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Detection of Mutation in *gyrA* and *parC* Gene on Resistant *Salmonella enterica* Serovars. Isolated from Two Hospitals in South East Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors NEO and OEA designed the study, while author NEO performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author OEA supervised the laboratory work. Authors NEO and TIM managed the analyses of the study. Authors NEO, TIM, GNU and JCO managed the literature searches. All authors contributed financially, read and approved the final manuscript.

Research Article

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ABSTRACT

Aim: To detect the resistant pattern and existence of the genes responsible for floroquinolone-resistant in the quinolone-resistant determining regions (QRDR's) of *S. enterica serovars.Typhi.*
Study Design: The Stool samples from Patients with symptoms of enteric fever, from different units and wards from two hospitals in Southeast region of Nigeria, were used for the surveillance.
Place and Duration of Study: The study was carried out in the Department of Microbiology and Biotechnology, Nigerian Institute for Medical Research, Lagos, between July and

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December, 2011.
Methodology: 50 isolates of *Salmonella enterica* serovar.*Typhi* were screened for the antibiotics susceptibility pattern, using multidisc agar diffusion and E-test. Double disc synergy Test (DDST) reported the presence of ESBL's strains. The DNA amplification was performed by PCR using HOT FIREPol ® DNA polymerase with 25mMMgcl$_2$. DNA – sequencing of the (QRDR'S) of *gyrA* (n= 32) and *parC* (n=3), was performed using sanger sequencing ABI 3730 x l, Applied Biosystems.
Results: A total of 39(78%) of the *S. enterica* produced *β*-lactamase. ESBL's positive strains were 17(34%) and 46(92%) isolates were Multi-Drug Resistant *S. typhi* (MDRST). Sequencing of the mutations in *gyrA* gene of the (QRDR's) was at Asp-87- Gly and Asp-87- Asn or at Ser-83- Tyr, while mutations in *parC* 3(6%), were at Asp-87- Gly.
Conclusion: Chromosomal encoded ESBL's and mutations were found to be responsible for the MDRST. Ceftriazone and levofloxacine were found to be significant alternatives in treating *S. enterica* serovar.*Typhi*. This is the first report of mutation in both *gyrA* and *parC* genes in *S. enterica serovar.Typhi* in Southeast Nigeria.

Keywords: S. enterica serovars.Typhi.; parC; gyrA; mutation; ESBL's; floroquinolone.

1. INTRODUCTION

Salmonella are gram negative, motile aerobic rods that characteristically ferment glucose and manose but fail to ferment lactose or sucrose; they are pathogenic for humans or animals by the oral route. DNA hybridization studies have now shown that all pathogenic *Salmonellae* belong to a single species, *Salmonella enterica* [3]. S. *enterica* subsp. *enterica* has over 2000 serovars which can cause disease in humans [3]. Antibiotics resistant *Salmonella* are of global concern because they affect both developed and developing countries due to increased international travel [27]. These concerns have been further reinforced in recent years by the emergence of antimicrobial resistance among the major groups of the enteric pathogens [27]. The resistance of gastroenteric *Salmonella* strains to these antimicrobial agents is in large part due to the production of extended-spectrum β-lactamases (ESBLs) encoded on plasmids, as well as on the chromosome, as reported by David and Frank; Yujuan and Ling; Yah [6,28,27].

The development of resistant *S. typhi* strains to antibiotics such as those historically used to treat *Salmonella* infections has forced physicians to prescribe fluoroquinolones or third-generation cephalosporin [17]. However, extrapolation from data in literatures suggests that quinolone resistance is likely to develop unless use of this drug class is restricted [17]. Among *Salmonellae*, and especially *Salmonella enterica* serovars *Enteritidis*, Hadar, *typhimurium* and Virchow, there have been reports of an increase in quinolone resistance [25], and in a previous study it was discovered a geographically dependent distribution of *gyrA* mutation at codon 83 and 87 in *S.hadar* [15]. Earlier studies have observed that in *Salmonellae*, the relative frequency of different mutations in *gyrA* was dependent on the quinolone antibiotic used for selection [14,12], and that the position and type of amino acid substitution in *gyrA* varied with the serovars [12]. The rate of fluoroquinolone resistance in south and Southeast Asia and to some extent, in East Asia is generally high and on the increase [1]. Fluoroquinolones have become the first-line drugs for the treatment of typhoid fever [13]. Thus they are active drugs against isolates of the *Salmonella species* [11]. There are several reports, however, of treatment failures when these antimicrobials have been used to treat *Salmonella* infections caused by strains with reduced susceptibility [11]. The

