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Molecular Detection of Plasmid-Mediated Quinolone Resistance in Salmonella Typhi Isolated from Stool of Patients Attending Selected Primary Health Centres in Kaduna Metropoilis, Nigeria

Attah Ambrose Abuh*, Ishaleku David, Nkene Istifanus Haruna, Igbawua Isaac Nyiayem, Datok Danladi Walong

Department of Microbiology, Nasarawa State University, PMB 1022, Keffi, Nasarawa State

***Corresponding Author**

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ABSTRACT

Fluoroquinolones have been the drug of choice for the treatment of *Salmonellla* infections in humans and animals. The emergence of fluoroquinolone resistant *Salmonella* strains has posed significant treatment challenges worldwide. This research is aimed at detecting the Plasmid mediated quinolone resistant genes of *Salmonella typhi* isolated from stool samples of patients attending selected Primary Health Centres in Kaduna metropolis. Methodology: A total of 200 stool samples were randomly collected from patients with suspected Typhoid fever using standard protocols. *Salmonella typhi* was isolated from the samples using standard culture and microbiological techniques. Antibiotics susceptibility testing was done using the disc diffusion method to investigate the ability of *Salmonella typhi* to resist the tested antibiotics. The bacterial genome was extracted and resistant genes were detected using PCR. Results. Out of the 200 stool samples, 61(30.5%) *Salmonella typhi* was isolated. Out of the 61 isolates, 44 (72.1%) carried resistant genes of which 6 of the isolates carried the Ciprofloxacin resistance genes. The genes that were discovered mainly were *QnrB, aac(6')-Ib-cr-R* and *QepA*. Conclusion: The antibiotic susceptibility testing of *Salmonella typhi* in this study showed an increasing trend of plasmid mediated quinolone resistance (PMQR). The allele *B* of *qnr* gene was found to be the predominant cause of PMQR in this study.

Key words: *Salmonella*, Fluoroquinolones, Plasmid, Resistance genes, Antibiotics.

INTRODUCTION

Salmonella typhi (*S. typhi*) and *Salmonella paratyphi* (*S. paratyphi*) are the major causes of enteric fever with *Salmonella typhi* predominating[1]. The organism (Salmonella) is transmitted by eating/drinking contaminated food/water or inadequate food hygiene and the feacal-oral route. It's (*Salmonella*) infections in humans is a major public health challenge globally, especially in developing countries like Nigeria (Kaduna, our study site not being an exception) where there is inadequate sanitation due to a large number of low-and middle-income homes. Reports have suggested that typhoid infections caused by *Salmonella typhi* are becoming increasingly resistant to many antimicrobial agents, possibly because there has been a repeat of treatment with same antibiotic over time. This has led to the emergence of antimicrobial-resistant strains, thereby worsening the situation of antibiotic resistance. Of the Salmonella spp infecting humans, *Salmonella typhi* is more prevalent and more virulent and is therefore chosen for this study.

Quinolones and Fluoroquinolones are a class of bactericides that include all synthetic drugs containing a quinolone or naphthyridone nucleus. Quinolones exert their antibacterial effect by inhibiting bacterial DNA synthesis[2]. These drugs (fluoroquinolones) are the most extensively used drugs for the treatment of *Salmonella* infections since the advent of multi-drug resistance *Salmonella* strains[3]. In developing countries like Nigeria, Ciprofloxacin continues to be the drug of choice for the treatment of enteric fever. This is because, it is orally effective and economical. In addition, independent mutations typically arise once per 10^7cell divisions or less, the likelihood for multiple mutations, which is important for fluoroquinolone resistance in a single clone is negligible[4]. The quinolones are also fully synthetic and therefore seemed

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impossible that resistance genes would be available for recruitment onto mobile elements[4]. The fluoroquinolones were thought or anticipated to arrest/thwart resistance but resistance to these agents by *Salmonella typhi* has become common and widely spread. Fluoroquinolones, in their mechanism of action, target 2 essential bacterial enzymes, DNA gyrase and topoisomerase IV. In *Enterobacteriaceae* including *Salmonella*, quinolone resistance is known to develop from the accumulation of chromosomal mutations in the quinolone resistance-determining region (QRDR) of the target enzyme genes (or targeted genes), *(gyrA/gyrB)* and/or topoisomerase IV *(parC/parE)* genes encoding the bacterial enzymes targeted by fluoroquinolones[2]. Three interesting types of Plasmid mediated quinolone resistance (PMQR) mechanisms have been identified: *qnr* genes, which protect target enzymes; *aac(6')-Ib-cr* gene, which is known to help in mediation and acetylation of certain quinolones; and *oqxAB* and *qepA* genes, which produce mobile efflux pumps[5]..According to[6], there are three different mechanisms found to be responsible for resistance to fluoroquinolones. Mutations in the quinolone resistance-determining region (QRDR) and these include: Mutations in gyrase *(gyrA/gyrB)* and /or topoisomerase IV *(parC/parE)* genes encoding the bacterial enzymes targeted by fluoroquinolones. Secondly, by reducing intracellular drug accumulation by up-regulaton native efflux pumps either alone or with reduced expression of outer membrane porins. The third one is the plasmid mediated quinolone resistance (PMQR) encoded by *qnr* genes, *A, B,* and *S.* Reduced susceptibility to ciprofloxacin in clinical bacterial isolates conferred by a variant gene encoding aminoglycoside acetyltransferase *aac(6')Ib*, has also been shown[7], [8].

