

Original Research Article

Detection of Plasmid-Mediated *qnr* Genes among the Quinolone Resistant *Salmonella typhi* From Patients Attending University of Abuja Teaching Hospital, Abuja, Nigeria

ABSTRACT

Aims: The aim of this study was to determine the presence of the *qnr* genes among *S. typhi* isolated from stool of patients with suspected typhoid fever, in University of Abuja Teaching Hospital, Abuja, Nigeria.

Study design:: Cross sectional study

Place and Duration of Study:Department of Microbiology, Nasarawa State University, Keffi, between November 2022 and November 2023.

Methodology:Stool samples of patients with suspected typhoid fever were collected by standard methods in sterile disposable containers. After analysis of stool, microscopic observations and culture analysis,*Salmonella typhi* was isolated, antibiotic susceptibility testing was carried out, and the bacterial genome was extracted by boiling method.PCR for detection of *qnr* genes including *qnrA*, *qnrB* and *qnrS* was done by specific primers, then PCR products were run using gel electrophoresis and visualized by gel documentation system.

Results: Out of 150isolates, 13 (8.7%) were positive for *S. typhi*. Antibiotic resistance among the isolates in decreasing order were as follows:imipenems (100.0%), cefuroxime (100.0%), cefotaxime (100.0%), nalidixic acid (92.3%), amoxicillin/ clavulanic acid (84.6%), ceftriaxone/sulbactam (84.6%), ciprofloxacin (84.6%), gentamicin (76.9%), levofloxacin (46.2%) and ofloxacin (46.2%).Themost common antibiotic resistant phenotype was AUG-CTX-IMP-OFX-CN-NA-CXM-CRO-CIP-LBC at 30.4%. Multiple antibiotic resistance (MAR) was observed in 100% (13/13) of the isolates with the common MAR indices being 1.0 (30.8%), 0.7 (23.5%), 0.8 (23.1%) and 0.9 (15.4%). The only positive *PMQR*genes were *qnrS* and *aac(6)-Ib-cr*with percentage occurrence of 50.0% respectively

Conclusion:The *S. typhi*isolates showed lower resistance to ofloxacin, levofloxacin, and gentamicin, and all isolates were MAR, with resistance to 10 antibiotics being the most predominant. In addition, *qnrS* resistancegene was the most common gene expressed.

Keywords: *Salmonella*, Typhoid fever, Gene, *qnrS*

1. INTRODUCTION

Quinolones are an important class of antibacterial drugs [1]. Initially, nalidixic acid, a member of the class of drugs, had limited clinical use. But with chemical modifications, especially the addition of fluorine, fluoroquinolones were birthed. Fluoroquinolones (such as levofloxacin, ciprofloxacin) are more potent, have a broader spectrum of activity, and a lower frequency of resistance as recorded in several studies [2]. Fluoroquinolones have been used to treat many infections [3], including *Salmonella* infections, which can be dangerous, and even life-threatening [4]. The introduction of quinolones into human medicine has led to the emergence and increase of resistance to this class of antibiotics [5, 3].

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), primarily *gyrA* and *parC* [6, 3]. Since the late 1990s, 3 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 [7, 8]. A PMQR gene alone usually offers decreased susceptibility to fluoroquinolones and has less effect on nalidixic acid susceptibility [2, 8].

The implications of PMQR in the treatment of infections and the dissemination of antimicrobial resistance are numerous and important. Plasmids harboring quinolone resistance genes can be horizontally transferred to other bacteria, thus spreading resistance. The aim of this study was to determine the prevalence of *S. typhi* and expression of *qnr* genes (*qnrA*, *qnrB*, and *qnrS*) in *S. typhi* isolated from the stool of patients in University of Abuja Teaching Hospital, Abuja, Nigeria.

2. MATERIAL AND METHODS

2.1 Media.

Bacteriological media that were used in this study include: Salmonella-Shigella (SSA) Agar; Nutrient agar (NA); Mueller-Hinton agar (MHA); Mueller-Hinton broth (MHB); Bismuth sulfite agar (BSA); Selenite F- Broth (SFB); Xylose Lysine Deoxychocolate agar (XLD); Simmons Citrate agar (SCA); Triple Sugar Iron agar (TSI); Peptone water (PW) all were obtained from Oxoid Ltd (U.K.).