mutations that are responsible for fluoroquinolone resistance is in the *gyrA*, *gyrB*, *parC*, and *parE* genes of *Salmonella enterica serovar.Typhi* and serovar *Paratyphi A* [13]. Fluoroquinolone resistance in *Salmonella enterica* is of clinical importance because ciprofloxacin is the drug of choice for treating invasive human salmonellosis [8]. Fluoroquinolone resistance in *S. enterica* is usually mediated by at least one mutation in a DNA topoisomerase gene. However, in clinical human and veterinary isolates of *Salmonella* spp., mutations are usually confined to *gyrA* [21]. A single mutation in *gyrA* on its own is not sufficient for clinical resistance to fluoroquinolones, though a *gyrA* mutation is a good marker indicating that fluoroquinolones should not be chosen for treating the respective infection [21]. For these reason, this research was designed to find current drugs of choice for the treatment of enteric fever caused by *S. enterica serovars.Typhi.,* and possible resistant factors due to mutation as often specified for *Salmonella* species and the presence of the genes responsible for this drug resistance in Nigeria, especially in the Southeast region where much data concerning the use of floroquinolone as its drug of treatment has not been documented or published.

2. MATERIALS AND METHODS

A total of 65 stool samples were randomly collected from patients diagnosed with enteric fever, using sterile plastic bottles from several units and wards in two hospitals. The samples are from the routine section of the Medical Microbiology Laboratory in the Federal Medical Centre, Owerri and Umuahia from the Southeast part of Nigeria between July and December, 2011.

After investigation, Fifty *Salmonella enterica* serovars were identified and screened (25 each from unrelated patients from both hospitals) by standard biochemical characterization, using microbact® identification kit-12E (oxoid-England). The antibiotic- sensitivity screening of the isolates (using macfarland standard of 0.5ml dilution) was carried out by the multidisc agar diffusion method and E-test (Oxoid, India) on 20ml molten muller Hilton Agar using antibiotics disc (oxoid, India) [4] with a standard ATCC 14028 strain of *S enterica* serovar typhi.

2.1 Beta-lacatamase Production Test Using Nitrocefin Sticks

The nitrocefin™ sticks (oxoid, India) is a chromogenic cephalosporin β-lacatamase indicator in a container [18]. The nitrocefin sticks was allowed to reach room temperature after removing it from the freezer. Then a well separated representative colony from the primary isolation medium was selected. The nitrocefin stick (colour coded black) is removed from the container and holding the coloured end, the colony is touched with the impregnated tip of the stick, which is rotated to pick up a small mass of cells. The inoculated tip of the stick is placed between the lid and the base of the inverted plate. The reaction requires moisture, so the inoculated tip of the stick is placed in the moisture condensate in the lid or if condensate is not available in the inverted lid, one or two drops of distilled water were added to the lid to moisten the tip of the stick. The reagent was examined (impregnated tip of the stick) for up to five minutes and, if negative it was re-examine after fifteen minutes. A positive reaction was shown by the development of a pink/red color. No color change was observed with organisms that do not produce beta-lactamase. The unused nitrocefine stick was used as control.

2.2 ESBL's Detection using Double Disc Synergy Test (DDST)

The test inoculum (0.5 McFarland turbidity) was spread onto Mueller-Hinton agar (MHA;Oxoid, India) using a sterile cotton swab. A disc of (20µg amoxycillin + 10µg Clavunalic acid) was placed on the surface of MHA; then discs of ceftriaxone (30µg), Ceftazidime (30µg) and Cefotaxime (30µg) were kept around it in such a way that each disc was at distance ranging between 10 and 15 mm from the amoxil-clavulanic acid disc (centre to centre). The plate was incubated at 37ºC overnight. The organisms were considered to be producing ESBL when the zone of inhibition around any of the Broad-spectrum cephalosporin discs showed a clear-cut increase towards the amoxil-clavulanic acid disc [27].