PMQR (Plasmid-Mediated Quinolone Resistance) genes encode proteins that reduce the susceptibility of bacteria to quinolones, thereby making them less effective in treating infections. It (PMQR) promotes higher level of resistance by their interaction with specific chromosomal mechanisms of quinolone resistance. PMQR is the most troublesome element of all the three, this is because .the plasmid determinants of resistance are capable of inter and intra species horizontal gene transfer, which will ensue wide spread dissemination. Therefore its emergence in *Salmonella typhi* strains is a cause of worry for Clinicians and Microbiologists as well as for patients[9].

In Kaduna, Nigeria, there have been few researches to characterize *S. typhi* isolates or look at the prevalence of Plasmid-mediated quinolone resistance among *S. typhi* isolated from patients. This study therefore focuses on Antimicrobial resistance profile and molecular detection of PMQR in *S. typhi* from stool of patients attending primary healthcare centers in Kaduna Metropolis, Nigeria.

MATERIALS AND METHODS

Sample collection

A total of 200 stool samples were collected from routine clinical specimens at Medical Laboratories of the selected Primary health care Centres namely; Rafin guza, Kabala Doki, Badikko, Kakuri, Barnawa, and Angwan Television respectively within Kaduna metropolis. All the samples collected were transported immediately to Gold specialist Diagnostic Services, JJ9 Ibadan street Kaduna. The samples were transported in an insulating foam box containing ice blocks to maintain the temperature between 4^0C to 6^oC.

Isolation, Identification and characterization of Salmonella typhi.

1. Isolation of Salmonella typhi

Salmonella typhi was isolated from stool samples using cultural characteristics as earlier described by[10]. Briefly, a loopful of stool samples was inoculated in 5ml of Selenite F broth and incubated at 37°C for 24h. The 24 h Selenite F broth colonies were sub-cultured into petri-dishes containing sterile Bismute Sulphite Agar by streaking. The plates were incubated at 37ºC for 24 hours. Colonies from the Bismute Sulphite Agar plates with dark growth/black spots were further subjected to biochemical tests to confirm the isolates as *Salmonella typhi* as described by[11].

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2. Antimicrobial Susceptibility Testing

The antibiotic susceptibility test of the isolates was carried out as earlier described by Clinical and Laboratory Standards Institute"[12]. Briefly, 3 pure colonies of the isolate from stool samples of patients in the selected hospital was inoculated into 5 ml sterile 0.85% (w/v) NaCl (normal saline) and the turbidity of the bacteria suspension was adjusted to the turbidity equivalent to 0.5 McFarland standard. The McFarland's standard was prepared as follows; 0.5 ml of 1.172% (w/v) BaCl2.2H2O was added into 99.5 ml of 1% (w/v) H2SO4 [12]. "A sterile swab stick was soaked in the standardized bacteria suspension and streaked on Mueller Hinton agar plates and the antibiotics disc were aseptically placed and allowed time to diffuse and the plates were incubated at 37°C for 24 h. The diameter zone of inhibition in millimeter was measured and the result of the susceptibility was interpreted in accordance with the susceptibility break point earlier described by Clinical and Laboratory Standards Institute[12].

The antibiotics that were used were obtained from India (India. HiMedia Laboratories Pvt. Ltd. Plot No; C-40. Rd. No 21Y, MIDC, Wagle Industrial Area, Thane, (West) 400604, Works:B/4-6, M.I.D.C, Dindori, Nashik, India) and include: Amoxyclav/Amoxicillin (AMC) - 30µg, Ofloxacin (OFL) - 5µg, Levofloxacin (LE) - 5µg, Netilmicin (NET) - 30µg, Ciprofloxacin (CIP) - 5µg, Chloramphenicol (CHL) - 30µg, Gentamicin (Gen) - 10µg, Cotrimoxazole (COT) - 25µg, Ceftriaxone (CTR) - 30µg and Streptomycin (S) - 30µg.

