

Laboratory Diagnosis of Typhoid Fever using Widal and Blood culture Methods in Aba, Southeastern Nigeria

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Abstract The emergence of multidrug resistance strains of *Salmonella typhi* poses a big challenge to the eradication of typhoid fever. Typhoid fever shares similar transmission factors with malaria, and require careful and accurate laboratory diagnosis for effective treatments of patients. Increased request for Widal test as a quick diagnosis of typhoid fever has produced exaggerated results since typhoid fever and malaria often show mimicking symptoms even in laboratory diagnosis. The main objective of this study is to investigate the rate of infection of typhoid fever in Aba, southeastern Nigeria, using Widal test and blood culture methods. Widal and blood culture were carried out on all the samples. Out of 400 blood samples examined, comprising of 126 (31.5%) males and 274 (68.5%) females, 98 (24.5%) were tested positive for *Salmonella typhi* using the Widal test while the blood culture method only recorded 37 (9.3%). On age-related prevalence, the age bracket 31-40 years showed the highest prevalence rate for both methods with 23 (32.4%) for Widal test and 9 (12.7%) for blood culture method. Sex-related prevalence also showed that more males (34.9% and 11.1%) were infected with *Salmonella typhi* than females (19.7% and 8.4%) for Widal and blood culture methods respectively. The result of this study shows a significant mean difference (t-value = 2.95, p-value = 0.026) between Widal and Blood culture at 5% level of significance. The blood culture test was found to be more sensitive than the widal test. It is therefore recommended that the assumingly high incidence of the disease using Widal test will be greatly reduced if blood culture technique is routinely adopted as a baseline for the diagnosis of typhoid fever.

Keywords: laboratory diagnosis, typhoid fever, widal test, blood culture

Cite This Article: Ezeigbo O.R., Agomoh N. G., and Asuoha-Chuks N., "Laboratory Diagnosis of Typhoid Fever using Widal and Blood culture Methods in Aba, Southeastern Nigeria." *American Journal of Microbiological Research*, vol. 3, no. 6 (2015): 181-183. doi: 10.12691/ajmr-3-6-1.

1. Introduction

Typhoid fever remains widely recognized as a major public health problem in most developing countries like India, Africa, South and Central America with rapid population growth, increased urbanization and limited safe water, infrastructure and health system [1,2]. Typhoid fever, also known as enteric fever, is an acute systemic infection caused by *Salmonella*, a gram-negative bacterium [3]. *Salmonella* serotypes include *Salmonella typhi*, *Salmonella paratyphi A*, *Salmonella paratyphi B* and *Salmonella paratyphi C* [4,5]. Human beings are the only reservoir host for typhoid fever and the disease is transmitted by the ingestion of food or water contaminated with faeces of infected persons which contain the bacteria parasite [6]. There have been an estimated number of 16 million typhoid cases with an associated 600,000 related deaths worldwide each year [5]. Globally, estimated burden of typhoid fever in 2010 was 26.9 million episodes while in Africa, crude incidence of 362 cases per 100,000 persons per year were reported [7].

The Widal test was the mainstay of typhoid fever diagnosis for decades. It is used to measure agglutinating antibodies against H and O antigens of *Salmonella typhi*. However, the major drawback of the Widal test is its cross-reactivity with some other bacteria of same genus [8]

and its mimicking symptoms with malaria which often produces false positive results. The common detection of high antibody titre of these *Salmonella* serotype in malaria patients has made some people to believe that malaria always co-infect with typhoid [9,10]. The isolation of *Salmonella typhi* from bone marrow is the current gold standard method for confirming a case of typhoid fever; however, this method is extremely painful and requires equipment and trained laboratory personnel which may not be available in the primary healthcare facilities in the developing world [11]. Blood culture, a more practical but less sensitive alternative is often used. This has its own lapses as it takes 2-3 days opposed to the quick diagnosis using Widal. As a result, diagnosis may appear delayed or overlooked and patients without typhoid may receive unnecessary and inappropriate antimicrobial treatment [12]. This study was carried out to evaluate the sensitivity and specificity of blood culture method against the quick-diagnosing Widal test in the accurate diagnosis of typhoid fever in Aba, Southeastern Nigeria.

2. Materials and Methods

Study Area: The study was conducted in Aba, Southeastern Nigeria. Aba lies between latitude 5° 07' N

and longitude 7° 22' E and 205m (673ft) above sea level. Aba is a commercial town in Abia State. The people are predominantly traders, artisans, farmers and public/civil servants. Aba is a major settlement and commercial centre in a region that is surrounded by small towns and villages. Aba falls within the rainforest zone and is usually flooded during the rainy season. This encourages water borne diseases and other infections.

Research Ethics: Ethical review and clearance of the research protocol were obtained from the Ethical Review Committee of the Department of Biology/Microbiology, Abia State Polytechnic, Aba. Samples were collected from different hospitals located at Aba with the permission of the hospital authorities. The patients' consent was obtained prior to sampling by filling the Patient Consent Form. All subjects who accepted provided their bio-data. Patients who are already on antibiotics were excluded from this study.

Sample Collection: Samples were collected from patients directed to the laboratories for Widal tests by attending physicians from five major hospitals in Aba. The blood was collected aseptically by venepuncture technique. Five milliliters of blood collected from each person were tested for *Salmonella typhi* O and H antibodies and also cultured for *Salmonella typhi*.