2.2 Antibiotic Discs

The antibiotics discs used in this study and their potency are as follows: Amoxicillin/Clavulanate (AMC: 30 µg), Cefotaxime (CTX: 25 µg), Imipenem/Cilastatin (IMP: 10 µg), Ofloxacin (OFX: 5 µg), Gentamicin (CN: 10 µg), Nalidixic acid (NA: 30 µg), Cefuroxime (CXM: 30 µg), Ceftriaxone/Sulbactam (CRO: 30 µg), Ciprofloxacin (CIP: 5 µg), Levofloxacin (LBC: 5 µg). All the discs were sourced from Oxoid Ltd. UK.

2.2 Chemicals and Reagents

The chemicals and reagents that were used in this study include: Acridine orange, Carbolfuschin, Crystal violet, Ethanol, Xylene solution, Creatinine, Potassium hydroxide and Kovac's reagents, obtained from BDH Chemical Ltd, England; Ethidium bromide, Iodine solution, EDTA and Glycerol obtained from Sigma Chemical Ltd, England; and Agarose gel, from Schwarz/ Mann Biotech.

2.4 Primers and their Amplicon Sizes

Primers were purchased from Inqaba Biotech(South Africa). The primers, sequences and amplicon sizes are as shown in Table 1.

Target gene	Primer Sequence	Annealing Temperature (°C)	Amplicon size (bp)	Reference
<i>qnrA</i>	5'- CCGCTTTTATCAGTGTGACT-5' 3'-ACTCTATGCCAAAGCAGTTG -3'	55	188	[9]
<i>qnrB</i>	5'- GATCGTGAAAGCCAGAAAGG -5'3'- ACGATGCCTGGTAGTTGTCC -3'	54	469	[9]
<i>qnrC</i>	5'-GGGTTGTACATTTATTGAATCG -5'3'- CACCTACCCATTTATTTTCA -3'	54	308	[9]
<i>qnrD</i>	5'-CGAGATCAATTTACGGGGAATA-5' 3'-AACAAAGCTGAAGCGCCTG - 3'	57	582	[9]
<i>QnrS</i>	5'- ACGACATTCGTCAACTGCAA- 5' 3'-TAAATTGGCACCCCTGTAGGC- 3'	55	417	[9]
<i>aac(6)- Ib</i>	5'- TTGCGATGCTCTATGAGTGGCTA-5' 3'-CTCGAATGCCTGGCGTGTTT- 3'	57	482	[9]
Class1I ntegron	5'-TCCACGCATCGTCAGGC -5' 3'-CCTCCCGCACGATGATC -3'	55	280	[9]

2.5 Study Location

The study was carried out at the University of Abuja Teaching Hospital (UATH). UATH is a 350 - bed hospital (tertiary health facility) located in, Gwagwalada, a town in the Federal Capital Territory of Nigeria.

2.5 Sample Collection

A total of 150 stool samples of patients with suspected cases of typhoid fever were collected using sterile container and transported using icepack to the Microbiology Laboratory, Nasarawa State University, Keffi for analysis.

2.5 Isolation and Identification of *Salmonella typhi*

Salmonella typhi was isolated and identified using Gram staining, indole test, methyl red test, Voges-Proskauer test, citrate test and oxidase test as described by [10] and further identified using KB003HI25TM identification kits following manufacturer's instruction.

2.6 Antimicrobial Susceptibility Testing

The antibiotic susceptibility test of the isolates was carried out as earlier described by Clinical and Laboratory Standards Institute [11]. 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 [11]. 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 at the centre of the plates and allowed to stand for 1 h for diffusion. 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 [11].

