) out of 390 cases were TyphiDot positive.

Conclusion

However, a larger prospective study would be required to fully evaluate the usefulness of this test in countries endemic to typhoid fever. Studies by Kiran *et al.*, 2015showed 92.5% false positive results of Widal titre which was associated with cross reaction of antibodies from serum of febrile patient other than typhoid fever. These imply that the diagnostic utility of Widal test has been limited due to low sensitivity, specificity and positive predictive value (sherwal *et al.*, 2004). In a study by Kiran *et al.*, 2015 Widal test was positive in 68% of the patients. The test therefore had sensitivity of 45% and specificity of 86%.Another study done in Kenya showed that widal testing done on acute phase serum of

patients suspected to have typhoid fever had limited diagnostic capability due to its low sensitivity in which among all typhoid cases only 26% had diagnostic titre (Omuse, *et al* 2010).

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