Antibiotics Susceptibilities and Plasmid Profiles of Salmonella typhi from Patients Attending Yobe State Specialist Hospital, Damaturu, Yobe State, Nigeria

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ABSTRACT

Salmonella infection remains a major public health concern worldwide. This study aimed to determine the antibiotics susceptibilities and plasmid profiles of Salmonella typhi from patients attending Yobe State Specialist Hospital, Damaturu, Yobe State, Nigeria. Two hundred and thirty three (233) samples (100 blood and 133 stool) were collected aseptically from patients diagnosed with enteric fever. The samples were processed using standard microbiological methods for identification of Salmonella typhi. The antimicrobial susceptibility patterns were determined using Kirby-Bauer disc diffusion techniques using ten antibiotics and the phenotypic expression of extended spectrum beta-lactamases (ESBLs) were determined using double disc diffusion test, and were further screened for plasmid DNA by standard method. Out of two hundred and thirty three (233) samples, thirty five isolates tested positive for S. typhi comprising of 20 (57%) from blood and 15 (43%) from stool. The stool isolates were highly (100%) resistant to tariivid and less (13.3%) resistance to pefloxacin while blood isolates showed highest (60%) resistant against chlorophenicol and least (20%) resistance to cefriaxone fourteen (14) Salmonella typhi showed ESBLs production; the Stool samples has the highest (n=8, 57.1%) ESBLs producers while least (n=6, 42.8%) were from the blood samples. plasmid size and number determine by bands were separated using agarose gel electrophoresis. This study reveals that both the resistant antibiogram and plasmid profile are still viable epidemiological tools for tracing the sources of Salmonella isolates.

Keywords: Blood; Stool; Salmonella typhi; Antimicrobial; Resistant; Plasmid

INTRODUCTION

The genus Salmonella belongs to the family Enterobacteriaceae. They are facultative anaerobic rods. Salmonella is mostly motile except Salmonella pullurun and Salmonella galarum, non-spore forming, Gram-negative bacterium (Salehi et al., 2015). There are currently over 2587 serotypes of Salmonella, responsible for salmonellosis, grouped into two basic species: Salmonella enterica and Salmonella bongori (Grimont et al., 2007). Salmonella spp. is mainly transmitted by the faecal-oral route mainly through contaminated food or water. Outbreaks of Salmonella -associated diseases are usually associated with contaminated water and the ingestion of contaminated food of animal origin like, fishes, poultry, meat and milk (WHO, 2014). Salmonella enterica is an important cause of human morbidity and mortality worldwide. Salmonella typhi
is the etiological agent of typhoid fever while Non-
Typhoid Salmonella species (NTS) are associated with
gastroenteritis and invasive infection in children, the
elderly and immuno compromised patients; these
include: Salmonella Typhimurium, Salmonella
enteritidis amongst other serovars of Salmonella
enterica that affect both human and animal, of which
S. Typhi affects human only. Both Salmonella typhi
and NTS are among the most frequent pathogens
causing blood stream infections (BSI) in tropical low-
resource settings (Reddy et al. 2010). The highest
incidence and prevalence of Salmonella infection
worldwide occur in Asia region (Crump et al. 2013).
Mainly in south and Southeast Asia, where isolates
show high rate of antibiotic resistance. It is estimated
that each year there are approximately 21.6 million
cases of typhoid fever which result in 200,000 deaths
worldwide (Adeshina et al., 2010). However, the
incidence of cases and death has been greatly
increased by combination of poor sanitation and
hygiene, unavailability of vaccines and high cost of
effective antimicrobial chemotherapy (Adeshina et al,
2010).

In Nigeria, the antibiotics mostly readily available for
treatment of typhoid and other Salmonella related
diseases are chloramphenicol, ampicillin,
tetracycline, third generation cephalosporin,
gentamycin, quinolones and trimethoprim/sulfamethoxazole (El – Sayed et al.,
2012).

Recently multi-drug resistant (MDR) strains have
emerged presumably due to the extensive use of
antimicrobial both in human and animals’ husbandry.
In veterinary medicine for instance, antibiotics are
being used in livestock production, disease
prevention and as growth-promoting feed additives.
The use of antibiotics in animals’ production disrupts
the normal flora of the intestine; prolongs faecal
shedding of these organisms into the environment
and emergence of antibiotics-resistant Salmonella
strains. MDR in Salmonella is a cause of great concern
in both clinical and veterinary medicine, as it may
limit the therapeutic options available for the
treatment of Salmonella-associated diseases (Davis,
2015).

