Investigation on the incidence of Salmonella species was carried out among presumptive typhoid fever patients attending Specialist Hospital Sokoto. A total of 120 blood samples were collected from the patients. The samples were examined for typhoid agglutinins using Widal agglutination test and blood culture technique respectively. The result obtained showed that 72 (60%) samples out of the 120 (100%) are positive based on Widal agglutination test and only 6 (5%) samples were positive on blood culture. Male patients had a higher incidence with 38 (52.8%) cases compared to their females counterparts with 34 (47.2%) cases. Based on the Widal agglutination test, Salmonella typhi had the highest occurrence in 60 samples followed by Salmonella paratyphi A (19), Salmonella paratyphi B (15), Salmonella paratyphi C (14) respectively while based on culture technique, Salmonella typhi appear in all the 6 positive samples. T test analysis of the Widal agglutination test and blood culture technique in the diagnosis of typhoid fever showed that the null hypothesis should be rejected and concludes that significant differences exist between Widal agglutination test and blood culture technique. It can therefore be concluded that, the incidence of typhoid fever based on Widal test is higher when compared with blood culture technique in the diagnosis of typhoid fever.

Key words: Salmonella species; Specialist Hospital; Sokoto; Typhoid fever.

INTRODUCTION

Salmonella infections of humans and animals continue to be a major public health problem worldwide and also have a large negative economic impact on food production (Chiang, 2008). The main sources of Salmonellosis in humans are food, animals and their products such as raw eggs, poultry meat and pork (Chaunchom, 2003). The true incidence of Salmonella-associated diseases (SADs) in humans is difficult to evaluate because of lack of an epidemiological surveillance systems, especially in developing countries. The annual incidence of typhoid is estimated to be about 17 million cases worldwide, and is highest in those between the ages of 5 and 12 years (WHO, 2014).

Typhoid fever (enteric fever) caused by Salmonella typhi is an endemic disease in the tropic and sub-tropic and has become a major public health problem in developing countries (WHO, 2008). In Nigeria, typhoid fever is among the major widespread diseases affecting both young children and young adults as a result of many interrelated factors such as inadequate facilities for processing human wastes and indiscriminate use of antibiotics (Akinyemi et al., 2005).

Detection of typhoid bacteria depends on the collection of appropriate samples from patients in the laboratory. Viable organisms can be detected from blood, stool and urine cultures, depending on the period of exposure to the pathogens (Cheesbrough, 2006). Serological (widal) test to determine typhoid agglutinins is the commonly used diagnostic method due to its rapidness, affordability and is easy to perform (Ley et al., 2010). Widal test has

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been the only rapid diagnostic assay used in developing countries like Nigeria, despite the fact that its reliability is controversial (Cheesbrough, 2006). The test is based on macroscopically visible agglutination reaction between antibodies present in the serum and somatic (O) and flagella (H) antigens of the Salmonellae, available as coloured preparations (Ley et al., 2010).

Typhoid fever remains a threat to many people in sub Saharan Africa for several reasons which includes increasing poverty, poor public health services, increase in HIV/AIDS spread, increase resistance of the organism to a wide range of antimicrobial agents and lack of portable water (Alhassan et al., 2012). It is often encountered in tropical countries including Nigeria due to the poor standard of hygiene and unavailability of portable drinking water and this constitute serious sources of morbidities and mortalities. The Widal test has been the only rapid diagnostic assay used in developing countries like Nigeria, despite the fact that its reliability is controversial (Okonko et al., 2010). The quality of Salmonella antigens and interpretation of results, specifically in the Widal agglutination test have been identified as areas of controversy. Hence, the suitability of blood culture which is the gold standard for the diagnosis of typhoid fever alongside serologic test in diagnosing active infections (Okonko et al., 2010). This study was carried out in specialist Hospital Sokoto to determine the incidence of Salmonella Spp among presumptive typhoid fever patients and also to access the reliability of widal test and blood culture in the diagnosis of typhoid fever.

MATERIALS AND METHODS

Study Area

Sokoto state is located to the extreme Northwestern part of Nigeria between the longitudes 4° 8’E and 6° 54’E and latitudes of 12°N and 13° 58’N. It forms boundaries with the Niger Republic to the north, Kebbi state to the west and southwest and Zamfara to the east. The study was carried out in Specialist Hospital Sokoto, Sokoto State, Nigeria. It is located in Sokoto south and was established by the colonial masters since 1937. It is one of the major referral centers for a number of privately owned hospitals and local government’s primary health care within the state. Most of the patients seen at the hospital come from the city metropolis and surrounding districts. Hence, it was suitable to use the center as a study area.

Study Population

The study population consists of presumptive typhoid fever patients recommended by the physicians for Widal test.

Samples Collection

A total of 120 Blood samples were collected from 120 presumptive typhoid fever patients (60 males and 60 females) recommended for Widal test attending Specialist Hospital Sokoto, by venepuncture in. Out of the 5ml collected, 3ml was inoculated directly into culture bottle thioglycolate broth for blood culture and 2ml for Widal test. The samples were transported in accordance with the best practices of collecting, transporting and handling of clinical material (Cheesbrough, 2000).

