Prevalence of Typhoid Fever among Patients Attending General Sani Abatcha Specialist Hospital Damaturu, Nigeria

Abstract: This research reports on the prevalence of typhoid fever among patients attending General Sani Abatcha Specialist Hospital Damaturu, Nigeria. Typhoid fever caused by Salmonella is an endemic disease in the tropics and sub-tropics and has become a major public health problem in developing countries of the world with an estimated annual incidence of 540 per 100,000. The annual incidence of typhoid is estimated to be about 17 million cases worldwide. It is often in tropical countries including Nigeria where they constitute serious sources of morbidity and mortalities. Blood samples were collected from 150 patients for appropriate bacteriological analysis which includes, Serological test (WIDAL), Blood culture on XLD agar, and Biochemical tests such as: oxidase, catalase, TSI and SIM: 122(81.33%) were found to be infected and 28(18.67%) were not infected i.e. negative. The overall number of males examined are 77 and 64(42.67%) were found to be positive. And for the females 73 were examined with 58(36.67%) infected. The prevalence is higher in males and among age group 26-30, where 11 examined and 8 (72.73%) infected. The prevalence of typhoid fever is considerable and most are due to four serotypes. The results imply the need for policy to promote public hygiene and regularly saved individuals in contact with food items for public consumption.

Keywords: typhoid fever, (WIDAL), oxidase, Nigeria.

INTRODUCTION

Typhoid fever is an ill life threatening sickness caused by salmonella typhi. The sickness is usually of acute onset with symptoms of septicaemia, yet one, which last for several weeks and many present with the problem of prolonged pyrexia of obscure origin. Salmonella typhi, which is peculiar to humans (men), this organism, is passes directly through contamination of water or food (pelczar et al.,2002).

Typhoid share common symptoms with malaria such as fever, headache, and weakness, in most cases, non-responds to malaria treatment lead to suspicion of typhoid infection. The general accurate procedure is to conduct widal test in order to confirm certain extend of typhoid infection. Typhoid fever is an endemic disease in the tropic and has become a major public health problem in developing countries of the world with an estimated annual incidence of 540 per 100,000. The annual incidence of typhoid is estimated to be about 17 million cases worldwide (World Health Organization WHO, 2008). It is often encountered in tropical countries including Nigeria where they constitute serious source of morbidities and mortalities (Ibekwe, 2008). Typhoid and paratyphoid fevers are infections caused by bacteria, which are transmitted from faeces to ingestion. Thus, clean water, hygiene and good sanitation prevent the spread of typhoid and paratyphoid. Contaminated water is of the pathways of transmission of the disease (World Health Organization WHO, 2008). Salmonella Typhi is gram-negative bacteria, which are motile, though non-flagellate variants occur. Capsules are not formed. They are intestinal pathogens, which cause enteric fever known as typhoid fever (Philip, 2006). It is pathogenic to both man and mammals with associates inflammatory reaction in the intestinal tract.

Typhoid fever is among the water-borne infections characteristics of environments with poor sanitation and hygiene. People become infected after eating or drinking beverages that have been handled by a person who is infected or drinking water that is contaminated by sewage containing bacteria. Once the bacteria enter the person’s body they multiply and spread from the intestine into the blood stream (World Health Organization WHO, 2008). Even after recovery from typhoid or paratyphoid, a small number of individuals (called carriers) continue to carry the bacteria. These people can be the source of infection for others. The transmission of typhoid in less-industrialized countries may be due to contamination of food or water. In some countries shell fish taken from sewage-contaminated beds is an important route of infection. The transmission is more likely to occur via food contamination by carriers handling food (World Health Organization WHO, 2008).
Typhoid fever is a global infectious disease with prevalence in Africa, South America and greatest risk in Indian sub-continent (Bhutta et al., 1996). The annual incidence is estimated to about 17 million cases worldwide (World Health Organization WHO, 2008). In Africa, it has an estimated crude incidence of 362 cases per 100,000 per person per year.

