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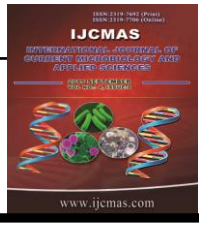

Original Research Article

Prevalence of Multidrug Resistant *Salmonella enterica* Serovar Typhi in Kaduna Metropolis, Kaduna, Nigeria

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Keywords

S. enterica serovar Typhi, Resistance, Imipenem, Amoxicillin

A B S T R A C T

A study was conducted here to determine the prevalence of multidrug resistant *Salmonella enterica* serovar Typhi among patients complaining of suffering from typhoid fever complications in Kaduna Metropolis, Kaduna. For this purpose, stool specimens of 220 male and female patients were collected from five different hospitals in Kaduna Metropolis, Kaduna. Seventy six presumptive Salmonella isolates were obtained from two hundred and twenty stool samples collected from male and female patients (51.8% from males and 48.2% from females) of varying ages in five selected hospitals in Kaduna metropolis. Forty seven isolates out of the presumptive isolates were *Salmonella* sp. identified using conventional biochemical tests, Microbact™ 12A/12E Gram negative identification system, and confirmed using molecular identification through chromosomal DNA extraction using Easypure bacteria DNA kit and PCR, where the Salmonella ompC gene codes for a major outer membrane protein in the *Salmonella* genus. Out of the forty seven *Salmonella* sp., sixteen were *S. enterica* serovar Typhi identified serologically using *S. enterica* serovar Typhi specific antiserum. All the sixteen *S. enterica* serovar Typhi isolates showed multidrug resistance to ciprofloxacin, ofloxacin, tetracycline, cotrimozaxole, amoxicillin, amoxicillin/clavulanic acid, nalidixic acid, ceftriazone, cefotaxime, nitrofurantoin and chloramphenicol with a record of high resistance to amoxicillin (100%) and low resistance to ciprofloxacin, ofloxacin and chloramphenicol (12.5%). All the isolates were sensitive to imipenem.

Introduction

Penicillins such as amoxycillin and ampicillin, cephalosporins such as ceftriaxone and cefuroxime, aminoglycosides such as streptomycin and gentamycin, macrolide such as erythromycin, fluoroquinolones such as ciprofloxacin, ofloxacin, and perfloxacin, and tetracyclines are used for treatment of *Salmonella enterica* serovar Typhi infection (Richard *et al.*, 2007).

Chloramphenicol has been the treatment of choice for typhoid fever since its discovery in 1947.Because of the alarming spread of plasmid mediated chloramphenicol resistant *S. enterica* serovar Typhi throughout the

world, newer antibiotics with good *in vivo* activity against *Salmonella typhi* are needed. Typhoid fever responds slowly to ampicillin, amoxicillin, cotrimoxazole or trimethoprim alone. Among fluoroquinolones, ciprofloxacin, ofloxacin and perfloxacin are most widely used antimicrobial agents. They act by inhibiting bacterial enzymes DNA gyrase which is responsible for division, coiling and supercoiling of bacterial DNA during multiplication. Of the third generation cephalosporins; ceftriaxone, cefotaxime and cefoperazone are effective therapeutic alternative in multidrug resistant *S. enterica* serovar Typhi infected cases (Arora, 2011).

The fluoroquinolones (ciprofloxacin and ofloxacin), third generation cephalosporins (ceftriaxone and cifixime), and azithromycin came up as the 2nd line of treatment for multidrug resistant strains. Aztreonam and imipenem are also potential third line drugs that have been used recently in serious infections. The azalide antimicrobial, azithromycin is also an option in the treatment of multidrug resistant enteric fever (Raveendran *et al.,* 2010).

Typhoid fever caused by multi drug resistant *S. enterica* serovar Typhi has become a significant cause of morbidity and mortality over recent years. These strains have also caused out breaks throughout the world, especially in South America, Indian subcontinent, Africa and South-East Asia (Basudha *et al.,* 2007). The period 1990 to the present has been a hallmark era in the history of enteric fever because of the emergence and dissemination of *S. enterica* serovar Typhi strains carrying resistance to multiple clinically relevant antibiotics (Shyamapada *et al.,* 2012).