This study aim to isolate, identify and plasmid profile
of Salmonella typhi from blood and stool samples
among patients attending Yobe State specialist
hospital Damaturu, Yobe State.

MATERIALS AND METHODS

Study Area

The study was carried out in Yobe State Specialist
Hospital (YSSH) Damaturu. Damaturu is the capital
city of Yobe State, Nigeria, located in Northeast
Nigeria. It’s located on coordinates: 12°00’N
11°30’E/12.000°N 11.500°E). Yobe state is mainly an
agricultural state, it was created on August 27, 1991
out of old Borno State. The state borders the Nigeria
states of Bauchi, Borno, Gombe, and Jigawa while to
the North of Niger Republic, it borders the Diffa
Region and the Zinder Region.

Study Design, Sample Collection and Preparation

During a six months period (April-september, 2023),
the sample populations were grouped into two with
a total of 233 clinical samples of 100 blood samples,
and 133 stool samples from patients attending the
Yobe State Specialist Hospital (YSSH) Damaturu that
shows symptoms of enteric fever. All patients were
instructed on how to collect appropriate specimen.

Blood Culture

Using a sterile syringe and needle, 4ml of whole blood
from the patient was collected and dispensed into
20ml tetrathionate. The mixture was incubated
overnight at 37°C for 18-24hrs in an incubator. Tubes
that show turbidity were sub-cultured each from each
of the containers unto freshly prepared sterile and
dried Salmonella-Shigella agar (SSA), and incubated at
37°C for 18-24 hours in an incubator. The sub
culturing was done three times before conclusion
that there is no growth (Sur et al., 2007).

Subculture and Characterization

The pure isolates was obtained after the subculture
and it was characterized using a gram stain and
biochemical tests. The pure isolates were presevered
in a slant vijour bottle for next usages (Chessbrigh,
2007).

Stool Culture

The purulent or mucoid parts of the stool samples
were picked using a sterile wire loop and inoculated
into selenite F broth medium and then incubated at
37°C overnight for 18 to 24hrs in an incubator. The
broth cultures were subcultured in SSA. The SSA,
and MCA plates were incubated overnight at 37°C for
18 to 2hrs in an incubator (Sur et al., 2007).

Antimicrobial Resistant Profiles

Antimicrobial resistance profile of Salmonella
isolates were determined by the disc diffusion
method of Kirby Bauer, (1966) and zones of inhibition
interpretation was carried out as described by the
Clinical Laboratory Standard Institute (CLSI, 2014). The antibiotic disks used are manufactured by using the disk diffusion method of Kirby Bauer as described by the Clinical Laboratory Standard Institute (CLSI, 2016). Each Salmonella isolates was transferred in to Muller hilton broth and incubated at 37οC for 24 hours. The turbidity of the suspension was adjusted aseptically with sterile saline to obtained turbidity of 0.5 McFarland standards. Then, pour on muller hinton agar plate to cover their surface and then drained the excess media. The antibiotic disks were placed on the surface of agar at equal distance, sufficient to separate them from each other to avoid overlapping of the inhibition zones. Each plate carries a maximum of ten disc. After 30 seconds of pre-diffusion, the plates were incubated at 37οC for 24 hours followed by the diameter of inhibition zones, measurement and the adjusted to the nearest rounded number. A total of 10 antimicrobial agents were used in this study namely: Ten (10) antimicrobial disks was be used in this study these include: chloramphenicol (30μg), cefazidime (30μg), ciprofloxacin (30μg), cefotaxime (30μg), amoxicillin (30μg), augmentin (10μg), gentamycin (30μg), pefloxacin (30μg), tarvid (10μg) and ceftriaxone (30μg) (CLSI, 2016).

**Determination of Extended Spectrum β-lactamase (ESBL) among the Isolates**

This test was carried out by placing augmentin (AMC 30μg) antibiotics disc at the centre of sterile Mueller Hinton agar plate containing the streaked colonies of the positive isolate of *Salmonella typhi* on agar culture, cefazidime (CAZ 30μg), cefotaxime (CTX 30μg) and ceftriaxone (CRO 30μg) were placed beside augmentin (AMC 30μg) disc with distances of 15mm from the centre and the plate was incubated overnight at 37°C for 24 hours in an incubator. All clear zone of inhibition towards the Augmentin (AMC 30μg) showed positive results for the production of ESBL (CLSI, 2016).