Typhoid fever begins 7-14 days after ingestion of the organism (salmonella). The fever pattern is stepwise, characterized by a rising temperature over the course of each day that drops by subsequent morning. The peaks and troughs rise progressively over time. Over the course of the first week of illness, the notorious gastrointestinal manifestation of the disease develops. These include diffuse abdominal pain and tenderness and, in some cases, fierce colicky right upper quadrant pain. Monocytic infiltration inflames peyer patches and narrows the bowel lumen, causing constipation that lasts the dry coughs, dull frontal headache, delirium, and an increasingly stuporous malaise (Bhutta et al., 1996).

At approximately the end of the first week of illness, the fever plateaus at 103-104 degree Fahrenheit (39-40 degree Celsius). The patient develops rose spots, which are salmon-colored, blanching, truncal, maculopapules usually 1-4cm wide and fewer than five (5) in numbers, these generally resolve within 2-5 days. These are bacterial emboli to the demis and occasionally develop in persons with shigellosis or non-typhoidal salmoniasis (Ibekwe, 2008). During the second week of the illness signs and symptoms above may progress. The abdomen becomes distended, and sub-splenomegaly (enlarge spleen) is common. Relative bradycardia and dicrotic pulse (double beat, the second beat weaker than the first) may developed.

In third week, the still febrile individually grows more toxic and anerobic with significant weight loss. The conjunctivae are infected, and the patient is tachypneic with a thread pulse and crackles over the lungs bases. Abdominal distention is severe. Some patients experience foul, green-yellow, liquid diarrhea (pea soup diarrhea). The individual may descend into the typhoid state, which characterized by apathy, confusion, and even psychosis. Necrotic peyer patches may cause perforation and peritonitis. This complication is often unheralded and may be masked by coticosteroids. At this point, overwhelming toxemia, mycarditis, or intestinal hemorrhage may cause death.

If the individual survives to fourth week, the fever mental state, and abdominal distention slowly improve over a few days. Intestinal and neurologic complications may still occur in surviving untreated individuals. Weight loss and debilitating weakness lasts for months. Some survivors become asymptomatic S. typhi carriers and have the potential to transmit bacteria indefinitely (Ibekwe, 2008).

To the best of my knowledge, there is limited public health surveillance data on prevalence of Typhoid among patients in General hospital Damaturu, Nigeria. This research is the first work to be conducted in the study area in order to phenotypically characterize and determine prevalence of Salmonella Typhi from patient’s Blood attending General Hospital Damaturu using standard bacteriological methods. This study examined the prevalence of typhoid fever among patients attending General Sani Abacha Specialist Hospital Damaturu. This paper therefore serves as a catalyst for the need for policy to promote public hygiene and regularly saved individuals in contact with food items for public consumption.

**METHODOLOGY**

**Study area**
The study was carried out among patients attending General Sani Abacha Specialist Hospital Damaturu. The hospital was formerly comprehensive health care centre, which later declared as General hospital in 27th, August, 1991, by former head of state General Ibrahim Badamasi Babangida. It present name is General Sani Abacha specialist hospital Damaturu. The hospital is located along Gujba road opposite college of nursing. It serves as referral centre in the state, and comprises about 7 wards which include; paediatric ward, female surgical, male surgical and male medical. With 12 departments which include; medical record, dental, dietary unit, medical laboratory department, general out patients department, ante-natal and pharmacy.

**Sample Size Determination**
The sample size is determined by using thnush field sample size calculation formulae
\[ Z = \frac{N}{(1 + Ne^2)} \]
Where,
\[ N = \text{population size}= unknown= 4000 \]
\[ e = 0.05 \text{ at confidence level of 95\%} \]
Thus, \( N=1000(1+3500\times0.0025)=99.97 \)
Approximately \( =100 \) (Thrushfield 1997)
Therefore,a number of one hundred and fifty samples were collected from patients attending General hospital Damaturu, Yobe state to increase precision of the study.