The choice of antibiotics above was based on the fact that while some are widely used for the treatment of common bacterial infections, some have specific advantages. For example, this combination of Amoxiclav extends the spectrum of amoxicillin to cover beta-lactamase producing organisms, which are resistant to amoxicillin alone. This makes it effective against a broader range of bacteria, including some strains of Salmonella typhi. Ofloxacin and Levofloxacin are fluoroquinolones, a class of antibiotics known for their high efficacy against a variety of bacteria, including *Salmonella typhi*. Fluoroquinolones are often preferred due to their ability to achieve high intracellular concentrations, which is important for treating infections like typhoid fever that involve intracellular bacteria. The emergence of extensively drug-resistant (XDR) typhoid strains which require alternative treatments therefore informed the choice of the fluoroquinolones included in the study.

3. Determination of Multiple Antibiotic Resistance (MAR) Index

The MAR index of the isolates was determined as described by [13]. using the formula:

$$\text{MAR index} = \frac{Number\ antibiotics\ isolate\ is\ resistant\ to}{Number\ of\ antibiotics\ tested}$$

Molecular detection of Quinolone Resistance Genes.

1. DNA Extraction

The bacterial DNA was extracted by a method earlier described by[5] with minor modifications. Briefly, ten (10) milliliters of an overnight broth culture of the bacterial isolate in 1ml Luria Bertani (LB) were spun at 14000rpm for 3 min. The supernatant was discarded and the harvested cell pellet was re-suspended in 1ml sterile distilled water and transferred into 1.5ml centrifuge tube and centrifuged at 14000rpm for 10 min. The supernatant was discarded carefully. The pellet was re-suspended in 100µl of sterile distilled water by vortexing. The tube was centrifuged again at 14000rpm for 10 min. and the supernatant was carefully discarded. The cells were then re-suspended in 500µl of normal saline and heated at 95^{o}C for 20 min. The heated bacterial suspension was cooled on ice for 10 min. and spun for 3 min at 14000rpm. The supernatant containing the DNA was transferred to a 1.5ml microcentrifuge tube and stored at -20^{o}C for other downstream reactions.

2. DNA quantification

The extracted genomic DNA was quantified using the Nanodrop1000 spectrophotometer as eralier described by[14]. Briefly, the software of the equipment was lunched by double clicking on the Nanodrop icon. The

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equipment was initialized with 2µl of sterile distilled water and blanked using normal saline. 2µl of the extracted DNA was loaded onto 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..

3. DNA Amplification by Polymerase Chain Reaction (PCR).

All the isolates were first confirmed by Polymerase Chain Reaction (PCR) through amplification of the *Salmonella* genes using primers earlier described by[15] Forward 5′-TGAAATTATCGCCACGTTCGGGCAA-3′ and Reverse 5′-TCATCGCACCGTCAAAGGAACC-3′, with an amplicon size of 284 bp. *S. enterica* ATCC® 13076 strain (ATCC, Manassas, VA, USA) was used as a positive control. A tube containing the diluent without the addition of the extracted DNA which was to serve as a negative control was run alongside with the test to rule out false positive results. The reaction was carried out in a total volume of 25 µL, composed of 14.87 µL of distilled-deionized water, 5 µL of 5× colorless GoTaq® Flexi Buffer (Promega, Madison, WI, USA), 1 µL of dNTPs (1.5 mM) (Invitrogen, Waltham, MA, USA), 1 µL of each primer (forward and reverse) (10 pmol/µL), 1 µL of MgCl2 (25 mM), 0.125 µL of GoTaq® Flexi DNA polymerase (Promega), and 1 µL of gDNA as a template. The amplification was carried out in a T100 thermocycler (Bio-Rad, Hercules, CA, USA) with an initial denaturation step at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 30 s, and a final extension step at 72°C for 7 min. Amplicons were revealed on 1.5% agarose gel by electrophoresis (Cleaver Scientific, Power Pro 700 MA 150W Bio-Rad, Hercules) using the 100 bp DNA ladder Load Ready™ (Amplyus, Cambridge, MA, USA). at 100-200Ma for one hour. The gel was stained with Bromo-phenol blue and visualized under UV light and documented in a gel-doc system with CCD camera attached to it (Hero-Lab).

4. Detection of Plasmid Mediated Quinolone Resistance (PMQR) genes in Quinolone resistant Salmonella typhi from Kaduna metropolos

Genomic DNA from above isolates were used as template for the detection of PMQR. The Primers used are as earlier described by[15] with their amplicon sizes are as shown in Table 1. The PCR conditions were as described above and the annealing temperature was adjusted depending on the melting temperature of each primer set.

Table-1: Primers Used to Evaluate the Presence of PMQR Genes in Salmonella spp. Strains.