The antibiotics that form the mainstay therapy for typhoid fever patients in developing countries are chloramphenicol, ampicillin, and cotrimoxazole. The resistance strains of *S. enterica* serovar Typhi to these antibiotics have emerged and continue to be of clinical significance. The efficacy of this antibiotic and other first line antibiotics such as tetracycline become doubtful following unprecedented upsurge in enteric fever in early 1990s. From 1997 through 1998, study on the prevalent of multidrug resistance in *S. enterica* serovar Typhi was conducted in Lagos, and most strains isolated were resistant to these antibiotics (Akinyemi *et al.,* 2005).

Since 1948, Chloramphenicol had been the mainstay of treatment of enteric fever until 1972 when chloramphenicol-resistant typhoid fever becomes a major problem. Although initially, susceptible to ampicillin and cotrimoxazole, *S. enterica* serovar Typhi strains resistant simultaneously to all the first line drugs emerged in 1970s. Since then, these multidrug resistant strains have spread in an epidemic form and have rapidly emerged worldwide. With the emergence of multidrug resistant strains, quinolone, particularly fluoroquinolones has been widely used and recommended as an alternative drug for typhoid fever where the first line drug no longer in use (Basudha *et al.,* 2007).

There is a changing trend in susceptibility pattern of *S. enterica* serovar Typhi worldwide with emerging resistance to fluoroquinolones, and the emergence of resistance to fluoroquinolones has reduced the therapeutic options available (Sehra *et al.,* 2013; Srirangaraj *et al.,* 2014). It has rapidly gain resistance to antibiotics like ampicillin, chloramphenicol, cotrimoxazole, and also to previously efficacious drugs like ciprofloxacin (Madhulika *et al.,* 2004). Since 1993, *S. enterica* serovar Typhi with decreased susceptibility to ciprofloxacin has

been isolated. Subsequently, an extensive outbreak of typhoid fever due to *S. enterica* serovar Typhi with decreased susceptibility to ciprofloxacin in Tajikistan was reported (Basudha *et al.,* 2007).

Drug resistance to Salmonella has been on the rise in India with the emergence of nalidixic acid-resistant *S. enterica* serovar Typhi (NARST) isolates. This along with the emergence of resistance to third and fourth generation cephalosporins, has diminished the therapeutic options available to newer quinolones, extended spectrum cephalosporins, azithromycin, and carbapenems (Srirangaraj *et al.,* 2014). The flouroquinolones such as ciprofloxacin and ofloxacin, third generation cephalosporins such as ceftriazone and cefixime, and azithromycin comes up as the 2nd line of treatment for *Salmonella typhi* multidrug resistant strains (Raveendran *et al.,* 2010). An antibiogram of *S. enterica* serovar Typhi isolated at Pondicherry shows resistance of this organism to ampicillin, gentamycin, chloramphenicol, co-trimoxazole and nalidixic acid (Madhulika *et al.,* 2004).

Results of in vitro antibiotic sensitivity test by Adabara *et al.* (2012) showed that isolates of *S. enterica* serovar Typhi were generally resistant to ceftriaxone, cefuroxime, amoxicillin, ampicillin, ciprofloxacin, and augmentin which are the drugs of choice routinely used in the study area (Minna, Nigeria) for the treatment of typhoid fever. Ceftriaxone-resistant *Salmonella typhi* have been reported (Basudha *et al.,* 2007), but surveillance data from many countries worldwide shows that ceftriaxone resistant to *S. enterica* serovar Typhi still remain low all over the world (Raveendran *et al.,* 2010). A few cephalosporin-extended resistant *S. enterica* serovar Typhi strains, which produce extended-spectrum β-lactamase, have been reported (Morita *et al.,* 2010).

The aim of this study was to determine the prevalence of *S. enterica* serovar Typhi in Kaduna Metropolis, Kaduna, Nigeria.

Materials and Methods

Study area

The study area is Kaduna Metropolis, Kaduna State, Nigeria. Kaduna State is located in North West region of Nigeria (Fig. 1). The state lies at latitude 10^0S and it metropolis politically covered Kaduna north and Kaduna south with fractional part of Chukun and Igabi. The indigenes of the state in the metropolis are mostly civil servants, farmers, traders, students, fishermen, and applicants. Sources of drinking water available to these areas include boreholes, public and private wells, rivers, streams and municipal water. The indigenes of Kaduna and environs are farmers, fishermen, civil servants and traders. Food generally consumed in the study area is made up of cereals, fish, beef/beef products, tuber crops and vegetables.

Selected hospitals for this research work were Gwamna Awan General Hospital Kakuri and General Hospital Sabon Tasha (located in Kaduna South), Yusuf Dantsoho Memorial Hospital Tudun Wada, Sefa Specialist Hospital, and Barau Dikko General Hospital (located in Kaduna North), all in Kaduna Metropolis (Fig. 1).

