

## DETECTION AND DIAGNOSIS OF *SALMONELLA TYPHI* FROM STOOL AND BLOOD SAMPLES USING WIDAL, TUBEX<sup>tf</sup> AND POLYMERASE CHAIN REACTION

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### ABSTRACT

*There are currently no effective, quick, and sensitive techniques for identification of Salmonella serovar Typhi. Typhoid fever is difficult to diagnose clinically in highly endemic areas, since the symptoms are vague and similar with other febrile disorders such as malaria, and dengue fever. Considering the challenges involved with typhoid diagnosis by blood culture and serology, the PCR approach has lately been used, however it is not the gold standard for typhoid diagnosis. The aim of this study is to determine the best diagnostic method used for detection of typhoid fever using Widal test, Tubex<sup>tf</sup> and fecal culture for the detection of typhoid. Blood and stool samples were collected from febrile patient and were screened by Widal and Tubex<sup>tf</sup> tests, while stool samples were screened for Salmonella Typhi by culture and PCR for confirmation. The results of stool samples obtained after screening by culture, biochemical tests and confirmation by PCR did not confirm Salmonella Typhi bacteria. The 28 blood samples have a corresponding rate of 0% Tubex<sup>tf</sup> and Widal was 93.3%. Therefore, this study suggests that Tubex<sup>tf</sup> should be offered in typhoid-endemic areas and also recommends its use in the diagnosis of typhoid fever because the results obtained correlate with stool culture leading to PCR Confirmation. Accurate diagnosis before establishing a case of febrile typhoid fever is very important, similarly, sensitivity should dictate the best of antibiotics to be used for treatment.*

**Keywords:** Typhoid fever, Tubex<sup>tf</sup>, Widal, PCR and Drug resistance

### INTRODUCTION

*Salmonella enterica serovar Typhi* causes typhoid fever, commonly known as enteric fever. Humans are the only hosts and reservoirs of infection, and the disease is primarily linked to low socioeconomic status and poor hygiene. (Durga *et al.*, 2021) Typhoid fever is involved in 21.5 million infections and 200,000 deaths worldwide each year (Abdulaziz and Hisham 2014). Estimate

by from the World Health Organization (WHO), states there are up to 21 million cases of enteric fever and 161,000 fatalities each year (Douye and Elijah 2017). Since this estimate was from a small number of surveillance studies using the most recent diagnostic tools, the actual disease burden is unknown (Baker *et al.*, 2010). In Africa, 4.36 cases per 427 million people are reported, and tropical nations such as Nigeria are regularly

impacted, where they are a major source of illness and mortality (Douye and Elijah 2017). There has been no comprehensive epidemiological study of the origin and dissemination of *S. Typhi* (Douye *et al.*, 2017a). This febrile sickness is one of the most common, affecting both children and adult in their reproductive age (Douye *et al.*, 2017b). Most tropical and underdeveloped nations, including Nigeria has been designated as a typhoid fever endemic zone (Laura *et al.*, 2017). Despite recent advancements in water and sanitation, it is therefore regarded as one of the most significant infectious disease hazards to public health on a worldwide scale (MacFadden *et al.*, 2016). Global fear about typhoid is mirrored in perceptions of the disease as a prevalent and highly infectious among children and adults where outbreaks have reinforced this impression among the general public and health professionals. As a result, the Widal test is commonly used to screen children and adults with fever in the hospital, because not many facilities have the capability to carry out blood culture, the recognised gold standard diagnostic (Chart *et al.*, 2000).

The overall objective of the study was to establish the best applied diagnostic technique for the detection of *Salmonella Typhi* involving from the Widal test, Tubex<sup>tf</sup> and stool culture leading PCR.

## **MATERIALS AND METHOD**

### **Collection of Samples**

Twenty-eight (28) blood samples and 28 stool samples were collected from the Lagos University Teaching hospital at the Department of Medical Microbiology and Parasitology. In accordance with standard laboratory procedures, samples were obtained in plain bottles for blood samples and universal bottles for stools, then immediately

transported to Nigerian Institute of Medical Research for storage of stool samples in a refrigerator at 4°C until needed for testing and blood samples centrifuged.