Target gene	**Primer sequence**	**Annealing temperature (°C)**	**Amplicon size (bp)**	**Reference**
qnrA	F- CCGCTTTTATCAGTGTGACT R- ACTCTATGCCAAAGCAGTTG	55	188	[15]
qnrB	F- GATCGTGAAAGCCAGAAAGG R- ACGATGCCTGGTAGTTGTCC	54	469	[15]
qnrC	F- GGGTTGTACATTTATTGAATCG R- CACCTACCCATTTATTTTCA	54	308	[15]
qnrD	F- CGAGATCAATTTACGGGGAATA R- AACAAGCTGAAGCGCCTG	57	582	[15]
qnrS	F- ACGACATTCGTCAACTGCAA R- TAAATTGGCACCCTGTAGGC	55	417	[15]

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Target gene	Primer sequence	Annealing temperature (°C)	Amplicon size (bp)	Reference
aac(6')-Ib	F- TTGCGATGCTCTATGAGTGGCTA R- CTCGAATGCCTGGCGTGTTT	57	482	[15]

RESULTS AND DISCUSSION

Isolation and Identification of Salmonella typhi

Salmonella typhi was isolated and identified by cultural morphological and biochemical characteristics. The organism grew and showed colorless colonies on *Salmonella-Shigella* Agar (SSA) and black metallic sheen on Bismuth Sulphite Agar. It is a Gram negative, rod shaped, nitrate-positive, Hydrogen sulphide-positive, and Methyl red-positive organism.

Occurrence of Salmonella typhi in relation to study centre, age and gender

The percentage occurrence of *S.* Typhi isolated from the study population was: 36.7 % > 35.0 % > 34.2 % > 32.0 % > (23.3 %) and 22.5 % from PHC Rafin Guza, PHC Television, PHC Barnawa, PHC Kabala Doki, PHC Badikko and PHC Kakuri respectively. This shows that the highest occurence was from PHC Rafin Guza while the lowest was from PHC Kakuri as shown on table 2.

Table 2: Occurrence of *Salmonella typhi* Isolated from Stool of Patients in Selected Primary Health Center in Kaduna Metropolis

Primary Health Center	No of Samples	No. (%) isolated
PHC Rafin Guza	30	11 (36.7)
PHC Kabala Doki	25	8(32.0)
PHC Badikko	30	7(23.3)
PHC Kakuri	40	9(22.5)
PHC Barnawa	35	12(34.3)
PHC Television	40	14(35.0)
Total	200	61(30.5)

In relation to age, *S. typhi* was highest at age 11 - 20 (44.4 %) in Rafin Guza and lowest at age 41-50yrs (20.0 %). from Badikko.

Occurrence in relation to gender showed highest occurrence (44.4%) in males from PHC Television and lowest in females (22.2%) from PHC Barnawa.

Antibiotics resistance profile of S. typhi from stool of patients from Kaduna metropolis

The *S. typhi* isolates showed antimicrobial resistance profile to the used antibiotics from highest to the lowest resistance level as follows: (100 %) > (81.8 %) > (72.7 %) > (63.6 %) > (36.6 %) > (27.2 %) against Cotrimoxazole, Chloramphenicol and Ceftriaxon, Amoxyclav/amoxycillin and Streptomycin, Netilmicin, Levofloxacin, Ciprofloxacin and Gentamicin respectively. From PHCKD, the isolates were highly resistance to Cotrimoxazole (100 %) followed by Amoxyclav/amoxicillin (75.0 %), Streptomycin (62.5 %) but less

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resistance to Ofloxacin (37.5 %) and Levofloxacin (25.0 %). Isolates from PHCB were highly resistance to Amoxyclav/amoxicillin (100 %) followed by Netilmicin(85.7 %), Chloramphenicol, Cotrimoxazole and Streptomycin (75.0 %) but less resistance to Ofloxacin, Ciprofloxacin and Gentamicin (28.5%). *S.* Typhi isolated from PHCK was resistance to Amoxyclav/amoxicillin (88.8 %) followed by Chloramphenicol and Streptomycin (66.6 %) but completely susceptible to Levofloxacin, Ciprofloxacin and Gentamicin. Isolates from PHCBA were highly resistance to Streptomycin (91.6 %) followed by Chloramphenicol and Cotrimoxazole (75.0 %), Netilmicin(66.6 %) but less resistance to Levofloxacin (16.6 %) and completely susceptible to Ofloxacin, Ciprofloxacin and Gentamicin. From PHCT *S. typhi* isolated were highly resistance to Chloramphenicol and Streptomycin (85.7 %) followed by Netilmicin and Cotrimoxazole (78.5 %), Ceftriaxone (71.4 %) but less resistance to Ofloxacin and Gentamicin (14.2 %) but completely susceptible to Levofloxacin. This result is presented in table 3.

Table 3: Antibiotics resistance profile of *Salmonella typhi* isolated from stool of patients in selected Primary Health Center in Kaduna metropolis.