2.3 DNA Extraction, Quantification and Purity Test

The 1.5ml of an overnight broth culture was pipetted into eppendorf tubes and centrifuged at 5xg. The pellets were washed twice using sterile distilled water. Then the mixture was resuspended in 100 µl sterile distilled water, vortexed and placed in a water block to boil for 10-20 mins at 100^{o}C. After boiling, the mixture was then centrifuged for 10 mins. The purified chromosomal DNA pellets were transferred into a new tube and stored on ice. The spectrophotometer lens (Nano drop ND1000) was used to measure the purity level of the DNA by adding 0.2 µl drop of the DNA sample on the spectrophotometer lens. The readings were viewed on the computer screen. Purity levels used were between 1.5 - 1.8 or 2 µl (data not shown).

2.4 PCR Amplification and Agarose gel Electrophoresis

The purified DNA template in PCR eppendorf tubes (2.0µl DNA) was mixed with the Master mix ready to load (HOT FIREPol $^{®}$ DNA polymerase with 2.5mM $Mgcl_2$, Solis Biodyne). The mixture of primers, DNA and the Master Mix was vortexed, to mix and then centrifuged before introducing it into the PCR machine (Eppendorf-Germany). After amplification, the PCR products were then taken for electrophoresis (CBS.Scientific Company Inc.) on the agarose gel at 80-100volts and finally viewed on the UV light for visible amplified image of the genes. A DNA-marker of 100 base pairs was used.

The Polymerase Chain Reaction were performed under the following conditions with the (Solis biodyne 5x HOT FIREPol ®, germany) Master mix Ready to load. The thermocycling conditions for *gyrA* were 30 cycles of 95ºC for 30 secs., 42ºC for1 mins, 72ºCfor 1 mins, and 95ºC for 30 sec., (PCR timing 12.38 - 2.34hrs.). The specific primers used to amplify the quinolone resistant determining regions (QRDR's) were designed from accession number sequences X78977 and M68936 as follows;

gyrA- F (5'CGT TGG TGA CGT AAT CGG- 3'), R(5'CCG TAC CGT CAT AGT TAT- 3', while *parC* is F(5'CTA TGC GAT GTC AGA GCT GG-3'), R(5'TAA CAG CAG CTC GGC GTA TT-3' (designed by Fermenters Inc.). The resulting amplicon sizes were 251 and 260 base pairs for *gyrA* and *parC* respectively.

2.5 DNA Sequencing

The PCR products containing the gene of interest were selected for DNA sequencing, with the same primer sequence at Gatc-Biotech, Germany using Sanger sequencing (ABI 3730 x

l, Applied Biosystems) automatically edited with PhredPhrap. The Mega-version 5 soft ware was used for the alignment of the gene and the blasting was done using the NCBI database BLAST [24].

3. RESULTS

The results of fifty clinical isolates of *Salmonella enterica* serovars. *Typhi*, obtained from stool sample from both male and female patients were as shown (Table 2). The isolates from the IPU department in the second hospital had the highest number of patients infected with *S. enterica*, as compared to the first, hospital. The GOPD had the highest isolates recovered from patients in the first hospital sampled, followed by isolates recovered from patients coming for the National Health Insurance Scheme(NHIS) unit, Children out patients (CHOP) and Emergency patients units (EPU) in the same hospital (Table 1).

Table 1. Prevalence of *S. enterica* in various departmental unit and hospitals in the south-east region of Nigeria

S/No	Unit/ Department	Location (Ow) *S. enterica (1-25)*	Location(Um) *S. enteric (26-50)*
1.	NHIS	5(20%)	0(0%)
2.	IPU	4(16%)	23(92%)
3.	GOPD	14(56%)	2(8%)
4.	CHOP	1(4%)	0(0%)
5.	EPU	1(4%)	0(0%)

KEY: NHIS = National Health Insurance Scheme, IPU= In - Patience Unit, GOPD= General Out Patience Department, CHOP= Children Out Patience, EPU= Emergency Patience Unit, SKIN= Skin Clinic, OW = Owerri, Um = Umuahia.