### **Location of Studies**

This study was carried out in Nigerian Institute of Medical Research (NIMR) Yaba Lagos State in Molecular Biology and Biotechnology laboratory unit.

### **Stool Samples**

The stool samples were inoculated into sterile containers with selenite F broth according to manufacturer's instructions and incubated for about 18- 20 hours for isolate recovery before being inoculated into *Salmonella-Shigella* agar prepared according to manufacturer's instructions using the streak plate method and incubated for 24 hours to observe suspected growth. Suspicious colonies were sub-cultured on *Salmonella-Shigella* agar to get distinct colonies and incubated for about one hour.

### **Chromosomal DNA Extraction**

The DNA was extracted straight from the isolated bacteria from the stool samples by boiling Briefly, 1.5ml of organisms in broth were centrifuged for 5 minutes at 10,000rpm. The supernatant was discarded, and the pellets were thoroughly rinsed with sterile water twice. Following that, 200 µl of sterile water was added to the pellets, which were vortexed to homogenize before being heated in a dry bath at 100°C for 10 minutes. This was followed by 5 minutes of vortexing and centrifugation at 12,000rpm. The supernatant containing DNA was transferred to another tube and stored at -20°C. The concentration and purity of the extracted DNA were determined using a Nanodrop spectrophotometer.

## PCR amplification of the *fliC* Gene

The *fliC* gene codes for protein components of bacterial flagella that can cause typhoid, a human systemic infection. The primer sets *fliC* F (aag-gaa-aag-atc-atg-gca) and *fliC*-R (tta-acg-cag-taa-aga-gag) were used for PCR amplification (Gerda and Patrick., 1993). The 25ml reaction mixture contained 1U Taq DNA polymerase, 1.5mM Magnesium Chloride, 200M of each dNTP, 20pmol of each primer, and x1 PCR buffer (Fermentas). For amplification in an Eppendorf Mastercycler gradient, the following cycling parameters were employed. After a 5-minute denaturation at 94°C, 40 cycles of 1 minute at 94°C, 1 minute at 36°C, and 1 minute at 72°C were performed. This was followed by a 10-minute extension at 72°C.

## Blood Samples

The blood samples after separation by centrifuging at 2000rpm for 5mins, serum was introduced into plain bottles to avoid lysis of blood, before test was carried on.

## Widal

The Widal agglutination test, proposed by Widal more than a century ago for the diagnosis of typhoid disease (John and Salih., 2008), identifies serum antibodies to *S. Typhi*'s O=9,12 somatic, H=d flagella, and "Vi" capsular antigens. The interpretation of the Widal test is still complex, with several studies reporting differing cut-offs (Olopoenia *et al.*, 2000), and the test has lost some favor in recent years as antigenic determinants of both typhoid and non-typhoid *Salmonella* species have been identified. (John and Salih 2008). The standard laboratory procedures were followed using the Widal test kits containing the somatic (O) and flagella (H) polyvalent antiserum of *S. Typhi*, and *S. Paratyphi* (A, B, C) on a clean, sterile white background tile and

observed for agglutination and recorded after which the titration was done, a titre of 1:160 and 1:320 was considered, H and O antibodies were both considered when interpreting the Widal test results.

## Tubex<sup>®</sup>

These fast typhoid antibody tests are carried out in accordance with the manufacturer's instructions, which are included with the kit. Before starting, all reagents were brought to room temperature and interpretation of the results for current typhoid detection was performed by moving the reaction wells on the colour scale by comparing the color of each supernatant with the Tubex-TF colour scale, readings are taken and considered positive if the blue tint is compared to a 4-point scale indicating weak positivity and a score of 6 to 10 is positive.

## Susceptibility Testing

*In vitro* susceptibility testing for 30 biochemically determined isolates was performed. The susceptibility of pure cultured bacterial strains to different antibiotics was determined using the Kirby-Bauer disc diffusion technique and interpreted based on the guidelines of the Clinical Laboratory Standards (Wayne, 2001). The Antibiotics (Abtek Biological Ltd) discs containing the following antibiotics; Augmentin (30µg), Ofloxacin (5µg), Gentamicin (10µg), Nalidixic (30µg), Nitrofurantoin (200µg), Cotrimoxazole (25µg), Amoxillin (25µg), Tetracycline (25µg) was used for sensitivity. Mueller-Hinton (MH) agar plates was seeded with bacterial cells preadjusted to the 0.5 McFarland turbidity standard then antibiotics were carefully placed on agar and incubated at 37°C for 24h. Interpretation of strains as susceptible or resistant based on zone of inhibition according

to current NCCLS criteria is consistent with WHO requirements (Wayne, 2002).