2.6.1 Determination of Multiple Antibiotic Resistance (MAR) Index

The MAR index of the isolates was determined as described previously [12] using the formula:

$$\text{MAR Index} = \frac{\text{No antibiotics isolate is resistant to}}{\text{No. of antibiotics tested.}}$$

2.7 Molecular detection of Quinolone resistance genes

2.7.1 DNA Extraction

The DNA was extracted by a method as earlier described by [13] with minor modification. Ten (10) milliliters of an overnight broth culture of the bacterial isolate in 1 ml Luria-Bertani (LB) were spun at 14000 rpm for 3 min. The supernatant was discarded, and the harvested cell pellet was resuspended in 1 ml sterile distilled water and transferred into 1.5-ml centrifuge tube and centrifuged at 14000 rpm for 10 min. The supernatant was discarded carefully. The pellet was resuspended in 100 µl of sterile distilled water by vortexing. The tube was centrifuged again at 14000 g for 10 min, and the supernatant was discarded carefully. The cells were re-suspended in 500 µl of normal saline and heated at 95°C for 20 min. The heated bacterial suspension was cooled on ice for 10 min and spun for 3 min at 14000 rpm. The supernatant containing the DNA was transferred to a 1.5-ml micro centrifuge tube and stored at -20°C for other downstream reactions [13].

2.7.2 Amplification of Target Genes

The DNA amplification of target plasmid-mediated Quinolone resistant genes in ciprofloxacin resistant *S. typhi* isolates was carried out using single plex method by modification of the method earlier described by [14]. Briefly, the reaction was carried out in 25 µl reaction volume in artificial tubes which is made up of 5 µl master mix, 2.4 µl primers (0.4 µl each of forward and reverse primers), 0.5 µl of MgCl₂, 1.5 µl of DNA template and 15.6 µl of nuclease free water. The reaction tubes were placed in the holes of the thermal cyclor was closed and the door was closed. Then *qnrA*, *qnrB*, and *qnrS* genes were amplified under the following conditions: Initial denaturation at 94°C for 5 min followed by 32 cycles of amplification at 94°C for 45 sec each, annealing at 53°C for 45 sec, with final extension at 72°C for 5 min [14].

The amplification condition for detection of *aac (6) -1b-cr* was carried out as follows; initial denaturation at 95°C for 20 min, annealing at 59°C for 40 sec and initial extension at 70°C for 30 sec and with final extension at 72°C for 5 min [14]. The primers used are listed in Table 1.

2.7.3 Agarose gel electrophoresis

The PCR products (10 µl) were evaluated on a 1.5% (w/v) Agarose gel (Gibco Life Technologies, Paisley, United Kingdom) at 100 mV for 60 min using BIO-RAD Power Pac 3000; and a molecular weight marker (1-kb DNA Ladder) was used as a standard. The DNA bands were then visualized and photographed under UV light using UVitec and Video copy processor after staining the gel with ethidium bromide as described by [15].

3. RESULTS AND DISCUSSION

3.1 Isolation and Identification of *Salmonella typhi*

The organism which grew with colorless colonies on Salmonella-Shigella (SSA) Agar, black metallic sheen on Bismuth Sulphite Agar, Gram negative, rod shape, nitrate-positive, Hydrogen sulphide-positive, and Methyl red-positive was identified as *S. typhi*.

3.1 Occurrence of *Salmonella typhi*

The isolation rate for *S. typhi* was 8.7 % (13/150). In relation to age of patients, the occurrence of *S. typhi* was highest at age 21-30yrs (16.7%), but lowest in age 41-50 (5.7%) as shown in Table 2.

Table 2. Occurrence of *Salmonella typhi* from stool of patients with suspected typhoid fever in University of Abuja Teaching Hospital, Abuja, Nigeria in relation to age

Age	No of Samples	Number (%) <i>Salmonella typhi</i>
≤ 10	10	1(10.0)
11- 20	20	2(10.0)
21- 30	30	5(16.7)
31- 40	40	2(5.0)
41- 50	35	2(5.7)
> 50	15	1(6.7)
Total	150	13 (8.7)

3.2 Antimicrobial Resistance Profile

The Antibiotic Resistance of *S. typhi* isolates from patients with suspected typhoid fever in University of Abuja Teaching Hospital, Abuja, is as given in Table 3. The isolates from UATH were more resistant to Cefotaxime (100.0%), imipenem (100.0%), and ceftriaxone/Sulbactam (100.0%), but were less resistance to Levofloxacin (46.2%), and ofloxacin (46.2%).