Table 2. Epidemiological distribution of *S. enterica* serovars in relation to age range and gender of patients infected in the two hospitals

Age limits	Number of Patients infected	
	Male	Female
0-10	0	3
11 -20	15	24
21- 30	1	3
31- 40	0	0
41- 50	1	2
51- 60	0	1
Total	17(34%)	33(66%)

The antibiotics susceptibility test reveals that the *S. enterica* were resistant to Co-trimoxazole 31(62%), Chloramphenicol 37(74%), Sparfloxacin 35(70%), amoxicillin-clavulanic acid 45(90%), Streptomycin 33(66%), Ceftazidime 21(42%), Pefloxacin 9(18%), Ofloxacin 8(16%), Amoxicillin 35(74%), Gentamycin 16(32%). It was observed that only a low percentage was resistant to Ciprofloxacin 6(12%), Levofloxacin 4(8%), Ceftriaxone 5(10%), and Cefotaxime 6(12%) (Table 3 and 4). The β-lactamase analysis shows that 39(78%) of the *S. enterica* serovars.Typhi produced the enzyme, while only 11(22%) were negative. Seventeen (17) of the isolates were positive for Extended Spectrum β-lactamase enzyme ESBL's (Table 3 and 4).

Table 3. Phenotypic resistance pattern in relation to *β*- lactamase, ESBL's and other resistant genes in *S. enterica* serovar.*Typhi* from FMC Owerri

Code S/no	Number of antibiotics	Resistance phenotypic pattern	β-lactamase/ ESBL positive	Presence *gyrA / parC*
OW1.	5	SP,AM,STR,CAZ,AMC	+ve	Nil/ Nil
OW2.	2	CH,AMC	+ve	Nil/ Nil
OW3.	2	CH,AMC	+ve / ESBL	+ve/ +ve
OW4.	3	SXT,CH,CAZ	-ve	Nil/ Nil
OW5.	7	SXT,CH,SP,AM,STR,CAZ,AMC	+ve / ESBL	Nil/ Nil
OW6.	4	LEV,CTX,CAZ,AMC	+ve	Nil/ Nil
OW7.	3	SXT,CH,AMC	+ve	Nil/ Nil
OW8.	2	SXT,CAZ	+ve	Nil/ Nil
OW9.	8	SXT,CH,SP,CPX,AM,STR,CAZ,AMC	+ve / ESBL	+ve/ Nil
OW10.	7	SXT,CH,SP,CPX,AM,CAZ,AMC	+ve / ESBL	Nil/ Nil
OW11.	9	SXT,CH,SP,CPX,AM,GEN,STR,CAZ,AMC	+ve / ESBL	+ve/ Nil
OW12.	9	SXT,CH,SP,CPX,AM,GEN,STR,CAZ,AMC	+ve / ESBL	Nil/ Nil
OW13.	7	SXT,CH,SP,AM,GEN,STR,AMC	+ve	+ve/ Nil
OW14.	10	SXT,CH,SP,AM,STR,LEV,CRO,CTX,CAZ,AMC	+ve / ESBL	+ve/ Nil
OW15.	6	SXT,CH,SP,AM,STR,AMC	+ve / ESBL	+ve/ Nil
OW16.	7	SXT,CH,SP,AM,STR,CAZ,AMC	+ve / ESBL	+ve/ Nil
OW17.	8	SXT,CH,SP,AM,GEN,STR,CAZ,AMC	-ve / ESBL	Nil/ Nil
OW18.	10	SXT,CH,SP,CPX,AM,GEN,PEF,OFX,STR,AMC	-ve	+ve/ +ve
OW19.	10	SXT,CH,SP,CPX,AM,GEN,PEF,OFX,STR,AMC	+ve	+ve/ +ve
OW20.	8	SP,AM,STR,LEV,CRO,CTX,CAZ,AMC	+ve / ESBL	Nil/ Nil
OW21.	5	SXT,SP,AM,STR,AMC	+ve	+ve/ Nil
OW22.	3	AM,STR,AMC	-ve	+ve/ Nil
OW23.	4	AM,STR,CAZ,AMC	-ve / ESBL	+ve/ Nil
OW24.	6	AM,STR,CRO,CTX,CAZ,AMC	+ve / ESBL	+ve/ Nil
OW25	4	CH,AM,CAZ,AMC	+ve	+ve/ Nil