**Plasmid Curing of the Isolates**

Single colonies of isolates were inoculated into nutrient broth supplemented with 2% SDS, 0.1% acridine orange (Electran) and incubated at 37°C for 24 h after which they were plated out on nutrient agar, incubated at 37°C for another 24 h (Durve et al., 2013). Colonies obtained were then harvested and subjected to plasmid extraction.

**Plasmid Extraction of Salmonella Isolates**

The DNA templates of each of the confirmed pure culture of *Salmonella typhi* isolates were generate by dispensing pure colonies of overnight grown culture unto 100-μL1X Tris- EDTA buffer, vortex mixed and boiled at 100°C for 10 minutes. Thereafter, transferred immediately to the freezer (-20°C) for 10 minutes, maintained at room temperature, vortex mixed again and centrifuged at 10,000 rpm for 10 minutes. The resulting supernatant containing DNA templates of the isolates were separated, stored at 4°C, and used as DNA template for PCR amplification (Yang et al., 2008).

**DNA Quantification of Extracted DNA**

The extracted genomic DNA was quantified using the Nano drop 1000 spectrophotometer. The software of the equipment was launched by double-clicking on the Nano drop icon. The equipment was initialized with 2 ul of sterile distilled water and blanked using normal saline. Thereafter, 2μl of the extracted DNA was loaded into the lower pedestal, the upper pedestal was brought down to contact the extracted DNA on the lower pedestal. The DNA concentration was measured by clicking on the “measure” button (Abimiku et al., 2019).

**Amplification of 16S rRNA Gene of the Isolates**

The 16S rRNA genes of the *Salmonella* isolates were amplified using the universal primer 27F: 5’-GGAACTGAGACACGGTCCAG-3’ and 1492R: 3’-CCAGGTAAAGGTTCGCGT-5’ (Zymo Research, USA) on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 50 μl for 35 cycles. The PCR mix included: X2 Dream Taq Master Mix (Taq polymerase, dNTPs, MgCl, Zymo research, USA), the primers at a concentration of 0.4 M and the extracted DNA as a template. The PCR conditions were as follows: 5 minutes at 95°C for initial denaturation, 30 cycles each 1 minute at 94°C for denaturation, 1 minute at 60°C for annealing and 30 second at 72°C for extension and final extension at 72°C for 10 minute. The product was resolved on a 1% agarose gel and visualized on a UV transilluminator.

**Detection of all the amplified genes by Agarose Gel Electrophoresis**

One percent (1%) agarose gel was used to resolve DNA fragment. This was prepared by combining 1g agarose in ten times concentration of tris-borate ethylene diamine tetracacetate (10ml 10XTB-EDTA) buffer and 90ml sterile distilled water in 250ml beaker flask and heating in a microwave for 2 minutes until the agarose is dissolved. Exactly 0.7μl of ethidium bromide was added to the dissolved agarose solution with swirling to mix. The gel was then poured onto a mini horizontal gel electrophoresis tank and casting combs was inserted.
The gel was allowed to set for 30 minutes. The casting combs were carefully removed after the agarose gel had solidified completely. One times concentration (1X) TBE buffer was added to the reservoir until it covered the agarose gel. Precisely 8µl of gel tracking dye (bromophenol blue) was added to 10µl of each sample with gentle mixing. The sample was loaded onto the wells of the gel at a concentration of 10µl, the mini horizontal electrophoresis gel setup was covered and electrodes connected. Electrophoresis was carried out at 100-200mA for one hour. At the completion of electrophoresis, the gel was removed from the buffer and visualized under UV light and documented.

**Conjugation Experiment**

*Salmonella* isolates harbouring the group of ESBLs were selected for the conjugation experiment using the broth mating technique described by Chen *et al.*, (2007) with rifampicin resistant *E. coli* as the recipient. Transconjugants were selected on a Brain heart infusion agar (BHI agar) plate containing rifampicin (200 µg/mL). Transfer of plasmids, and co-dissemination of antibiotic resistance was confirmed by PCR, plasmid isolation, and antibiotic susceptibility testing for the transconjugants and recipient, as previously done for the donor.

**Data Analysis**

The data obtained in this research were analysed using descriptive statistics: plates, figures, percentages and tables using Microsoft word 2013, and the blood and stool was compared by using Chi-square at level of significance p<0.05 using SPSS.

**RESULTS AND DISCUSSION**

The main aim of this study was to investigate the antibiotics susceptibilities and plasmid profile of *Salmonella typhi* isolated among patients attending Yobe State Specialist Hospital Damaturu.