## RESULT

30 blood samples collected from febrile patients were tested using the Widal and Tubex<sup>tf</sup> tests. of the 30 blood samples. Widal screening test shows positive for 28 (93.3%), while using the Tubex<sup>tf</sup> assay blood samples tested negative for all 30 (0%) because there

was no separation of coloured particles in solution.

Figure 1 below shows the 30 stool samples from febrile patient screened for *Salmonella Typhi* using stool culture and confirmation with PCR. Results indicated zero amplification, Indicating negative for *Salmonella Typhi* just like that of the Tubex<sup>tf</sup>

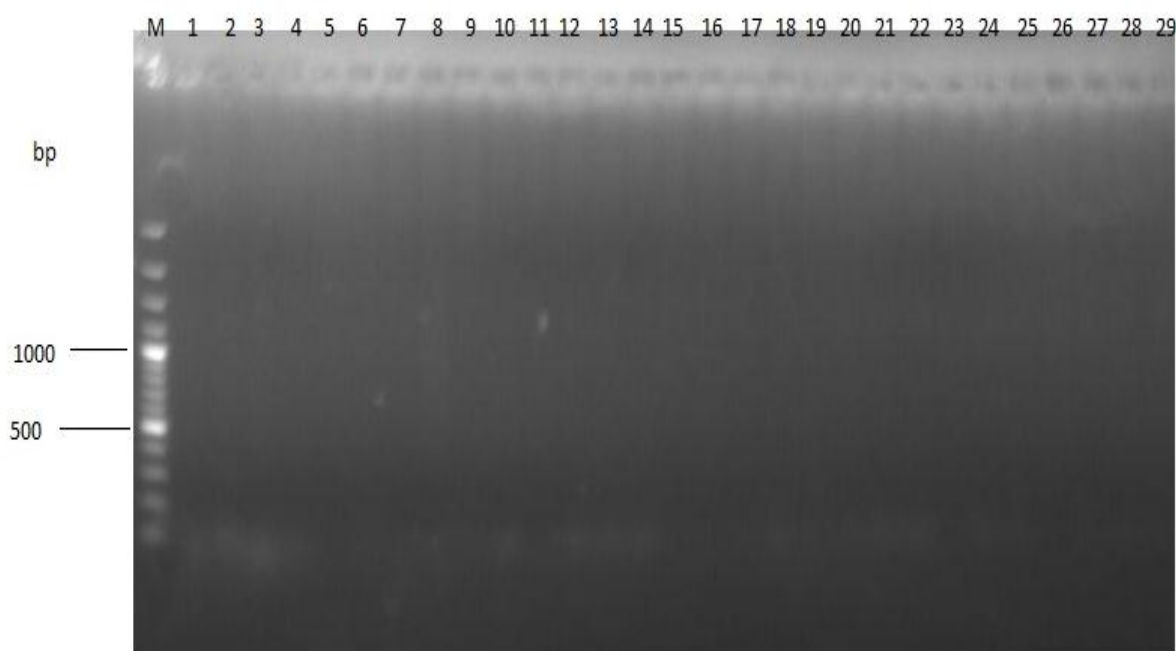


Figure 1: Amplification products by multiplex PCR of selected bacteria strains analysed by Agarose (1%) Gel Electrophoresis

KEY: bp- base pair, M- markers, n (1-29)- number of samples

Table 1; shows the percentage susceptibility and resistance of bacteria isolate confirmed at the level of biochemical test, the results of antibiotic resistance profile testing on *Salmonella* sp on following antibiotics includes Augmentin (30 µg) is 100%, Ofloxacin (5 µg) is 10.7%, Gentamicin (10 µg) is 32.1%, Nalidixic (30 µg) is 64.3%, Nitrofuratoin (200 µg) is 14.3%, Cotrimoxazole (25 µg) is 75%, Amoxillin (25 µg) is 100% and Tetracycline (25 µg) 92.9%.