***KEY**: **SXT**= Co-trimozaxole, **CH**= Chloramphenicol, **SP**= Sparfloxacin , **CPX**= Ciprofloxacin, **AM**= Amoxicillin , **GEN**= Gentamycin, **PEF**= Pefloxacin, **OFX**= Ofloxacin, **STR**= Streptomycin, **LEV**= Levofloxacin, **CRO**= Ceftriaxone, **CTX**= Cefotaxime, **CAZ**= Ceftazidime, **AMC**= Amoxicillin-clavulanic acid, **+ve** = Positive, -ve = Negative, **ESBL**= Extended Spectrum Beta Lactamase, Nil = Not present, OW= Owerri.*

Table 4. Phenotypic resistance pattern in relation to *β*-lactamase, ESBL's and other resistant genes in *S. enterica* serovar.*Typhi* from FMC Umuahia

Code S/no	Number of antibiotics	Resistance phenotypic pattern	β-lactamase/ ESBL positive	Presence *gyrA /parC*
U26.	5	CH,AM,STR,CAZ,AMC	-ve/ ESBL	+ve/ Nil
U27.	6	SXT.CH,SP,AM,STR,AMC	+ve	Nil/ Nil
U28.	7	SXT,CH,AM,GEN,PEF,OFX,STR	+ve	+ve/ Nil
U29.	3	AM,CAZ,AMC	-ve	Nil/ Nil
U30.	6	SXT.CH.SP,AM,STR,AMC	-ve	+ve/ Nil
U31.	1	AM	+ve	+ve/ Nil
U32.	6	SXT,CH,SP,AM,STR,AMC	+ve	+ve/ Nil
U33.	7	SXT,SP,AM,PEF,OFX,CAZ,AMC	+ve/ ESBL	+ve/ Nil
U34.	4	CH,GEN,STR,AMC	-ve	Nil/ Nil
U35.	5	SXT,CH,SP,AM,AMC	+ve	+ve/ Nil
U36.	4	SXT,CH,SP,AMC	+ve	+ve/ Nil
U37.	5	CH,SP,AM,STR,AMC	-ve	+ve/ Nil
U38.	6	SXT,SP,AM,STR,CAZ,AMC	-ve /ESBL	Nil/ Nil
U39.	6	CH,SP,AM,GEN,PEF,STR	+ve	+ve/ Nil
U40.	11	SXT,CH,SP,AM,GEN,PEF,OFX,STR,CRO,CTX,AMC	+ve/ ESBL	+ve/ Nil
U41.	5	SXT,CH,SP,GEN,AMC,	+ve	Nil/ Nil
U42.	4	CH,SP,GEN,AMC,	-ve	+ve/ Nil
U43.	5	SXT,CH,SP,GEN,AMC	+ve	Nil/ Nil
U44.	9	SXT,CH,SP,AM,GEN,PEF,OFX,STR,AMC	+ve	+ve/ Nil
U45.	3	CH,SP,AMC	+ve	+ve/ Nil
U46.	5	SXT,CH,SP,STR,AMC	+ve	+ve/ Nil
U47.	4	SP,AM,STR,AMC	+ve	+ve/ Nil
U48.	7	SXT,CH,SP,PEF,OFX,STR,AMC	+ve	Nil/ Nil
U49.	11	CH,SP,GEN,PEF,OFX,STR,LEV,CRO,CTX,CAZ,AMC	+ve	+ve/ Nil
U50	7	SXT,CH,SP,AM,GEN,STR,AMC	+ve	+ve/ Nil

KEY: SXT= Co-trimozaxole, CH= Chloramphenicol, SP= Sparfloxacin , CPX= Ciprofloxacin, AM= Amoxicillin , GEN= Gentamycin, PEF= Pefloxacin, OFX= Ofloxacin, STR= Streptomycin, LEV= Levofloxacin, CRO= Ceftriaxone, CTX= Cefotaxime, CAZ= Ceftazidime, AMC= Amoxicillin-clavulanic acid, +ve = Positive, -ve = Negative, ESBL= Extended Spectrum Beta Lactamase, Nil = Not present, U = Umuahia.