Table 1 showed the numbers of samples collected for each of the specimen and the numbers of *S. Typhi* isolated from each of the specimen. A total number of 233 samples where collected out of which blood is 100(43%) and stool is 133(57%), 15 samples (57.1%) from blood samples confirmed the presence of *S. typhi* while 20 samples (42.9%) from stool showed growth of *S. typhi*. The results agreed with the findings of Melita (2011) and Kabir *et al.* (2007) who also reported high positive results of *Salmonella typhi* from blood samples. This study also agrees with the study of Okonko *et al.* (2010) where they reported less number of *Salmonella typhi* from stool samples than the blood samples.

Table 2. The multidrug resistance (MDR) pattern of 35 *Salmonella typhi* isolates found to be resistance to three and above antibiotics. The isolates where highly resistant was against tarivid (100%) less resistance was against pefloxacin (13.3%) in stool and on other Isolates where highly resistant was against chloropenicol (60%) less resistance was against ceftriaxone (20%) in blood.

Table 3 The total number of positive samples for extended beta lactamase production *Salmonella typhi* among the MDR isolates. Fourteen (14) *Salmonella typhi* isolates shows ESBL production; the Stool has the highest ESBL production with (57.1%) while least blood with (42.8%)

Table 4 The plasmid sizes and number of plasmids detected in *Salmonella typhi* isolate show by Agarose gel electrophoresis in this study, various plasmids of different molecular weight ranging from 2.4kb to 23.1kb were isolated *Salmonella typhi* from stool and Isolates harboured single to multiple (1-4) plasmids, Occurrence of plasmid was more in isolates from stool compared to those from blood Only one isolate isolated from blood harboured 3 plasmids.

The plasmid transfer by conjugation from the donor *Salmonella typhi* isolates to the *Escherichia coli* recipient show by agarose gel electrophoresis the donor transfer only one plasmid.

Thirty five 35 isolates tested positive for *S. typhi* comprising of 20 (57%) culture from blood and 15 (43%) culture from stool. The highest level of resistance was against tarivid (60%) follow by cefotaxime (51.4%) ceftazidine and chloropenicol with (48.5%). Fourteen (14) *Salmonella typhi* isolates shows ESBL production; the stool has the highest ESBL producer with (57.1%) and blood has (42.8%). The PCR amplification of resistant genes were detected those that shows positive ESBL and *Salmonella typhi* harboured plasmids with sizes ranging from 2.4 to 23.1 kb. And Four of fourteen *S. typhi* ESBL-producing strains harboured 23 kb self-transmissible plasmid that was co-transferred with cefotaxime resistance to *Escherichia coli* transconjugants the bands were separated using agarose gel electrophoresis.
Table 2. Antibiotics susceptibility patterns and occurrence of Multi Drug resistant *Salmonella typhi* isolated from patients attending Yobe State Specialist Hospital Damaturu

<table>
<thead>
<tr>
<th>Antimicrobial Agents (Concentrations)</th>
<th>Symbols</th>
<th>Stool Isolates (% n = 15)</th>
<th>Blood Isolates (% n = 20)</th>
<th>Multi Drug Resistant Isolates Combined (% n = 35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin (10ug)</td>
<td>AM</td>
<td>10 (66.6)</td>
<td>5 (25)</td>
<td>15 (42.8)</td>
</tr>
<tr>
<td>Ceftazidine (30ug)</td>
<td>CAZ</td>
<td>8 (53.3)</td>
<td>7 (35)</td>
<td>17 (48.5)</td>
</tr>
<tr>
<td>Chloramphenicol (10ug)</td>
<td>CH</td>
<td>5 (33.5)</td>
<td>12 (60)</td>
<td>17 (48.5)</td>
</tr>
<tr>
<td>Cefotaxine (30ug)</td>
<td>CTX</td>
<td>7 (46.6)</td>
<td>11 (55)</td>
<td>18 (51.1)</td>
</tr>
<tr>
<td>Ciprofloxacin (30ug)</td>
<td>CPX</td>
<td>10 (66.6)</td>
<td>6 (30)</td>
<td>16 (45.7)</td>
</tr>
<tr>
<td>Augmentin (30ug)</td>
<td>AU</td>
<td>10 (66.6)</td>
<td>5 (25)</td>
<td>15 (42.8)</td>
</tr>
<tr>
<td>Gentamycin (10ug)</td>
<td>CN</td>
<td>9 (60)</td>
<td>7 (35)</td>
<td>16 (45.7)</td>
</tr>
<tr>
<td>Pefloxacin (30ug)</td>
<td>PEF</td>
<td>2 (13.3)</td>
<td>8 (40)</td>
<td>10 (28,5)</td>
</tr>
<tr>
<td>Tarvid (10ug)</td>
<td>OFX</td>
<td>15 (100)</td>
<td>6 (30)</td>
<td>21 (60)</td>
</tr>
<tr>
<td>Ceftriaxone (30ug)</td>
<td>CPO</td>
<td>9 (60)</td>
<td>4 (20)</td>
<td>13 (37.1)</td>
</tr>
</tbody>
</table>