Widal test

Sample Processing

A Blood sample collected for Widal test in a test tube were allowed to stand on rack at room temperature and was later centrifuge at 3000rpm for 5 minutes to separate the blood into serum and red cells.

Reagents

Reagents that were used in this test include
1. Antigen suspension (Omega diagnostics)
   a. O antigens: Salmonella typhi (TO), Salmonella paratyphi A (AO), Salmonella paratyphi B (BO), Salmonella paratyphi C (CO).
   b. H antigens: Salmonella typhi (TH), Salmonella paratyphi A (AH), Salmonella paratyphi B (BH), Salmonella paratyphi C (CH).
2. Physiological saline

Technique

Using a grounded pipette a drop of patients serum was placed eight times, one for each antigen (TO, AO, BO, CO and TH, AH, BH, CH) on a clean slide respectively. A drop of appropriate antigen to each serum was added. The serum and antigen suspension was mixed using a clean applicator. The slide was rock manually by hand for three minutes and examined the slide for agglutination (Ochei and Kolhatkar 2008). A positive Widal test was considered for any serum sample with antibody titre of 1 in 80 for both somatic O and flagella H antigen.

Isolation of Salmonella from Blood Sample

About 3ml of blood samples was inoculated into culture bottle containing about 27ml of thioglycolate broth and incubated for 24 hours at 37°C. At the end of the
Table 1: Incidence of widal positive and negative sera in relation to sex.

<table>
<thead>
<tr>
<th></th>
<th>No. of sera tested (%)</th>
<th>No. of widal positive</th>
<th>No. of widal negative (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>60 (50%)</td>
<td>38 (52.8%)</td>
<td>22 (45.8%)</td>
</tr>
<tr>
<td>Female</td>
<td>60 (50%)</td>
<td>34 (47.2%)</td>
<td>26 (54.2%)</td>
</tr>
<tr>
<td>Total</td>
<td>120 (100%)</td>
<td>72 (60%)</td>
<td>48 (40%)</td>
</tr>
</tbody>
</table>

Figure 1: Incidence of widal positive and negative sera in relation to sex

incubation period, sterile wire loop was used to pick from thioglycolate broth and streak into Salmonella-Shigella agar. Inoculated blood in a culture bottle were discarded as negative if no growth was observed after 7 days. The Salmonella-Shigella agar plates were incubated overnight at 37ºC and were examined after 24 hours for growth. Suspected colonies of Salmonella were subculture on a fresh Salmonella Shigella agar to obtain a pure culture. The pure culture was stored in the slant bottles for storage and further experiment (Bayeh et al., 2010).

Identification of the isolates
The isolates were identified using Gram staining, biochemical and serological test.

Method of Data Analysis
Widal agglutination test and blood culture technique results were subjected to T test (paired) analysis.

Results
Table 1 shows the results of laboratory diagnosis of typhoid fever using Widal agglutination test. Typhoid fever was found in 72 (60%) of the patients, out of which 38 (52.8%) were males and 34 (47.2%) were females. Typhoid fever is absence in 48 (40%) of the patients, out of which 22 (45.8%) were male and 26 (54.2%) were females.

Table 2 present the occurrence of Salmonella somatic (O) and flagella (H) antigen from widal test result. Somatic (O) antigen Salmonella typhi has the highest occurrence of 81 (67.5%) followed by Salmonella paratyphi B 45 (37.5%), Salmonella paratyphi A 39 (32.5%) and Salmonella paratyphi C 30 (25%) respectively. Salmonella typhi also have highest occurrence of flagella (H) antigen with 69 (57.5%) followed by Salmonella paratyphi A 33 (27.5%), Salmonella paratyphi C 29 (24.2%) and Salmonella paratyphi B 24 (20%) respectively.
Table 2: Occurrence of Salmonella agglutinin titres in 120 presumptive typhoid fever patients.

<table>
<thead>
<tr>
<th>Salmonellae</th>
<th>No. of sera tested</th>
<th>No. of widal + (%)</th>
<th>No. of widal - (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. typhi O</td>
<td>120</td>
<td>81 (67.5%)</td>
<td>39 (32.5%)</td>
</tr>
<tr>
<td>S. paratyphi A-O</td>
<td>120</td>
<td>81 (67.5%)</td>
<td>39 (32.5%)</td>
</tr>
<tr>
<td>S. paratyphi B-O</td>
<td>120</td>
<td>45 (37.5%)</td>
<td>75 (62.5%)</td>
</tr>
<tr>
<td>S. paratyphi C-O</td>
<td>120</td>
<td>30 (25%)</td>
<td>90 (75%)</td>
</tr>
<tr>
<td>S. typhi H</td>
<td>120</td>
<td>69 (57.5%)</td>
<td>51 (42.5%)</td>
</tr>
<tr>
<td>S. paratyphi A-H</td>
<td>120</td>
<td>33 (27.5%)</td>
<td>87 (72.5%)</td>
</tr>
<tr>
<td>S. paratyphi B-H</td>
<td>120</td>
<td>24 (20%)</td>
<td>96 (96%)</td>
</tr>
<tr>
<td>S. paratyphi B-O</td>
<td>120</td>
<td>29 (24.2%)</td>
<td>91 (76.8%)</td>
</tr>
</tbody>
</table>

Table 3: Occurrence of Salmonella species based on widal agglutination test.