Collection of samples

The period of collection of stool samples was between September, 2014 and April, 2015. A total of 220 stool samples were collected from patients with clinical syndromes of enteric fever attending five hospitals in Kaduna metropolis based on

ethical clearance and permission obtained from ministry health, Kaduna State. Clean sterile wide-necked leak proof screw cap bottles were used to collect the samples from the patients with the help of the hospital Laboratory Staff. The samples collected were then transported in thermo flasks packed with ice to Kaduna State University Microbiology laboratory for immediate examination or analysis within 2 hour of collection.

Samples analysis

Macroscopic examination

The samples collected were carefully examined macroscopically in the laboratory according to Ochai and Kolhatkar (2008), and Cheesbrough (2010) in order to note the color appearance, consistency and presence of mucus, blood or pus.

Isolation of *Salmonella* spp.

All clinical samples collected were cultured aerobically for isolation of *Salmonella spp* in the laboratory as described in Cheesbrough (2010). Each sample was inoculated by direct streaking on Salmonella Shigella agar, and a portion of the same sample transferred to Selenite Cysteine broth and incubated for 24–48hour and 12hours respectively at 37°C.

The Selenite Cysteine broth incubated samples were heavily inoculated on Salmonella Shigella agar plates after the 12 hours enrichment period and incubated for 24 hours at 37^0C. Black doted center colonies with or without transparent borders were identified and subculture on MacConkey agar plates, and non-lactose fermenters colonies were subsequently subcultured into nutrient agar slants and were kept at 4^oC for identification purpose.

Morphological and biochemical identification of isolates

Morphological and biochemical identification of the pure isolates obtained was carried out as described by Aneja (2007), Ochaiand Kolhatkar (2008) and Cheesbrough (2010).

Biochemical test using Microbact™ 12A/12E Gram-negative identification system

The procedure was carried out using the Microbact™ 12A/12E (Oxoid) gram negative identification system.

Molecular Identification

Chromosomal DNA extraction

DNA extraction was carried out by using EasyPure Bacteria DNA kit (TransGen Biotech Co., Ltd, Beijing, China), following the protocol for gram negative bacteria supplied by the manufacturer. The concentration and purity of the extracted DNA was estimated using a Nanodrop spectrophotometer (Nanodrop Technologies, Wilmington, DE). DNA samples were stored at -20°C until required for PCR analysis.

PCR amplification of the ompC

The Salmonella ompC gene codes for a major outer membrane protein (OMP) in the *Salmonella* genus. Polymerase chain reaction (PCR) amplification was carried out using the primer set ompC F 5´ATC GCT GAC TTA TGC AAT CG 3´ and ompC R 5´ CGG GTT GCG TTA TAG GTC TG 3´ for detecting S*almonella* species. *Salmonella typhimurium* 14028, a reference strain obtained from Nigerian Institute of Medical research Yaba, was used as positive control.

Procedure: The PCR was performed using the TranStart TopTaq DNA Polymerase Kit (TransGen Biotech Co., Ltd, Beijing, China). The 25µl reaction mixture contained x1 PCR buffer, 1.5mMMagnesium Chloride, 0.2mM of each dNTP, 20µM of each primer and 2 units Taq DNA polymerase. 1µl of genomic DNA was used. PCR amplification reaction was performed using BIO-RAD Thermal Cycler with an initial denaturation at 94^{O}C for 3 minutes, followed by 34 temperature cycles of heat denaturation at 94^{O}C for 30 seconds, primer annealing at 55^{O}C for 30 seconds, and a final step of extension at 72°C for 1 minute and a final step of extension at 72°C for 5 minutes. PCR products were separated by electrophoresis in 3% agarose gel, stained with ethidium bromide and visualized by UV transilluminator.

Forty seven (47) isolates were obtained as *Salmonella* sp. These were subjected to serological (serotyping) identification test for *S. typhi.*

Serological test

Serological test was carried out by using antisera agglutinating antisera (Murex Diagnostics, Dartfort, United Kingdom) according to Srirangaraj *et al.* (2014).