**Experimental Design**
A total of 150 blood samples were collected in three batches from patients attending medical laboratory to diagnosis, and transferred into EDTA and plain containers each. The blood samples were placed in EDTA container for culture and in plain container to obtain serum, arranged in Ice Park cooler and transported immediately to Biology Laboratory, Yobe State University Damaturu, for analysis. The samples were collected in a period of 12 weeks from July to October, 2019.
Sampling

Convenient sampling of 150 patients was collected in three batches from patients attending medical laboratory for diagnosis. Each patient, before taking the samples, was interviewed orally to obtain information on sex and age of the patient, and was told the type of research that will be done.

Collection of blood sample

Three milliliters (3ml) of the blood sample were collected from each patient using vein puncture and transferred into plain container and EDTA container each. Those in plain Vacutainer tubes were centrifuged at high speed (at 3000rpm) for 5 minutes in order to separate the serum from blood cells, whereas those in EDTA containers used for blood culture. The serum formed was separated from packed cell into clean container in each Pasteur pipette. The samples were processed immediately.

Laboratory Culture and Identification

The laboratory identification in this study involves: enrichment, selective plating, preliminary identification and complete biochemical identification.

Media Preparation

Standard media were prepared base on the manufacturer instruction. The specified media used for this study is: Oxoid™ Media, (Xylose Lysin Deoxycholate agar, Rappaport Vassiliadis and Nutrient Broth) manufactured by Thermo-Fisher Scientific, Waltham, Massachusetts, USA.

Enrichment medium

Blood samples (from EDTA containers) were analyzed by using semisolid modified Rappaport Vassiliadis medium as the selective enrichment medium, where the presumptive *Salmonella* isolates from pre-enrichment (buffered peptone water) transport medium were inoculated unto test tubes containing prepared Rappaport vassiliadis medium (Duerden et al., 1998).

Isolation of *Salmonella enterica* (selective plating)

The sample from enrichment medium were streaked into xylene lysine deoxycholate agar medium (XLD selective solid medium) and incubated at 37°C for 24h. The *Salmonella* isolates colonies, appears red with dark centers on XLD medium (Duerden et al., 1998).

Preliminary Identification

The preliminary identification will involve Gram staining, Oxidase test, and Catalase test.

Gram staining

Gram staining method is most frequently used in diagnostic bacteriology. Clean slides with heat fixed smears were placed on a staining tray, the smears were flooded with crystal violet gently and let stand for 1 minute, the slides were tilted slightly and gently rinsed with tap water or distilled water using a wash bottle, the smears were flooded with lugals/Gram's iodine and let stand for 1 minute, the slide were tilted slightly and gently rinsed with tap water or distilled water using a wash bottle. The smears were appeared as purple circle on the slide. Decolorized using 95% ethyl alcohol. The slides were tilted and applied alcohol drop by drop for 5 to 10 seconds until the alcohol runs almost clear. Careful not to over-decolorize, the slides were rinsed immediately and flooded gently with safranin to counterstain and let stand for 45 seconds. The slide were tilted slightly and gently rinsed with tap water using a wash bottle and blot dry slide on blot paper. Finally the smears were viewed using a microscope under oil immersion at 100x Magnification (Duerden et al., 1998). *Salmonella* organisms are gram negative. Thus, they appeared pinkish red (Duerden et al., 1998).

Catalase production test

The enzyme catalase mediates the breakdown of hydrogen peroxide into oxygen and water. This principle is used for detection of catalase enzyme in a bacterial isolate. A loopful of 10% hydrogen peroxide was putted on colonies of the test organism on nutrient agar. Alternatively, a few colonies of the organism were picked up with platium loop from nutrient agar plate and dipped in a drop of 10% hydrogen peroxide on a clean slide. The production gas bubbles from the culture, indicates a positive reaction. A false positive result may be obtained if the growth is picked up from the medium containing catalase e.g blood agar or if an iron wire loop is used (Duerden et al., 1998).