<table>
<thead>
<tr>
<th>Salmonellae</th>
<th>No. of sera tested</th>
<th>No. of widal + (%)</th>
<th>No. of widal - (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. typhi</td>
<td>120</td>
<td>60 (50%)</td>
<td>60 (50%)</td>
</tr>
<tr>
<td>S. paratyphi A</td>
<td>120</td>
<td>19 (15.8%)</td>
<td>101 (84.2%)</td>
</tr>
<tr>
<td>S. paratyphi B</td>
<td>120</td>
<td>15 (12.5%)</td>
<td>105 (87.5%)</td>
</tr>
<tr>
<td>S. paratyphi C</td>
<td>120</td>
<td>14 (11.7%)</td>
<td>106 (88.3%)</td>
</tr>
</tbody>
</table>

Figure 2: Occurrence of Salmonella species based on widal agglutination test.

Table 4: The isolation frequency of Salmonella species from blood sample.

<table>
<thead>
<tr>
<th>Sex</th>
<th>No. of blood tested (%)</th>
<th>No. of culture positive</th>
<th>No. of culture negative (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>60 (50%)</td>
<td>4 (3.3%)</td>
<td>56 (96.7%)</td>
</tr>
<tr>
<td>Female</td>
<td>60 (50%)</td>
<td>2 (1.7%)</td>
<td>58 (98.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>120 (100%)</td>
<td>6 (5%)</td>
<td>104 (95%)</td>
</tr>
</tbody>
</table>

Table 3 shows the occurrence of Salmonella species based on widal agglutination test. Salmonella typhi have the highest occurrence affecting 60 (50%) of the patients followed by Salmonella paratyphi A 19 (15.8%), Salmonella paratyphi B 15 (12.5%) and Salmonella paratyphi C 14 (11.7%) respectively.

Table 4 present the isolation frequency of Salmonella species from blood sample. Out of 120 samples tested only 6 (5%) were positive in which 4 (3.3%) were males and 2 (1.7%) were females while the remaining 114 (95%)
are negative.

**DISCUSSION**

Typhoid fever also called enteric fever is an endemic disease in the tropic and sub tropical countries. This study reveals high incidence of (60%) seropositive cases of typhoid fever based on widal agglutination test (table 1). Male patients have a higher occurrence (52.8%) compared to the females patients (47.2%). This is probably due to the reflection of different eating habits and level of personal hygiene. In addition males happened to be more infected because they tend to be involved in activities like drinking water from any source, eating outside, and contact with carries as a result of shaking and animals or animals product which exposed them to the infection. This study agrees with the finding of Isah et al. (2013) and Okonko et al. (2010).

Diagnosis of typhoid fever based on culture method has proven the unreliability of widal agglutination test which is mostly used as a diagnostic tool for typhoid fever in Sokoto, most part of Nigeria and most developing countries. Definitive diagnosis of typhoid fever defends on the isolation of Salmonella typhi from blood, bone marrow, stool, and other body fluids (Okonko et al., 2010). In this study out 120 blood samples collected 72 were positive for typhoid fever based on widal agglutination test and only 6 were positive based on blood culture method. The unreliability and uncertainty of the widal agglutination test has been reported in many literatures. The Widal test is plagued with many controversies involving the quality of the antigen used and interpretation of the result particularly in the Widal test has been identified as areas of controversy. Hence, the suitability of blood culture alongside serological test in diagnosing active infection (Adeleke et al., 2006).

Similarly Okonko et al. (2010) reveals that the reliability of widal test in solely diagnosing typhoid fever has suffered doubt, it has been reported to remain positive month after effective therapy of the infection such that a positive may not necessarily indicate active infection, making the test relevance in diagnosing post infection complications. He concluded that diagnosis of typhoid fever based on serology (widal agglutination test) alone is frequently inaccurate. Ibekwe et al. (2008) also suggest that only bacteriological isolation of the enteric bacteria from the patient's blood, bone marrow, stool and urine constitute unequivocal infection.

According to the world health organization (WHO), 2003 Widal test can lead to false positive result because Salmonella typhi shares O and H antigen with other Salmonella serotype and has cross reacting epitopes with other entero bacteriaceae. In addition such result can occur in other clinical condition such as malaria, typhus, bacteremia caused by other organisms and cirrhosis. Blood culture which is the gold standard for diagnosis of typhoid fever is not routinely requested by most physicians may due to the limitation of the laboratory media, volume of the blood cultured, presence of antibiotics and final result can only be obtained at the earliest of three days after specimen collection (WHO, 2003).

**CONCLUSION**

The incidence of typhoid fever in this study was observed to be high (60.8%) using widal agglutination test compared to blood cultural isolation with (5%). The prevalence of typhoid fever and resistant of Salmonella typhi to clinically useful antibiotics would be drastically reduced if diagnosis of typhoid fever will be solely based on blood culture method.

**REFERENCES**