Antibiotics	**Disc content (µg)**	No. (%) Resistant of *Salmonella typhi* isolates					
		PHCR (n=11)	PHCKD (n=8)	PHCB (n=7)	PHCK (n=9)	PHCBA (n=12)	PHCT (n=14)
Amoxyclav/Amoxycillin (AMC)	30	8(72.7)	6(75.0)	7(100)	8(88.8)	6(50.0)	9(64.2)
Ofloxacin (OFL)	5	6(54.5)	3(37.5)	2(28.5)	4(44.4)	0(0.0)	2(14.2)
Levofloxacin (LEV)	5	4(36.4)	2(25.0)	3(42.8)	0(0.0)	2(16.6)	0(0.0)
Netilmicin (NET)	30	7(63.6)	5(62.5)	6(85.7)	5(55.5)	8(66.6)	11(78.5)
Ciprofloxacin (CIP)	5	3(27.2)	4(50.0)	2(28.5)	0(0.0)	0(0.0)	4(28.5)
Chloramphenicol (CHL)	30	9(81.8)	5(62.5)	6(75.0)	6(66.6)	9(75.0)	12(85.7)
Gentamicin (Gen)	10	3(27.2)	4(50.0)	2(28.5)	0(0.0)	0(0.0)	2(14.2)
Cotrimoxazole (COT).	25	11(100)	8(100)	6(75.0)	5(55.5)	9(75.0)	11(78.5)
Ceftriaxone (CTR)	30	9(81.8)	4(50.0)	5(62.5)	4(44.4)	7(58.3)	10(71.4)
Streptomycin (S)	30	8(72.7)	5(62.5)	6(75.0)	6(66.6)	11(91.6)	12(85.7)

Key: PHCR= Rafin Guza, PHCKD= Kabala Doki, PHCB= Badikko, PHCK= Kakuri, PHCB= Barnawa, PHCT=Television

Multiple Antibiotic Resistance (MAR) Index

The MAR index of *S. typhi* isolated from stool of patients in selected Primary Health Center in Kaduna metropolis is given in Table 4. All the *S. typhi* isolated from selected Primary Health Center had MAR index of > 0.2 and the most common MAR index from PHCR was 0.3 (27.7 %). From PHCKD was 0.7 (37.5 %). From PHCB was 0.6 (71.4 %). From PHCK was 0.6 (44.4 %). From PHCBA was 0.8 (33.3 %). From PHCT was 0.6 (35.7 %).

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Table 4: Multiple Antibiotic Resistance Index (MARI) of *Salmonella* Typhi isolated from stool in selected Primary Health Center in Kaduna Nigeria.

			No. (%) Resistant isolates					
No. of antibiotics resistance (a)	No. of antibiotics tested (b)	MAR Index (a/b)	PHCR (n=11)	PHCKD (n=8)	PHCB (n=7)	PHCK (n=9)	PHCBA (n=12)	PHCT (n=14)
10	10	1.0	0(0.0)	1(12.5)	0(0.0)	1(11.1)	1(8.3)	1(7.1)
9	10	0.9	1(9.0)	1(12.5)	0(0.0)	0(0.0)	1(8.3)	2(14.2)
8	10	0.8	0(0.0)	1(12.5)	1(14.2)	0(0.0)	4(33.3)	1(7.1)
7	10	0.7	1(9.0)	3(37.5)	0(0.0)	1(11.1)	1(8.3)	1(7.1)
6	10	0.6	1(9.0)	0(0.0)	5(71.4)	4(44.4)	1(8.3)	5(35.7)
5	10	0.5	2(18.1)	1(12.5)	0(0.0)	0(0.0)	2(16.6)	1(7.1)
4	10	0.4	2(18.1)	1(12.5)	0(0.0)	1(11.1)	1(8.3)	2(14.2)
3	10	0.3	3(27.7)	1(12.5)	1(14.2)	1(11.1)	1(8.3)	0(0.0)
2	10	0.2	1(9.0)	1(12.5)	0(0.0)	0(0.0)	0(0.0)	1(7.1)

PHCR = Rafin Guza, PHCKD = Kabala Doki, PHCB = Badikko, PHCK = Kakuri, PHCBA = Barnawa, PHCT =Television.

Molecular Detection of plasmid mediated Quinolone Resistance genes in Quinolone resistant Salmonella typhi from Kaduna metropolos

The detection of plasmid mediated quinolone resistant genes from quinolone resistant isolates is as shown in table 5. The most common quinolone resistance gene detected from 6 isolates resistance to quinolone antibiotics was *QnrB* (83.3%) and the lowest was *aac(6')-Ib-cr-R* and *QepA* (50.0 %) as shown in table 5.

Table 5: Genotypic detection of quinolone resistance genes in *Salmonella t*yphi isolated from stool of patients in selected Primary Health Care in Kaduna metropolis.