Table 3. Antimicrobial resistance profile of *S. typhi* from stool of patients with suspected typhoid fever University of Abuja Teaching Hospital, Abuja, Nigeria

Antibiotics	Disc Content (µg)	No. (%) resistance in <i>S. typhi</i> (n=13)
Amoxicillin/Clavulanate (AMC)	30	11 (84.6)
Cefotaxime (CTX)	25	13 (100.0)
Imipenem/Cilastatin (IMP)	10	13 (100.0)
Ofloxacin (OFX)	5	6 (46.2)
Gentamicin (CN)	10	10 (76.9)
Nalidixic acid (NA)	30	12 (92.3)
Cefuroxime (CXM)	30	11 (84.6)
Ceftriaxone/Sulbactam (CRO)	30	13 (100.0)
Ciprofloxacin (CIP)	5	11 (84.6)
Levofloxacin (LBC)	5	6 (46.2)

3.3 Multiple Antibiotic Resistance (MAR) Index

Multiple antibiotic resistance (MAR), which is the resistance of microorganisms to at least two (2) antibiotics was observed in all 13 isolates (100.0%). This suggests the possibility that most of the isolates originated from an environment where abuse of antibiotics was regular [12]. The most common indices were 1.0 (30.8%), 0.8 (23.1%), and 0.9 (15.4%).

3.4 Molecular Detection of Plasmid Mediated Quinolone resistant genes

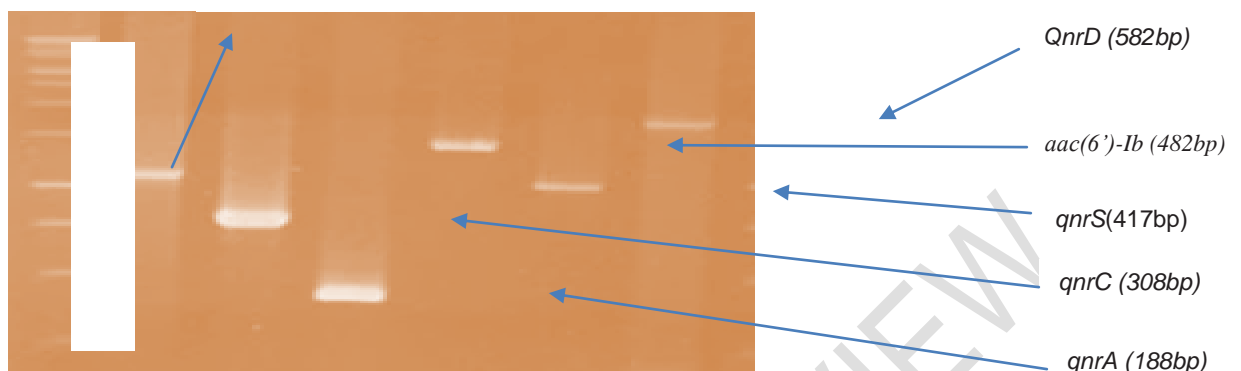
The plasmid mediated quinolone resistant genes detected in quinolone resistant isolates is as shown in table 4. The only positive PMQR genes were *qnrS* and *aac(6)-Ib-cr* with percentage occurrence of 50.0% respectively.

Table 4. Multiple Antibiotics Resistance (MAR) Index of *Salmonella typhi* isolates from stool of patients from University of Abuja Teaching Hospital, Abuja, Nigeria

No. of Antibiotic Resistance (a)	No of Antibiotics Tested (b)	MAR Index (a/b)	No (%) of MAR Isolates (n =17)
10	10	1.0	4(30.8)
9	10	0.9	2(15.4)
8	10	0.8	3(23.1)
7	10	0.7	1(7.7)
6	10	0.6	1(7.7)
5	10	0.5	1(7.7)
4	10	0.4	0(0.0)

Table 5. Molecular Detection of plasmid mediated Quinolone Resistance genes in Quinolone resistant *Salmonella typhi* University of Abuja Teaching Hospital, Abuja, Nigeria.