**Table 1:** Percentage of Resistant and Susceptibility Pattern of *Salmonella* Isolates

ANTIBIOTICS	Susceptibility %	Resistance %
AUG	0	100
OFL	89.3	10.7
GEN	67.9	32.1
NAL	35.7	64.3
NIT	85.7	14.3

COT	25	75
AMX	0	100
TET	7.1	92.9

KEY: Aug- augmentin, OfI- Ofloxacin, Gen- Gentamicin, Nal- Nalidixic acid, Nit- nitrofurantoin, Cot-Cotrimoxazole, Amx- amoxicillin, Tet- tetracycline.

## DISCUSSION

According to the findings of this study, blood samples exhibited 93.3% positivity and 6.7% negativity the widal test however Tubex-tf have 0% detection for blood samples. The advantage of these test is that they are available same but the possibility of false positive agglutination is common among semiquantitative slide tests. The analysis by Olopoenia *et al.* (2000) and Adeleke *et al.* (2006) that claims the Widal agglutination test is plagued by disputes over the caliber of *Salmonella* antigens and how the results should be interpreted is also pertinent. It is important to emphasize that a single Widal agglutination test is not diagnostic. According to Okonko *et al.* (2010) as a result, serologic testing, blood cultures, and stool cultures from each patient are very relevant for a more precise diagnosis of typhoid fever. However, more must be done than is currently the case to confirm the diagnosis through paired sera investigation. Tubex<sup>tf</sup> is a different straight forward and quick test that was used in this study, despite the fact that it is not frequently used in Nigerian laboratories it takes advantage of the Widal agglutination tests in the easy and simplicity of use while enhancing resolution and sensitivity through the separation of coloured particles in solution by using an inhibition assay format and only detecting antibodies to one antigen in *S. Typhi*. Therefore, specificity is increased as compared to Widal (WHO, 2003). Although the Tubex-tf test cannot identify which group D *Salmonella* as the culprit, a positive result always indicates a *Salmonella* infection

(Khanna *et al.*, 2015). Given the problems associated with the diagnosis of typhoid fever by stool culture and serological methods, PCR methods was used for the amplification the flagellin gene (*fliC*) of *S. Typhi*. The result in this study was negative for *S. Typhi*, due to the sensitivity and specificity results had zero amplification. These studies reported excellent sensitivity and specificity when compared to positive serological cases. PCR has not become an established method for diagnosis typhoid fever However PCR tests is expensive and time consuming, its application in endemic regions limited. In a similar study performed in India, sensitivity of PCR-based diagnosis was 95% compared to the Widal test which has a sensitivity of only 63%. Abdulaziz and Hisham, 2014).

This study also observed a high rate of resistance of *Salmonella* isolates to antibiotics such as amoxicillin, co-trimoxazole, as well as resistance to nalidixic acid reduced susceptibility to fluoroquinolones that was observed, similar findings was observed in a study by Adeshina *et al.*, (2009). However, the increasing emergence of drug-resistant *Salmonella* species can be attributed to the use of quinolones in animal feed in this country and also to the use of animal manure to enrich the soil for raw fruits. Although ampicillin, chloramphenicol, gentamicin, ofloxacin, ceflaximide, polymixin B, carbencillin, and tetracycline were previously reported to be ineffective against *Salmonella* spp., ofloxacin had the highest susceptibility (93%), followed by nitrofurantoin (83.3%) and gentamicin (70%) in this study.

## CONCLUSION

Despite the relatively small sample size in this study, there appears to be a correlation between PCR and the tubex-tf typhoid rapid antibody tests. This study suggest more studies should be carried out with larger sample size comparing stool culture, blood culture and other rapid diagnostic test. Tubex<sup>tf</sup> appears to have comparable performance and is more specific, although more sensitive, than the semi-quantitative slide agglutination assay. The semi-quantitative slide agglutination assay and the Tubex<sup>tf</sup> assay have shorter turnaround times than stool cultures leading to PCR. As a result of this study, in regions where typhoid fever is an endemic disease, it is recommended that Tubex<sup>tf</sup> be made available. And that a proper diagnosis be made before confirming a case of typhoid fever. It is also advised that antibiotic sensitivity testing should direct the decision of medication to be administered.

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