Quinolone resistance Genes	No. (%) of S. typhi
	n = 6
QnrB	5 (83.3%)
aac(6')-Ib-cr-R	3 (50.0%)
QepA	3 (50.0%)

Agarose gel electrophoresis of the amplified Plasmid mediated Quinolone resistance genes

The PMQR genes of *S. typhi* were visualized by Agarose gel electrophoresis as shown in plates 1, 2 and 3.

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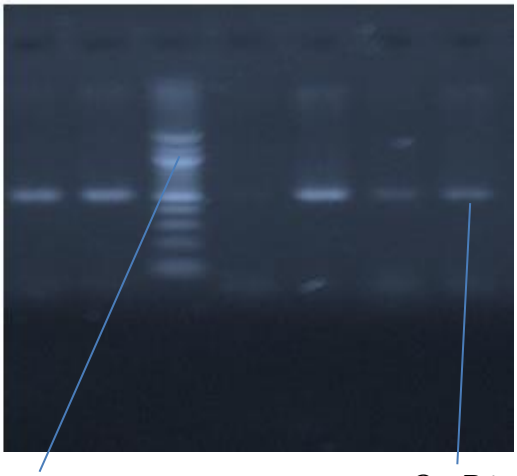
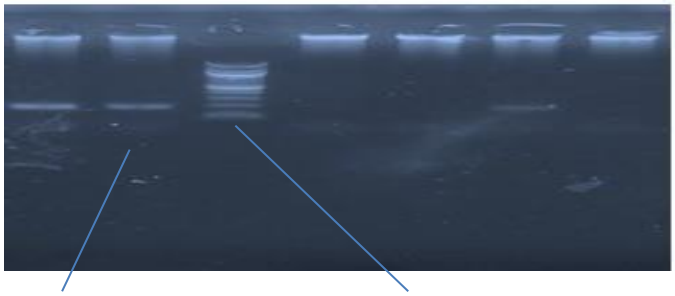
1 2 M 3 4 5 6



500bp *QnrB* (350bp)

Plate 1: Agarose gel electrophoresis of the amplified *QnrB* genes from the *S. tyiphi* isolates. Lanes 1, 2, 4, 5 and 6 represent the *QnrB* band, Lane M represents the 1500bp molecular ladder, while lane 3 shows no band.

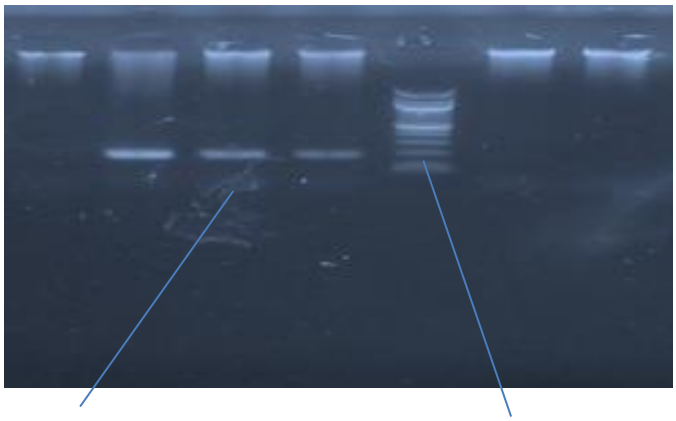
1 2 M 3 4 5 6



aac(6')-Ib-cr-R (200bp) 500bp

Plate 2: Agarose gel electrophoresis of the amplified *aac(6')-Ib-cr-R* genes from the *S. typhi* isolates. Lanes 1, 2 and 5 represent the *aac(6')-Ib-cr-R* band, Lane M represents the 1000bp molecular ladder, while lane 3, 4 and 6 shows no band.

1 2 3 4 M 5 6



QepA(260bp) 500bp

Plate 3: Agarose gel electrophoresis of the amplified *QepA* genes from the *S. typhi* isolates. Lanes 2, 3 and 4 represent the *QepA* band, Lane M represents the 1000bp molecular ladder, while lane 1,5 and 6 shows no band.

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DISCUSSION

Salmonella is a food-borne illness transmitted by consumption of contaminated food and water. From the 200 stool samples analyzed in this study, 30.5% (61/200) samples isolated *Salmonella typhi* following cultural, morphological and biochemical characteristics. This finding is higher than those conducted elsewhere in Nigeria by[5] who reported 8.7% occurrence and[16] who reported 20.9% in a study in Iraq. Occurrence in relation to gender showed higher occurrence of 44.4% in males from PHC Television than their female counterparts who showed a lower occurrence of 22.2% from PHC Barnawa. In relation to age, *S. typhi* was higher among participants aged 11 - 20 at 44.4 % in Rafin Guza and lower at age 41-50 yrs with 20.0 % from Badikko.