Oxidase test

This test depends on the presence, in bacteria, of certain oxidases that catalyze the oxidation of reduced tetramethyl-p-phenylene-diamine dihydrochloride (oxidase reagent) by molecular oxygen. A drop of freshly prepared 1% solution of oxidase reagent was putted on a piece of filter paper. The a few colonies of the test organism were rubbed on it. Oxidase positive isolates, produced a deep purple colour within 10 seconds. Alternatively, oxidase reagent will be poured over the colonies of the test organism on culture plate. The colonies of oxidase positive rapidly develop a deep purple colour (Duerden et al., 1998).

Complete Biochemical Characterization: Various biochemical identification tests used for this study for pathogenic identification and confirmation of *Salmonella enterica* isolates includes: urease test, citrate test, TSI test, SIM test, aesculin hydrolysis, indole test, Methyl red test, and Voges-Proskauer.

Urease test is performed to check the capability of microbes to produce urease. The urea agar slant consists of urea and pH indicator phenol red which causes changing the medium color to yellow in an acidic
environment and fuchsia in an alkaline environment. If urease is produced, it will hydrolyze urea to ammonia and carbon dioxide which creates alkaline environment. During the test, the straight wire containing pure culture was streaked over the surface of urea agar slant. The tubes were further kept in the incubator overnight at 37°C (Magee et al., 1993).

Citrate test: is carried out in labs in order to check the ability of microbes to utilize citrate as a sole source of carbon and energy. Citrate agar medium contains a pH indicator called bromothymol blue, which is green at normal pH, yellow at acidic pH and blue at basic pH. If citrate is utilized by the microbes, alkaline by-products will be formed which changes the medium colour from green to blue. Pure culture was taken using sterile straight wire and streaked over the surface of citrate agar slant. The tubes were further kept in the incubator overnight at 37°C (Magee et al., 1993).

Triplate sugar iron (TSI) agar is used to test the ability of microbes in sugar fermentation and hydrogen sulfide production. TSI agar consists of glucose, sucrose, lactose, pH indicator phenol red and ferrous sulfate. The sugar fermentation products results in acidic environment turns both the butt and slant yellow. If hydrogen sulfide is produced, it will react with the iron in the agar to form ferrous sulfide, were observed as a black precipitate in the butt (Magee et al., 1993). TSI agar was kept in the butt and the slant form in a test tube. The bacterial cultures from the colonies formed in agar medium were taken using a sterile straight wire. Then the needle containing cultures were stabbed into the butt of the TSI agar tube and streaked the needle back and forth along the surface of the slant. The tubes were further kept in the incubator overnight at 37°C (Magee et al., 1993).

Methyl Red (MR) Test this test detects the production of sufficient acid by fermentation of glucose so that the pH of the medium falls and it’s maintained below 4.5. The isolates were inoculated in glucose phosphate broth and incubated at 37°C for 2-5 days. Then five drops of 0.004% solution of methyl red were added, mixed well and the result was read immediately. Positive tests are bright red (indicating lo pH) and the negative are yellow. If the test is negative after 2 days the tests were repeated after 5 days (Duerden et al., 1998).

Indole production certain bacteria which possess enzyme tryptophanase, degrade amino acid tryptophan to indole, pyruvic acid and ammonia. Indole production was detected by inoculating the isolates into peptone water and incubating it at 37°C for 48-96 hours. Then 0.5 ml of Kovac’s reagent was added gently. A red colour in the alcohol layer indicates a positive reaction (Duerden et al., 1998). Kovac’s reagent consist of; paradimethylaminobenzaldehyde 10g, Amyl or isoamyl alcohol 150ml, and Concentrated hydrochloric acid 50ml (Duerden et al., 1998).

Voges-Proskauer (VP) test for acetoin production many bacteria ferment carbohydrates with the production of acetyl methyl carbinol (acetoin). In the presence of potassium hydroxide and atmospheric oxygen, acetoin is converted to diacetyl, and α-naphthol serves as a catalyst to form a pink complex. This test is usually done in conjugation with the methyl red test. An organism of the family Entrobacteriaceae is usually either methyl red positive and Voges-Proskauer negative e.g E. coli or methyl red negative and Voges-Proskauer positive Salmonella.