The polymerase chain reaction analysis shows that 32(64%) of the *S. enterica serovar.Typhi* possessed mutation in the *gyrA* gene, coding for point mutation in the quinolone resistant determining region QRDR's, while 3(6%) of the total isolates had mutation in the *parC* gene that codes for double mutation (topoisomerase iv enzyme), out of which 15(30%) isolates were resistant to at least two to four antibiotics. It was also discovered that only three (3) of the isolates had mutation in both *gyrA* and *parC* gene each on their chromosomes, while others have either mutation in *gyrA* or *parC* gene as chromosomally distributed (Figs.1 & 2).

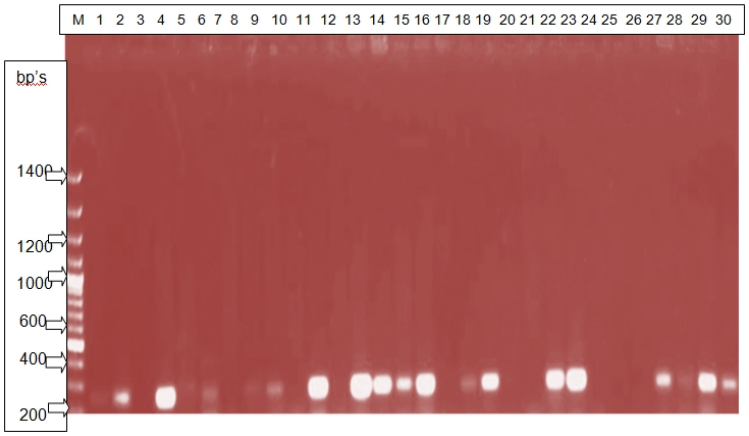

Fig. 1. Agarose gel electrophoresis pattern showing single PCR amplified products of gene mutation of *gyrA* from *S. enterica* serovar. Typhi. Lane1-30 showed some of the isolates from both hospitals with lane 1,2,4,6,8,9,11,13,14,15,16,18,19,22,23,27,28,29, and 30 having amplicons size (251bp's) positive for *gyrA* genes (codes for point mutation) in the DNA of *S. enterica* serovar. *Typhi*. The Lane M shows DNA molecular marker (100- bp ladder).

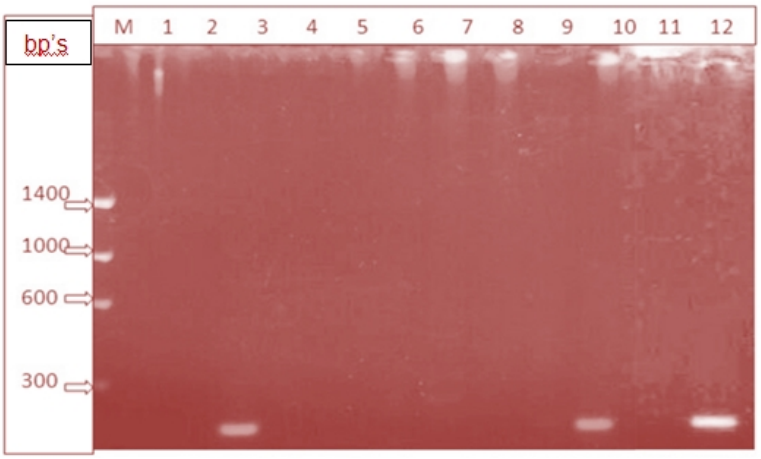

Fig. 2. Agarose gel electrophoresis pattern showing single PCR amplified products of gene mutation of *parC* from *S. enterica* serovar. Typhi. Lane 1-12 with lane number 3, 10 and 12 having amplicons size (260bp's) positive for *parC* (coding for double mutation) genes in the DNA of *S. enterica* serovar. *Typhi*. Lane M shows DNA molecular marker (100- bp ladder).