Salmonella is known as one of the most important causes of gastrointestinal disease in the world. Quinolones and fluoroquinolones are used successfully in the treatment of Salmonellosis particularly for infections that have become resistant to several antibiotics. But non-susceptible isolates to quinolones have been reported[17]. The *Salmonella typhi* isolated from stool of patients in selected Primary Health Centers in Kaduna metropolis as shown in table 3 were highly resistant to Amoxyclav/Amoxycillin and Cotrimoxazole at 100%, followed by Chloramphenicol and Ceftriaxon at 81.8 % from PHCB and PHCR respectively. The organism was however less resistance to Ofloxacin and Gentamycin at 14.2%, Levofloxacin at 16.6% and Ciprofloxacin at 28.5% in PHCT, PHCBA and PHCT respectively. The high resistance of the isolates to ceftriaxone which could be as a result of abuse and misuse of the antibiotic was similar to findings from a study in Abuja by[5]. The low resistance of the isolates to antibiotics including Ofloxacin (14.2%), levofloxacin (16.6%) and Ciprofloxacin (28.5%) were also lower than findings in Iran by[17]. This low resistance of the isolates to quinolone and fluoroquinolone antibiotics favours their use for the treatment of infections caused by *S. typh*i in the study area.

The preferred and extensive use of quinolones and fluoroquinolones in the treatment of *S. typhi* infections paved way for the emergence of resistance strains of the organism. The Multiple antibiotic resistance (MAR), which denotes the resistance of microorganisms to at least two (2) or more antibiotics as observed in this study further confirms that continuous use of antibiotics could results to multiple resistance of antibiotics by microbes.

Quinolone resistance genes detected from the 6 isolates in this study were *QnrB* (83.3%), *aac(6')-Ib-cr-R* (50%) and *QepA* (50.0 %). The presence of Qnr B and other PMQR genes in Salmonella can lead to reduced susceptibility to fluoroquinolones, making infections harder to treat. This is similar to a research carried out in selected General hospitals in Abuja, Nigeria's capital by[18]. The spread of Qnr B and other PMQR genes is a significant public health concern because it can lead to treatment failures and increased mortality rates in infections caused by resistant strains[19]. This result is comparable with earlier reports where PMQR genes were detected in Salmonella resulting to reduced susceptibility to Ciprofloxaxin as earlier reported by[20]. The high prevalence of qnr B detected in this study (83.3%) is similar to a study from South Korea where qnr B was also the most common resistant gene detected[21].

CONCLUSION

Salmonella typhi was detected from stool samples of the study participants with the occurrence more in males than in females and higher in age group of 11 - 20 years and less in age group 41 - 50years. The Salmonella isolates were highly resistant to Amoxyclav/Amoxycillin and Cotrimoxazole but less resistant to Ofloxacin, Levofloxacin and Ciprofloxacin. These less resistant antibiotics (Ofloxacin, Levofloxacin and Ciprofloxacin) could be used in the treatment of infections caused by *Salmonella typhi* since they are more susceptible. All the *Salmonella t*yphi isolates were Multiple Antibiotics Resistant. The qnrB, a PMQR gene was the most common gene detected in this study and its presence portends danger and calls for continuous surveillance with a view to mitigating treatment failures in infections caused by resistant strains.