Antimicrobial profile of the pure isolates using selected conventional antimicrobial agents used for treatment of *Salmonella typhi* infection in hospitals

Antimicrobial susceptibility test against *S.enterica* serovar Typhi was carried out using Kirby-Bauer disc diffusion techniques described by Arora (2011). A loopful of growth of each isolate on agar medium was suspended in sterile water and then was diluted in steps of 1:10 to give turbidity equivalent to the 0.5 McFarland standards (a density of $1x10^{8}$ cells/ml) before inoculation. The Muller-Hinton agar medium was prepared according to manufacturer's instructions and was poured (about 25ml of the media) into each of sterile petri-plates, the plates were allowed to solidify and a sterile swab stick was used to pick0.5 ml suspension of each isolate adjusted to $1x10^{8}$ cells/ml and was spread uniformly on the agar.

Sterile cotton swab were dipped in inoculums, remove the excess fluid by pressing and rotating the swabs against the wall of the tubes and then streaked on the surface of Muller Hinton plates. The inoculated plates were allowed to dry for about 5minutes. Using disc dispenser, single disc antimicrobial agents (Oxoid, Basingstoke United Kingdom): Ciprofloxacin (5µg), Ofloxacin (5µg), Tetracycline (25µg), Cotrimozaxole (25µg), Imipenem (10µg), Amoxicilin (25µg), Amoxicillin/clavulanic acid (3µg), Nalidixic acid (30µg), Ceftriazone (30µg), Cefotaxime (30µg), Nitrofurantoin (300µg), and Chloramphenicol (30µg) were dispensed onto the surface of the agar plates. After 30minutes of applying the discs, the plates were incubated aerobically at 37^{0}C for 18hours. The results were interpreted as either susceptible, intermediate, or resistant according to Clinical and Laboratory Standard Institute (CLSI) guidelines for 2014 (CLSI, 2014).

Results and Discussion

Description and distributions of clinical Samples

Two hundred and twenty stool (220) samples from in and out patients of varying sex and age complaining typhoid fever complications were collected for isolation and identification of *S. enterica* serovar Typhi isolates. Number of samples per sex collected from selected hospitals in Kaduna

state was shown in table 1. Distribution of stool samples based on consistency, sex and age was shown in table 2.

Morphological and biochemical characteristics of the presumptive isolates

The morphological and cultural characteristics of isolates revealed that seventy six isolates were *Salmonella* isolates. *Proteus* and *Citrobacter* sp were also isolated from the stool specimens. All the isolates were maintained on MacConkey agar plates for further research work.

Biochemical test using Microbact™ 12A/12E Gram-negative identification system

76 isolates were identified as *Salmonella* sp., 5 were identified as *Proteus* sp.and 1 was identified as *Citrobacter* sp.

Molecular identification of *Salmonella* Sp

Out of 76 isolates, 47 isolates were identified as *Salmonella* sp. after PCR amplification which was carried out using the primer sets ompC F 5´ATC GCT GAC TTA TGC AAT CG 3´ and ompC R 5´ CGG GTT GCG TTA TAG GTC TG 3´.

The PCR products confirming the presence of 204bp ompC genes of some *Salmonella sp*. on a 50kb marker are shown in plate 1.

Identification of *S.enterica* serovar Typhi using specific antiserum

Out of 47 isolates, isolates S1, S7, S10, S17, S20, S21, S24, S27, S32, S33, S36, S37, S40, S43, S45and S47 were identified as *Salmonella typhi*. These were relabeled as S1-S16.

Distribution of *Salmonella* sp. and *S. enterica* serovar Typhi among patients from the selected hospitals

Forty seven isolates were identified as *Salmonella* sp using biochemical and molecular identification system. Distribution of *Salmonella* sp. and *S.enterica serovar* Typhi among male and female patients attending different hospitals was shown in Table 3.Distribution of *Salmonella* sp. and *S. enterica* serovarTyphi among male and female patients of different age groups was shown in Table 4.

In vitro activity of antimicrobial agents against *Salmonella typhi* strains

The result of the *invitro* activity of antimicrobial agents against the *S. enterica serovar* Typhi strains (Table 5 and Table 6) shows a multidrug resistance trend. Out of the sixteen (16) isolates, four showed 58.33% resistance to the twelve conventional antimicrobial drugs, eight showed 41.67% resistance, two showed 33.33% resistance, and another two showed 25% resistance.

The antimicrobial drugs resistant profile (Table 5) showed that Amoxicillin had high resistance 16(100%), follow by amoxicillin/clavulanic acid 13(81.25%), tetracycline 12(75%), cotrimoxazole 11(68.75%), cefotaxime 9(56.25%), ceftriaxone 7(43.75%), nitrofurantoin 4(25%), nalidixic acid 3(18.75%), chloramphenicol 2(12.5%), ofloxacin 2(12.5%), ciprofloxacin 2 (12.5%) and imipenem 0(0.00%).