Widal Test: 3-5 mL of blood sample was collected into a sterile test tube and centrifuged for 5 minutes to separate the serum from the blood. A drop of the serum (0.08mL) was pipette and dropped on a sterile slide in four different parts for *Salmonella typhi* O and another for *Salmonella paratyphi* H antigens. Antigens O and H were shaken and dropped into the serum accordingly. It was then mixed and rocked gently for 2 seconds. The results were recorded as thus: depending on the gravity of the agglutination, $1/20$ is negative while $1/80 - 1/360$ is positive.

2.1. Blood Culture

Two millimeters of blood sample was aseptically introduced into 18mL thioglycolate broth and incubated at 37°C for an initial period of 48 hours and sub-cultured on Salmonella-Shigella Agar (SSA). *Salmonella typhi* were identified on the basis of standard culture, microscopic and biochemical characteristics. Inoculated blood culture medium was discarded as negative if there is no growth after 7-10 days.

2.2. Morphological and Biochemical Tests

Morphological and Biochemical tests were carried out on blood culture isolates of each sample to confirm the

presence of *Salmonella*. The tests include gram reaction, motility test and biochemical test such as catalase, coagulase, lactose fermentation, glucose fermentation test, citrate utilization test etc. The procedures for the test are according to Cheesbrough [13].

3. Results

Table 1 shows the age-related prevalence of typhoid fever using the Widal test and blood culture. Out of 400 individuals sampled, 126(31.5%) males and 274(68.5%) females, 98(24.5%) and 37(9.3%) were positive for *Salmonella typhi* using Widal test and Blood culture respectively. Forty four males (34.4%) were infected using Widal test while 14(11.1%) were infected using blood culture. The rate of infection among the females were 54 (19.7%) using Widal test and 23(8.4%) using blood culture. The result shows a significant mean difference (t-value = 2.95, p-value = 0.026, df = 6) between Widal and Blood culture at 5% level of significance.

Table 1. Prevalence of Typhoid fever using Widal test and Blood culture

Sex	Number Examined	Number Positive using Widal Test (%)	Number Positive using Blood Culture (%)
Male	126	44(34.9)	14(11.1)
Female	274	54(19.7)	23(8.4)
Total	400	98(24.5)	37(9.3)

Table 2 shows the prevalence of typhoid fever in relation to age. The highest prevalence of 17(40.5%) was obtained from the ages above 50 years using Widal test and 9(12.7%) for age bracket 31-40 years using Blood culture. The least prevalence of 5(12.2%) was obtained from ages less than 10 years using Widal test and 2(3.3%) from age bracket 41-50 years using blood culture.

Table 2. Prevalence of Typhoid Fever in Relation to Age

Age Group (years)	Number Examined	Number Positive using Widal Test (%)	Number Positive using Blood Culture (%)
< 10	41	5(12.2)	3(7.3)
11-20	83	16(19.3)	7(8.4)
21-30	102	26(25.5)	11(10.8)
31-40	71	23(32.4)	9(12.7)
41-50	61	11(18.0)	2(3.3)
>50	42	17(40.5)	5(11.9)
Total	400	98(24.5)	37(9.3)

The morphological and biochemical tests confirmed the presence of *Salmonella*.

Table 3. The Morphological and Biochemical Characteristics of the Blood culture Isolates

Morph	GR	Catal	Coag	Citr	Oxid	Ind	Ure	Methyl	V.P.	Glu	Lac	Suc	Organism
Black colonies on SSA	Negative, rod	+	-	-	-	-	-	+	-	A/G	A/-	A/-	<i>Salmonella typhi</i>

4. Discussion

Typhoid fever is a major health problem in developing countries and its diagnosis in clinical ground is difficult. Culture diagnosis of *Salmonella typhi* has revealed the unreliability of Widal agglutination test, which is basically the diagnostic method used in many suspected cases of

typhoid fever in Nigeria. For accurate and reliable diagnosis of typhoid fever, the use of blood culture method should highly be taken into consideration. Blood culture is considered to be a gold standard in the laboratory diagnosis of typhoid fever [14]. The present study evaluated the results obtained using Widal test and blood culture method. Out of 400 samples analyzed, 24.5% showed positive with Widal test while 9.3% showed positive for blood culture. The results agree with

the findings of other authors like [8] who obtained 62.5% and 55.0% positive results for typhoid fever using Widal and blood culture methods respectively. Also, positive results obtained using Widal test gave 95% negative results with blood culture [15]. Such misleading results using Widal test may keep one away from the true diagnosis because of cross reaction of antigen from other infections with *Salmonella* antibody [16]. An erroneous interpretation of rapid diagnostic tests delays the treatment of actual infection and increases morbidity. Increased request for Widal test as a quick diagnosis of typhoid fever has produced exaggerated results [17], since typhoid fever and malaria often show mimicking symptoms even in laboratory diagnosis. It is therefore recommended that the assumingly high incidence of the disease using Widal test will be greatly reduced if blood culture technique is routinely adopted as a baseline for the diagnosis of typhoid fever.

5. Conclusion

A reliable diagnosis of typhoid fever is based on culture of blood, bone marrow and stool. The result obtained from this study has shown blood culture to be the best method of diagnosing early *Salmonella typhi* infections in the absence of other culture methods. Widal test is found to give false positive results and therefore health personnel should not totally depend on widal test alone for the diagnosis of enteric fever, but should use other diagnostic methods (like blood culture) to differentiate *Salmonella* infection from other infections.

Acknowledgement

The authors wish to acknowledge the assistance of the hospital authorities in the collection of samples.

Competing Interest

Authors have declared that no competing interests exist.

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