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REFERENCES

1. Kapil A, Kagal A. Monchanda V. Chitnis DS. Veeraraghavan B Sharma A,et al Antibiogram of S. enteric Serovar Typhi and S. enterica serovar paratyphi A: A multi-centre study from India. WHO South-East Asia Public Health 2012:1:182-8.
2. Shaheen A, Tariq A, Iqbal M, Mirza O, Haque A, Walz T, and Rahman M. (2021). Mutational Diversity in the Quinolone Resistance-Determining Regions of Type-II Topoisomerases of Salmonella Serovars. Antibiotics (Basel, Switzerland), 10(12), 1455. https://doi.org/10.3390/antibiotics10121455
3. Harish BN, Menezes GA, Sarangapani K, & Parija SC. (2008). A case report and review of the literature: ciprofloxacin resistant Salmonella enterica serovar Typhi in India. Journal of infection in developing countries, 2(4), 324–327. https://doi.org/10.3855/jidc.229
4. Robicsek, A., Jacoby, G. A., & Hooper, D. C. (2006). The worldwide emergence of Plasmid-mediated quinolone resistance. The Lancet. Infectious diseases, 6(10), 629–640. https://doi.org/10.1016/S1473-3099(06)70599-0
5. Fasema R, Ngwai YB., Ishaleku D, Nkene IH, Abimiku RH, Tama SC and Igbawua IN (2024). Detection of Plasmid-mediated qnr Genes among the Quinolone Resistant Salmonella typhi from Patients Attending University of Abuja Teaching Hospital, Abuja, Nigeria. Asian Journal of Advanced Research and Reports, 18(5) : 80-89
6. Chiou, C. S., Lauderdale, T. L., Phung, D. C., Watanabe, H., Kuo, J. C., Wang, P. J., Liu, Y. Y., Liang, S. Y., & Chen, P. C. (2014). Antimicrobial resistance in Salmonella enterica Serovar Typhi isolates from Bangladesh, Indonesia, Taiwan, and Vietnam. Antimicrobial agents and chemotherapy, 58(11), 6501–6507. https://doi.org/10.1128/AAC.03608-14
7. Menezes GA,Harish BN, Parija SC. A case of fatal acute pyogenic meningitis in a neonate caused by extended spectrum beta-lactamase producing Salmonella group B. Jpn J Infect Dis 2008;61:234-5.
8. Govender N, Smith AM, Karstaedt AS, Keddy KH. Group for Enteric, Respiratory and Meningeal Disease surveillance in South Africa(GERMS-SA). Plasmid mediated quinolone resistance in Salmonella from South Africa. J Med Microbiol 2009; 2009 Oct; 58(Pt 10):1393-4.
9. Bitar R. Tarpley J. Intestinal perforation in typhoidal fever. A historical and state-of-the-art review. Rev Infect Dis.1985:7:257-71.
10. Al-Abadi IKM, and Al-Mayah AAS.(2011). Isolation and identification of Salmonella spp from chicken and chicken environment in Basrah province. African Journal of Biological Science, 7(1):33-43.
11. Cheesbrough M. District laboratory practice in tropical countries: Cambridge University Press; 2006.
12. CLSI. Performance standards for antimicrobial susceptibility testing. 27th ed. CLSI Informational Supplement M100-S22. Wayne, PA: Clinical and Laboratory Standards Institute; 2017.
13. Abimiku RH, Ngwai YB, Nkene IH, Bassey BE, Tsaku PA, Ibrahim T, Tama SC, Ishaleku D and Pennap GRI. (2019). Molecular Diversity and Extended Spectrum Betalactamase Resistance of Diarrheagenic Escherichia coli from Patients Attending Selected Health Care Facilities in Nasarawa State, Nigeria; International Journal of Pathogen Research, 3(1): 1-18.
14. Igbawua IN and Ngwai YB (2024). Kelch 13 gene polymorphisms of Plasmodium falciparum among human population in Nasarawa-West Senatorial District, Nasarawa State, Nigeria. Open Access Research Journal of Science and Technology, 2024, 10(01), 128–140
15. Herrera-Sánchez MP, Castro-Vargas RE, Fandiño-de-Rubio LC, Rodríguez-Hernández R, & Rondón-Barragán IS. (2021). Molecular identification of fluoroquinolone resistance in Salmonella spp. isolated from broiler farms and human samples obtained from two regions in Colombia. Veterinary world, 14(7), 1767–1773. https://doi.org/10.14202/vetworld.2021.1767-1773
16. Aljanaby AA, Medhat AR. Research article prevalence of some antimicrobials resistance associated-genes in Salmonella typhi isolated from patients infected with typhoid fever. J. Biol. Sci. 2017;17(4):171- 84.
17. Malehmir, S., Ranjbar, R., & Harzandi, N. (2017). The Molecular Study of Antibiotic Resistance to Quinolones in Salmonella enterica Strains Isolated in Tehran, Iran. The open microbiology journal, 11, 189–194. https://doi.org/10.2174/1874285801711010189

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18. Fasema, R, Bassey BE, Ngwai Y, Nkene I, Abimiku RH, Parom S, Yahaya I. (2020). Plasmid-mediated Quinolone Resistance Genes in Salmonella typhi from Patients Attending Selected General Hospitals in Abuja Municipal, Nigeria. Asian Journal of Biochemistry, Genetics and Molecular Biology. 10.9734/AJBGMB/2020/v4i230103.
19. Punchihewage-Don AJ, Hawkins J, Adnan AM, Hashem F, & Parveen S. (2022). The outbreaks and prevalence of antimicrobial resistant Salmonella in poultry in the United States: An overview. Heliyon, 8(11), e11571. https://doi.org/10.1016/j.heliyon.2022.e11571
20. Pribul, B. R., Festivo, M. L., de Souza, M. M., & Rodrigues, D.dosP. (2016). Characterization of quinolone resistance in Salmonella spp. isolates from food products and human samples in Brazil. Brazilian journal of microbiology : [publication of the Brazilian Society for Microbiology], 47(1), 196–201. https://doi.org/10.1016/j.bjm.2015.04.001
21. Kim, N. H., Choi, E. H., Sung, J. Y., Oh, C. E., Kim, H. B., Kim, E. C., & Lee, H. J. (2013). Prevalence of plasmid-mediated quinolone resistance genes and ciprofloxacin resistance in pediatric bloodstream isolates of Enterobacteriaceae over a 9-year period. Japanese journal of infectious diseases, 66(2), 151–154. https://doi.org/10.7883/yoken.66.151