In this current investigation, two hundred and twenty stool (220) samples from in and out patients of varying sex and age complaining typhoid fever complications were collected for isolation and

identification of *S. enterica* serovar Typhi isolates. As shown in table 1, one hundred and thirty five (135) samples were collected from male patients and eighty five (85) from female patients. Abdullahi (2010) reported that the frequency of salmonellosis was higher in males' children than their female counter parts, As shown in table 2, 73 samples were collected from patients of age 10 years and below, 35 samples from patients at the age group of 11–20 years, 30 from patients of age 21–30 years, 22 from m of age 31–40 years and 60 from patients of 41 years and above collected from five different hospitals. According to Buch *et al.* (1994), maximum cases of multidrug resistant typhoid fever are seen in children less than five years of age. Frequent diarrheal disease in children specially under five years malnutrition and indiscriminate use of antibiotics provides an ideal milieu for the emergence of typhoid fever. Presence of *Proteus* sp. and *Citrobacter* sp. supports the growing evidence that the typhoid fever does not always present with a distinct clinical feature and other pathogens may also present (Akinyemi *et al.*, 2005)

Seventy six presumptive *Salmonella* isolates were isolated from two hundred and twenty stool samples of varying consistency from male and female patients of varying ages presented with clinical syndrome of typhoid fever in five selected hospitals in Kaduna metropolis. Forty seven isolates out of the presumptive isolates were *Salmonella* spp identified using conventional biochemical tests, Microbact^{TM} 12A/12E Gram negative identification system, and confirmed using molecular identification through chromosomal DNA extraction using Easy pure bacteria DNA kit and PCR, where the Salmonella ompC gene codes for a major outer membrane protein (omp) in the *Salmonella* genus. Ngan *et al.* (2010) used the ompC gene, which is described by Alvarez *et al.* (2004) as a *Salmonella*-genus-specific protein for Salmonellae identification. Out of the forty seven *Salmonella* sp., sixteen were *S. enterica* serovar Typhi identified serologically using *S. enterica* serovar Typhi specific antiserum. *S. enterica* serovar Typhi, *S. enterica* serovar Paratyphi A, B and C are primarily human pathogens. All the sixteen isolates produced lactose non fermenting colonies on Salmonella Shigella agar, Deoxycholate agar and Xylose-lysine deoxycholate agar, In KIA medium, these isolates produced hydrogen sulfide (weak) but no gas. Man acquires infection by ingestion of contaminated water and food. Water becomes polluted by the introduction of feces from human or animals excreting salmonellae. Infection by food usually results either from ingestion of contaminated meat or by way of the hands of carriers. Poultry and eggs also comprise an important source of Salmonella for human because a large percentage of chickens are routinely infected with Salmonellae, and therefore a man can acquire these organisms through direct contact with uncooked chickens or ingestion of undercooked chicken. The infective dose of most serotypes including *S. enterica* serovar Typhi varies from 10^6 to10^9 cells (Arora, 2011). Distribution of isolate based on location and sex study showed spread of *S. enterica* serovar Typhi (responsible for typhoid or enteric fever) and other *Salmonella* spp (responsible for either paratyphoid fever, gastroenteritis or food poisoning) across the study population, indicating endemicity of these infection in the metropolis. Isolation of the *S. enterica* serovar Typhi and other *Salmonella* spp from patients' stool samples who were presented with clinical syndrome of enteric fever revealed the present of Salmonellae in the intestinal tract as either carriers or infectious persons.

Table.1 Number of samples per sex collected from selected hospitals in Kaduna state

Location	**Number of samples**		
	Male (%)	**Female (%)**	**Total (%)**
Gwamna Awan General Hospital	48(21.8)	22(10.0)	70(31.8)
Dantsoho Memorial Hospital	60(27.3)	45(20.5)	105(47.8)
General Hospital Sabon Tasha	13(5.9)	10(4.6)	23(10.5)
Sefa Specialist Hospital	6(2.7)	4(1.8)	10(4.5)
Barau Dikko General Hospital	8(3.6)	4(1.8)	12(5.4)
Total	**135(61.4)**	**85(38.6)**	**220(100.0)**