4. DISCUSSION

Salmonella typhi, a potentially lethal organism was successfully managed with introduction of chloramphenicol. Since then, the emergence of resistant strains began and now Multi-Drug resistant *Salmonella typhi* (MDRST) has become a real challenge especially in the developing world like Nigeria. There have been reports from different parts of the world about resistance pattern. In this study some of the isolates were found to be resistant to the three fist- line anti-salmonella drugs such as Co-trimoxazole, Chloramphenicol, and Amoxicillin-clavulanic acid. Some of the isolates were susceptible to Levofloxacin 46(92%), Ciprofloxacin 44(88%), Ofloxacin 42(84%) and Pefloxacin 41(82%). However, reduced susceptibility of some strains to floroquinolone and also multi-drug resistant *Salmonella enterica serovar.Typhi* (MDRST) were found (Table 3 and 4). Thus this trend of result supports the work of some authors [22], when they stated that most reports from developing countries are showing MDRST strain [22]. The survey also showed that the female patients were more infected with *S.enterica*, especially with the age range between 11-20 years (Table 2) in this study. The high rate of isolates from the in-patients units (IPU) and General out patients department (GOPD) (Table 1), revealed that there could have been easy clonal dissemination of individual multidrug resistant *S. enterica serovar.Typhi* or from transfers of R-plasmids-encoding genes that produces Extended Spectrum β-lactamase Enzyme such as CTX-M, TEM or SHV type of this enzymes [19]. Also the production of β-lactamase enzymes have been reported to destroys Cephalosporin by hydrolyzing their β-lactam nucleus [20].

It has been reported [23], that the first CTX-M-type *β*-lactamases were identified as plasmid-encoded enzymes in clinical isolates from the Enterobacteriaceae, and that all 30 isolates of *Klebsiella pneumonia* in their study in Nigeria, produced at least one β-lactamase and 17 (57%) produced a CTX-M *β*-lactamase [23].

From the report observed in this study (Table 3 and 4), most of the resistant were mediated through these β-lactamases and the ESBL's enzymes identified, either encoded on the plasmids or in the chromosomes, therefore confirming the reports of other authors [28,26,27]. Many reports have been put forward by several authors [1], that floroquinolones are highly effective against susceptible organism, yielding a better cure rate than Cephalosporins. They also stated that unfortunately, resistance to first generation floroqninolones is widespread in many parts of Asia [1]. Furthermore, they stated that in recent years, third generation Cephalosporins have been used in regions with high floroquinolones resistance rates, particularly in South Asia and Vietnam [1]. In this study, analysis showed that both the third generation cephalosporin and the floroquinolones are still maintaining their choice as a replacement for the treatment of chloramphenicol -resistant strain of *S. enterica* serovars typhi in this part of Nigeria, as ceftriaxone was observed to exhibit a very high effect on this isolates. Nevertheless, it has been reported that fluoroquinolone resistance in *S. enterica* is usually mediated by at least one mutation in a DNA topoisomerase gene. Also no organisms was observed to be resistant to ciprofloxacin in the isolates from FMC Umuahia (Table 4), and none of the *S. enterica* serovars possessed the mutation in *parC* gene. However, in clinical human and veterinary isolates of *Salmonella* spp., mutations are usually confined to *gyrA* [21]. In this study the mutation detected were found in *gyrA* and in *parC* resistant genes in the chromosomes of the *S.enterica* serovars which codes for point and double mutations respectively (Table 3). It was observed that two of the strains that harbored the two resistant gene of *gyrA* and *parC* showed complete resistant to ciprofloxacin, ofloxacin and other quinolones except levofloxacin used in this study (Table 3). It is also interesting to note that one of the isolates

harboring both mutation in *gyrA* and *parC* were not resistant to any of the quinolones in this study, and it also showed susceptibility to all antibiotics screened, except chloramphenicol and amoxicil-clavulanic acid. This may be as a result of the presence of the enzyme β-lactamases, harbored by this organism or any other resistant gene not detected. Secondly, it could have been that the gene been expressed was not coding for any resistant in the floroquinolone resistant determining region or thirdly, the organism exhibited an efflux pump mechanism of resistant to these two drugs. This trend of results showed that there have been some forms of resistant mutants along the *S. enterica serovars'* family, and that the presence of mutations in *gyrA* gene of the isolates showed that the resistance to floroquinolone is evolving in an ominous direction as reported by Brusch et al. [1].