Salmonella isolates were inoculated in glucose phosphate broth and incubate at 37°C for 48 hours. Then 1ml of potassium hydroxide and solution of α-naphthol was added in absolute alcohol. A positive reaction is indicated by the development of pink colour in 2-5 minutes and crimson in 30 minutes (Duerden et al., 1998).

Slide agglutination test (WIDAL)
One drop of positive control was placed on one reaction circles of the slide; another drop of isotonic saline was Pipette on the next reaction circle. (ve Control). Then, one drop of the patient serum was Pipette onto the remaining four reaction circles, one drop of Widal TEST antigen suspension ‘H’ was added to the first two reaction circles. Finally, one drop each of ‘O’, ‘H’, ‘AH’ and ‘BH’ antigens were added to the remaining four reaction circles. More so, the contents of each circle were mixed uniformly over the entire circle with separate mixing sticks and the slides were rocked, gently back and forth and observed for agglutination macroscopically within one minute (Krista 2018).

Interpretation of Widal Test-Slide Method
Agglutination is a positive test result and if the positive reaction is observed with 20 ul of test sample, it indicates presence of clinically significant levels of the corresponding antibody in the patient serum. No agglutination is a negative test result and indicates absence of clinically significant levels of the corresponding antibody in the patient serum.

RESULT
The prevalence of typhoid fever among patients attending General Sani Abacha Specialist Hospital Damaturu is presented in tables 1-5.
Table1 shows the overall number of patients examined during the study, 130 people were examined out of which 122 were found to be infected, i.e. about 81.33% of typhoid fever, while 28 people about 18.67% were not infected i.e. are negative.
Table2 shows the prevalence of typhoid in relation to sex (gender), a total of 77 males were examined and 64 were found to be infected, about 83.12%. Whereas, 73 females were examined and out of which 58 were found to be infected with typhoid, i.e. about 79.45%.
Table 3 shows age group and frequency of infection. The highest infection rate observed in age 26-30, where 20 were found to be infected out of 24 individuals with percentage of 83.33%. This was followed by age group >40 which shows removable infection, 18 were examined and all of them were infected with percentage of 100% infection. Then followed by age group 6-10 and 16-20, 18 were examined and 15 each were found to be infected with percentage of 83.33%, again followed by the age group 21-25 with 13 examined and 9 infected with 69.23%. The lowest prevalence is found in age group 36-40, 10 examined and 6 were infected with 60%.

Table 4 show the prevalence of typhoid among male that were examine during the study. The age group 6-10 and >40 has the highest prevalence or infection with 10 examined and all were infected with 100% infection. Followed by age group 11-15 and 26-30, 9 were examined 8 get infection with 88.89% while 26-30, 11 examined, 8 infected with 72.73%. Then followed by age group 21-25 with 13 examined and 9 infected with 69.23%. The lowest prevalence is found in age group of 36-40, 5 examined 3 get infected with 60%.

Table 5 shows, all the 122 positive isolates are positive for catalase, sulfur, mortality and Tripple sugar ion tests. While negative result for Citrate, oxidase and indole tests.

| Table 1: Prevalence of typhoid fever among patients attending General Sani Abacha Specialist Hospital Damaturu. |
|---|---|---|---|---|
| Number sampled | Number of infected | No. of un infected | % of +ve | % of -ve |
| 150 | 122 | 28 | 81.33% | 18.67% |

| TABLE 2: Prevalence of typhoid in reaction to sex of patients attending General Sani Abacha Specialist Hospital Damaturu. |
|---|---|---|---|---|
| Number males examined | Number of female examined | No. of infected males | No. of infected females | % of infected males/females |
| 77 | 73 | 64 | 58 | 42.67/38.67% |