Table.2 Distribution of stool samples based on consistency, sex and age

Age (Years)	**Consistency**						
	Watery (%)		**Semi-formed (%)**		**Formed (%)**		**Total (%)**
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**(Male & Female)**
≤ 10	10(4.6)	9(4.1)	11(5.0)	13(5.9)	19(8.6)	11(5.0)	73(33.2)
11-20	10(4.6)	5(2.3)	3(1.4)	2(0.9)	10(4.6)	5(2.3)	35(15.9)
21-30	4(1.8)	7(3.2)	4(1.8)	3(1.4)	8(3.6)	4(1.8)	30(13.6)
31-40	7(3.2)	5(2.3)	3(1.4)	2(0.9)	3(1.4)	2(0.9)	22(10.0)
41-above	20(9.1)	4(1.8)	13(5.9)	8(3.6)	10(4.6)	5(2.3)	60(27.3)
Total	**51(23.2)**	**30(13.6)**	**34(15.5)**	**28(12.72)**	**50(22.7)**	**27(12.27)**	**220(100.0)**

Table.3 Distribution of *Salmonella* sp. and *S. enterica* serovar Typhi among male and female patients attending different hospitals

Location	*Salmonella sp.(%)*		*Salmonella typhi (%)*		Total (Male and Female)(%)	
	Male	**Female**	**Male**	**Female**	***Salmonella* sp**	***Salmonella typhi***
Gwamna Awan General Hospital	10(21.3)	8(17.0)	3(18.7)	3(18.7)	18(38.3)	6(37.4)
Dantsoho Memorial Hospital	10(21.3)	6(12.7)	3(18.7)	2(12.5)	16(34.1)	5(31.2)
General Hospital Sabon Tasha	4(8.5)	3(6.4)	1(6.3)	2(12.5)	7(14.9)	3(18.8)
Sefa Specialist Hospital	2(4.3)	1(2.1)	1(6.3)	0(0.0)	3(6.4)	1(6.3)
Barau Dikko Specialist Hospital	2(4.3)	1(2.1)	1(6.3)	0(0.0)	3(6.4)	1(6.3)
Total	**28(59.7)**	**19(40.3)**	**9(56.3)**	**7(43.7)**	**47(100.0)**	**16(100.0)**

Table.4 Distribution of *Salmonella* sp. and *Salmonella typhi* among male and female patients of different age groups

Age	*Salmonella sp.*		*Salmonella typhi*		Total (Male and Female)	
	Male	**Female**	**Male**	**Female**	***Salmonella* sp**	***Salmonella typhi***
≤ 10	12	8	4	1	20	5
11-20	2	3	2	1	5	3
20-30	3	1	1	0	4	1
30-40	4	1	0	1	5	1
41- above	7	6	2	4	13	6

Table.5 *In vitro* activity of conventional antimicrobial agents against *Salmonella typhi* strains

S/N	Antibiotic	Disc content (µg)	Isolates Susceptibility Interpretative Trend															
			ST1	ST2	ST3	ST4	ST5	ST6	ST7	ST8	ST9	ST10	ST11	ST12	ST13	ST14	ST15	ST16
1	AMX	25	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
2	AMC	3	R	R	R	R	R	R	R	R	R	R	R	R	I	I	I	R
3	CRO	30	I	I	R	I	R	R	R	S	I	S	R	R	S	I	S	R
4	CEf	30	R	R	R	R	S	R	R	R	R	S	S	R	S	S	S	S
5	C	30	S	S	S	S	I	S	I	R	S	I	R	S	S	S	S	I
6	TET	25	R	R	S	R	R	S	R	R	R	R	R	S	R	R	R	S
7	COT	25	R	R	R	S	S	R	R	R	R	S	R	R	R	R	S	S
8	NA	30	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	S
9	OFL	5	S	S	S	S	S	S	S	S	S	S	R	S	R	S	S	S
10	CIP	5	I	I	S	I	S	I	S	R	I	S	I	S	I	R	S	S
11	IPM	10	S	I	S	S	S	S	I	I	S	I	I	S	S	I	S	S
12	NIT	300	S	S	S	S	R	S	R	R	S	S	I	S	S	S	I	R

Key**:** ST = *Salmonella typhi*, S/N = Serial number, R = Resistant, I = Intermediate, S = Susceptible, AMX = Amoxicillin, AMC = Amoxicillin/Cluvalanic acid, CRO = Ceftriaxone, CEF = Cefotaxime, C = Chloramphenicol, TET = Tetracycline, COT = Cotrimoxazole, NA = Nalidixic acid, OFL = Ofloxacin, CIP = Ciprofloxacin, IPM = Imipenem, and NIT = Nitrofurantoin