Quinolone resistance Genes	No. (%) of <i>S. typhi</i> (n = 6)
<i>QnrA</i>	1(16.7)
<i>QnrC</i>	1 (16.7)
<i>QnrD</i>	1 (16.7)
<i>QnrS</i>	2 (3.34)
<i>aac(6)-Ib-cr</i>	1 (16.7)
<i>qnrA+qnrC</i>	2 (3.34)
<i>qnrC + qnrD</i>	2 (3.34)
<i>qnrD + qnrS</i>	3 (50.0))
<i>qnrS + aac(6)-Ib-cr</i>	3 (50.0)
<i>qnrA + qnrC + qnrD +qnrS + aac(6)-Ib-cr</i>	6 (100.0)



Typhoid fever is caused by *Salmonella typhi*, which is known for being resistant to several antibiotics. It is one of the leading causes of death and infections in developing nations, and also a frequent cause of illnesses in Africa, particularly Nigeria. A study by Jubair *et al* in Iraq [16], stated a low prevalence of the *S.typhi* strain in Iraq (13%). This isn't consistent with another study by Rahman *et al* [17] where other Middle Eastern countries had an 80.0% of *S.typhi* -infected patients, suggesting that most *S. typhi* infections originate from the Asian continent, while the remainder is primarily from Africa and Latin America [17].

This study used 150 stool samples collected from patients with suspected typhoid fever to isolate and diagnose *S.typhi*. In the current study, only 13(8.7%) positive samples identified as *S.typhi* based on colony morphology on culture medium, biochemical testing amongst others. This is lower than reports from a study of hospitals in Najaf, Iraq, where a higher rate of culture-positive typhoid fever isolates was reported in 2017 and 2020 [18, 19].

The occurrence of *S. typhi* from stool of patients in relationship to their age was higher at age 21-30 years, and this is in agreement with a study which reported high occurrence of *S. typhi* in patients of age > 10-41 years [17] and 21-31 years [20].

The high resistance of the isolates to antibiotics such as cefotaxime, imipenem, and ceftriaxone/Sulbactam as observed in this study was not surprising and may be due to abuse of the antibiotics. The high resistance of the isolates to imipenem, cefotaxime and ceftriaxone/Sulbactam (all 100.0%) was similar to findings from a study in Abuja by Fasema *et al* [21]. Furthermore, the low resistance of the isolates to antibiotics including Ofloxacin (46.2%), and levofloxacin (46.2%) is higher than findings by Donget *et al* [22], but lower than reports from a study in Iraq and Iran [23, 24]. The low resistance of these isolates to antibiotics makes a case for them to be presented as the foremost option for treatment of infections caused by *S. typhi* in this region.

Quinolones were the best option for antibiotics when classical MDR *S.typhi* first appeared, However, *S.typhi* isolates that are resistant to quinolones have emerged due to the extensive use of quinolones to treat typhoid fever[25]. This is no surprise as the Multiple antibiotic resistance (MAR), which is the resistance of microorganisms to at least two (2) antibiotics was observed in all 13 isolates (100.0%). The three most common PMQR genes (*qnrA*, *qnrB*, and *qnrS*) that have led to the persistent spread of quinolone-resistant *S.typhi* were chosen in this study. However, the only positive PMQR genes were *qnrS* and *aac(6)-Ib-cr* with percentage occurrence of 50.0% respectively. A study on *qnr* genes in *S.typhi* isolates from South Korea found that *qnrB* was the most common *qnr* gene[26]. In India, the *qnrB* gene was amplified in 70.0% of *S.typhi* strains isolated[27]. In Iran, *qnrA* was found in 30.4% of the *S.typhi* isolates, *qnrS* was detected in 56.5%, while *qnrB* was detected in 1.1%[20].

4. CONCLUSION

This study showed that *S. typhi* is a cause of infection among typhoid patients, ofloxacin, and levofloxacin are fully effective against *S.typhi* clinical isolates from typhoid fever patients. All isolates considered were multiple drug resistant, and this is significant. The *qnrS* gene was present in some isolates. This gene may contribute to the quinolone resistance of *S. Typhi*. The appearance of a PMQR gene in *S. typhi* means it is necessary for there to be a limit to the use of quinolones to reduce the spread of resistant strains.

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

ETHICAL APPROVAL

Appropriate ethical committee approval was obtained prior to start of the research and is available for review.

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