However, a single mutation in *gyrA* on its own is not sufficient for clinical resistance to fluoroquinolones [1], but a *gyrA* mutation is a good marker indicating that fluoroquinolones should not be chosen for treating the respective infection [21]. Though none of the *S. enterica* serovars was from animals, the presence of this genes on the chromosomes of this *S. enterica serovar.Typhi* could be as a result of ingestion of Livestock feeds, poultry bird, fish etc, that has been treated with this drug in animal husbandry [10], or the patients may have been using this drugs based on indiscriminate prescription by the doctors or indiscriminate administration of the quinolones drugs, as also reported by some authors [5] in their study. This implies that these patients in whom this isolates were recovered from, especially the female patients may have been on floroquinolone treatment for a long time. Also it could be as a result of nosocomial infection as most of the patients were from the GOPD and IPU units of the hospitals. Also reports have it that *S.typhi* most commonly develops floroquinolone resistance through specific mutations in *gyrA* and *parC*, which codes for the binding region of DNA gyrase and topoisomerase iv respectively [1].

Also, it has been stated that transferable resistant to quinolones is sometimes rare in bacteria invivo, but clonal or resistance due to mutation in chromosomal gene remains the potential mechanisms accounting for high level of reduced floroquinolone susceptibility in Southeast Asia [11]. These trends of results or reports are applicable to the analysis obtained in this study, as the mutations found were chromosomally mediated resistant gene. In this study, the mutations that are responsible for the floroquinolone resistance in the *gyrA* and *parC* genes of the *Salmonella enterica* serovars were investigated and the sequences for the Quinolone Resistant Determining Region (QRDR) of the *gyrA* gene of the isolates which showed reduced susceptibility to some floroquinolone were detected and analysis revealed single mutation at the Ser-83 – Tyr , Ser-87- Gly and Ser-83- Phe, while some were found in Asp-87- Gly or Asp-86- Gly in *parC* gene and sequence analysis also revealed that some of the position of the amino acids in the *gyrA* mutation were identified as Asp-87- Asn or at Ser-83- Tyr. This reports were in line with the works of some authors [13], when they stated that a single mutation at either the Ser-83 or the Asp-87 codon were found after sequencing the genes, but no mutations were found in the *gyrB, parC*, and *parE* genes of *S.enterica* serovar typhi and Serovar paratyphi A [13], but in this study, *parC* mutations was found. This indicates that *gyrA* mutations are of principal importance for the floroquinolone resistance of serovars typhi and paratyphi A among the *Salmonellae* [13]. In another study, it was reported that a single *gyrA* mutation in Ser-83-Phe or Ser-83- Tyr [9] was associated with reduced susceptibility to Ciprofloxacin with MIC's of 0.125- 1.0 mg/L [9]. Similarly, in this study reduced susceptibility were observed in Ciprofloxacin and Levofloxacin with MIC ranging from 1.56- 1.25µg/L (data not shown) in both drugs which also correlates with the mutation in *gyrA* detected in this study.

5. CONCLUSION

The emergence of antimicrobial resistance in any part of the world may have a global bearing and thus deserves universal attention [11]. However, in this study it could also be suggested that extensive use and abuse of floroquinolone in human diseases could be responsible for the rapidly increasing quinolone resistance of *Salmonella enterica* in this part of Nigeria as observed. It is also interesting to know that from this study, one of the floroquinolones and a Cephalosporine still retains their potency and thus may be the drug of choice for *Salmonella* infections. Therefore, prescription drugs should be restricted to only medical personnel, and appropriate dispensing techniques should be adopted in every hospital to avoid under dosage or over dosage. Finally, hand washing in the hospital or at home is also recommended as a useful, safe and aseptic technique to patients, in order to prevent the transmission of nosocomial infections or diseases such as Typhoid fever caused by *Salmonella enterica* species, especially in Southeast Nigeria.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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