Table.6 Resistance pattern of *S. enterica* serovar Typhi isolates against the conventional antimicrobial agents

Isolates	Resistance patterns
ST1, ST2, ST4,ST9	AMX, AMC, CEF, TET, COT
ST3, ST6,ST12	AMX, AMC, CRO, CEF, COT
ST5	AMX, AMC, CRO, TET, NIT
ST7	AMX, AMC, CRO, CEF, TET, COT, NIT
ST8	AMX, AMC, CEF, C, TET, COT, CIP, NIT
ST10	AMX, AMC, TET
ST11	AMX, AMC, CRO, C, TET, COT, OFL
ST13	AMX, TET, COT, NA, OFL
ST14	AMX, TET, COT, NA, CIP
ST15	AMX, TET, NA
ST16	AMX, AMC, CRO, NIT

AMX - Amoxicillin, AMC- Amoxicillin/Cluvalanic acid, CRO - Ceftriaxone, CEF = Cefotaxime, C = Chloramphenicol, TET = Tetracycline, COT = Cotrimoxazole, NA = Nalidixic acid, OFL = Ofloxacin, CIP = Ciprofloxacin, IPM = Imipenem, and NIT = Nitrofurantoin

Figure.1 Map of Kaduna metropolis showing selected hospitals

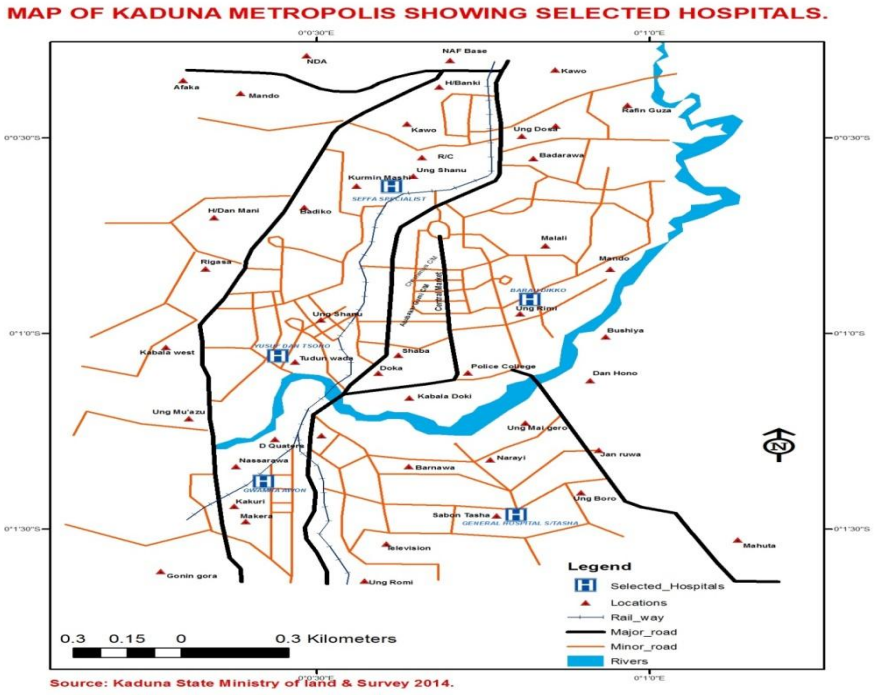
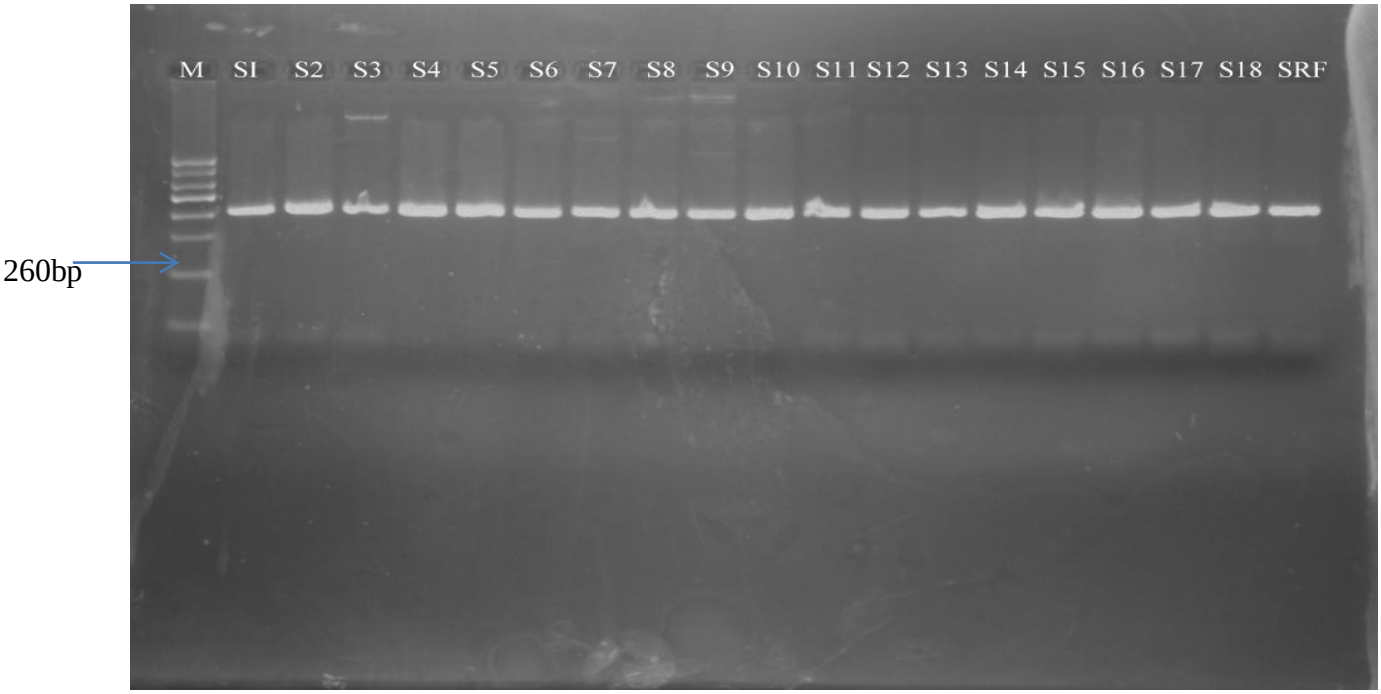