Table 3. Total number of extended beta lactamase producing *Salmonella typhi* isolated from patients attending state specialist hospital Damaturu

<table>
<thead>
<tr>
<th>Source</th>
<th>Number of Extended beta lactamase producing (%) n=14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>6 (42.8)</td>
</tr>
<tr>
<td>Stool</td>
<td>8 (57.1)</td>
</tr>
<tr>
<td>Total</td>
<td>14 (100)</td>
</tr>
</tbody>
</table>

Table 4. Plasmid sizes and number of plasmids detected in *Salmonella typhi* isolates

<table>
<thead>
<tr>
<th>Isolate number</th>
<th>Number of Plasmid</th>
<th>Estimated molecular sizes of Plasmid (Kb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>20.5 &amp; 7.8</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>20.5 &amp; 7.8</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>19.8, 14.6, &amp; 3.4</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>23.1, &amp; 19.2</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>4.7</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>27.1, 12.3, 8.7, &amp; 6.2</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>23.1, &amp; 19.2</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>18.9, 14.6 &amp; 3.4</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>19.2 &amp; 6.6</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>23.1, 19.8 &amp; 4.7</td>
</tr>
<tr>
<td>11</td>
<td>2</td>
<td>14.6 &amp; 12.3</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
<td>2.4</td>
</tr>
<tr>
<td>13</td>
<td>2</td>
<td>20.2 &amp; 17.8</td>
</tr>
<tr>
<td>14</td>
<td>2</td>
<td>20.2 &amp; 17.8</td>
</tr>
</tbody>
</table>
Table 5. Plasmid transfer by conjugation from the donor *Salmonella typhi* isolates to the *Escherichia coli* recipient

<table>
<thead>
<tr>
<th>Donor <em>S. typhi</em> isolate</th>
<th><em>E. coli</em> transconjugants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasmid profile, kb</td>
</tr>
<tr>
<td>1</td>
<td>23, 7.8</td>
</tr>
<tr>
<td>4</td>
<td>23, 19.2</td>
</tr>
<tr>
<td>7</td>
<td>23, 19.2</td>
</tr>
<tr>
<td>10</td>
<td>23, 19.8 &amp; 4.7</td>
</tr>
</tbody>
</table>

Plate 1. Agarose gel electrophoresis of the amplified *16SrRNA gene* Salmonella gene. Lane M represents 1500bp DNA molecular ladder, Lane PC represents Positive control; Lane NC represents Negative, Lane 1, 2 3 to Lane 14 represent the expression of the *16SrRNA* (660bp) gene for the Salmonella genus.

Plate 2: Agarose gel electrophoresis of the Plasmids of molecular weight of 0.4kb to 35.3kb detected in *Salmonella* isolates. Lane M represent Lambda DNA Hind III digest, Lanes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, and 14 represent the expression of the Hind III plasmid digested with their corresponding bands.
Plate 3. Plasmid transfer by conjugation from donor *Salmonella* typhi strains to recipient *Escherichia coli* j53-2. Lanes 1, 3, 5, and 7 are *S. typhi* donors Lag-003, -004, -007, and -010. Lanes 2, 4, 6, and 8 are *E. coli* j53-2 trans conjugants. Lane M represents Lamda DNA Hind III markers.

CONCLUSION

*Salmonella* infection remains a distressing public health concern worldwide. The findings of this study showed that ceftriaxone and pefloxacin are sensitive on *Salmonella typhi*. The laboratory culture of organism and antibiotic sensitivity testing should be encouraged in health institutions to avoid emergence of drugs resistance bacteria and effective and efficient management and control of disease.

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**Conflicts of Interest:** The authors declare that they have no competing interest.

**REFERENCES**