| Table 3: Age group and frequency of sample infected. |
|---|---|---|---|
| Age group | Number of examined | No. of infected | % of +ve | % of -ve |
| 0-5 | 116 | 11 | 68.75% | 31.25% |
| 6-10 | 18 | 15 | 83.33% | 16.67% |
| 11-15 | 18 | 14 | 77.78% | 22.22% |
| 16-20 | 18 | 15 | 83.33% | 16.67% |
| 21-25 | 13 | 09 | 69.23% | 30.77% |
| 26-30 | 24 | 20 | 83.33% | 16.67% |
| 31-35 | 15 | 14 | 93.33% | 6.67% |
| 36-40 | 10 | 06 | 60.00% | 40.00% |
| >40 | 18 | 18 | 100% | 0.00% |
| Total | 150 | 122 | 81.33% | 18.67% |

| Table 4: Age group and sex related infection |
|---|---|---|---|---|
| Age group | Number of examined males | No. of infected males (%) | No. of examine females | No. of infected females (%) |
| 0-5 | 07 | 06 (85.71%) | 08 | 05 (62.50%) |
| 6-10 | 10 | 10 (100.0%) | 08 | 05 (62.50%) |
| 11-15 | 09 | 08 (88.89%) | 09 | 06 (66.67%) |
| 16-20 | 10 | 07 (70.00%) | 08 | 08 (100.0%) |
| 21-25 | 08 | 06 (75.00%) | 05 | 05 (60.00%) |
| 26-30 | 11 | 08 (72.73%) | 13 | 12 (92.30%) |
| 31-35 | 07 | 06 (85.71%) | 09 | 08 (88.89%) |
| 36-40 | 05 | 03 (60.00%) | 05 | 03 (60.00%) |
| >40 | 10 | 10 (100.0%) | 08 | 08 (100.0%) |
| Total | 77 | 64 (83.12%) | 73 | 58 (79.45%) |
DISCUSSION

*Salmonella* typhi caused typhoid fever. The disease is among the common infectious diseases that are seen in our hospital. From the research conducted at General Sani Abacha Specialist Hospital on the prevalence, out of 150 samples analyzed 122 were found to be infected 81.33% and 22 were not infected 18.67%. This agrees with Wikels et al report (1981) that 1 in 100 patients coming under care in an adult medical ward has typhoid and 15% on paediatrics. In subtropical and tropical where the prevalence is much the incidence can be 1 out of 20 as a result of poor hygienic and quality of water available. Among 77 males examined, 64 were infected 42.67% and the prevalence were found to be highest in age group 6-10 and >40 with the prevalence rate of 100% infection. In females the total number examined are 73 and 58 were infected with 38.67% and the prevalence is highest in age group 16-20, 31-35 and >40with the prevalence rate of 100%, 88.89% and 100% respectively. This also agrees with Stuart and Pollen (1996) that the prevalence is much among young and adolescent. The infection is readily transmitted among very young and old. The new born babies and breast-feeding were prone to typhoid fever infection because of the antibodies and immunization gotten from the mother’s milk and colonization of gut by normal flora provides much more protection. Among the infected males and females of different age group to see there is significant difference from chi square test X2 test, X2=1.06 from the table under 5% level of probability at degree of freedom 4.d.f =9.488 and 1% level of probability at 4 degree of freedom 4.d.f=7.779. The table value were greater than the calculated value, therefore, there is significant difference between the infected males and females. This show that the rate of infection is not dependent on either males or females and this shows age is examined; thus, typhoid is a disease of older people than young ones.

CONCLUSION

Based on investigation and samples examined the prevalence of typhoid is more in males than females, this may be because males have unusually feeding habit, they can eat in such as restaurant. In females they are more conscious in their personal hygiene than males.

In this research of 150 samples were examined, 122 were infected. The age group 26-30 has the highest prevalence followed by 40, 6-10 and 16-20, 11-15 and 31-35, 36-40. The age group 36-40 is the lowest in prevalence firm the chi x2 tot there is significant differences. Typhoid is a disease of both young’s and olds of both sexes.

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