Plate.1 Gel electrophoresis showing 204bp ompC genes of some *Salmonella* sp. on a 50kb marker



Arora (2011) recorded that *Salmonella* sp. is shed in the feaces throughout the course of the disease and even in convalescence, with varying frequency, and that during the first week of the illness, salmonellae can be isolated from the feces in about half the cases and they are most easily isolated between third to fifth weeks of illness.

All the sixteen *S. enterica* serovar Typhi isolates showed multidrug resistance to ciprofloxacin, ofloxacin, tetracycline, cotrimozaxole, amoxicillin, amoxicillin/ clavulanic acid, nalidixic acid, ceftriazone, cefotaxime, nitrofurantoin and chloramphenicol with a record of highest (100%) resistant to amoxicillin and lowest resistant (12.5%) to ciprofloxacin, ofloxacin and chloramphenicol and zero (0%) resistant to imipenem. The resistance of *S. enterica* serovar Typhi isolates to these antimicrobials agents is of clinical significance. According to Arora (2011) and Srirangaraj *et al.* (2014), ceftriaxone, cefotaxime, and cefoparazone of the third generation cephalosporins are effective therapeutic alternative in multidrug resistant *S. enterica* serovar Typhi infected cases and there is a changing trend in susceptibility pattern of *S. enterica* serovar Typhi worldwide with emergence of resistance to fluoroquinolone, and consequently reduced the therapeutic options available. Resistance strains of *S. enterica* serovar Typhi to chloramphenicol and cotrimoxazole have emerged and continue to be of clinical significance (Akinyemi *et al.*, 2005; Kariuki *et al.*, 2010). Also, an antibiogram of *Salmonella typhi* in Pondicherry shows resistance of *Salmonella typhi* to chloramphenicol, cotrimoxazole, and nalidixic acid (Madhulika *et al.*, 2014).Resistance is transferable to other organisms, and they are metabolically normal. Transferable drug resistance involves all antibiotics in common use and transfer of drug resistance occurs *in vitro*, it also occurs *in vivo* but in normal intestines it is inhibited by several factors like anaerobic condition, bile salts, alkaline pH, and abundant anaerobes. But in intestines of persons on oral antibiotics therapy, transfer occurs readily due to selection pressure provided by the drug. However, because of the overuse of antibiotics in the hospitals it is said 'hospital is the heaven for drug resistant bacteria. Most resistance of concern are associated with R plasmid (Panigrahi *et al.*, 1996).

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