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# Molecular Identification of Plasmid-Encoded *qnr* Genes in Quinolone-Resistant *Salmonella typhi* from Patients at National Hospital, Abuja, Nigeria

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Author NYB and FR designed the study. Author ID managed the literature searches while author OJE and NIH performed the statistical analysis, and wrote the protocol. Author TSC and TJA wrote the first draft of the manuscript and Author AAAD managed the analyses of the study. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** The study aimed to investigate and document the presence of qnr genes in S. typhi isolated from stool samples of patients suspected of having typhoid fever at the National Hospital, Abuja, Nigeria.

**Study Design:** Cross sectional study.

**Place and Duration of Study:** Department of Microbiology, Nasarawa State University, Keffi, between October 2020 and April 2022.

**Methodology:** Stool samples from patients suspected of having typhoid fever were collected in standard sterile disposable containers. After stool analysis, which included microscopic examination and culture, *Salmonella typhi* was isolated. Antibiotic susceptibility testing was conducted on the isolates, and bacterial genomes were extracted using the boiling method. PCR with specific primers was used to detect qnr genes (qnrB, qnrA and qnrS). The PCR products were then analyzed by gel electrophoresis and visualized using a gel documentation system.

**Results:** Results showed that 6.6% (10/150) of samples were positive for S. typhi. Antibiotic resistance among the isolates was observed as follows, in descending order: imipenem (100.0%), cefuroxime (100.0%), cefotaxime (100.0%), nalidixic acid (90.0%), amoxicillin/clavulanic acid (90.0%), levofloxacin (80.0%), ceftriaxone/sulbactam (70.0%), ciprofloxacin (70.0%), gentamicin (70.0%), and ofloxacin (60.0%). The most prevalent antibiotic-resistant phenotype was AUG-CTX-IMP-OFX-CN-NA-CXM-CRO-CIP-LBC, observed in 20.0% of cases. Multiple antibiotic resistance (MAR) was detected in all isolates (10/10), with common MAR indices being 0.8 and 0.9 (30.0%). The only positive genes related to plasmid-mediated quinolone resistance (PMQR) were qnrA and aac(6′)-Ib-cr.

**Conclusion:** The *S. typhi* isolates exhibited significant resistance to all tested antibiotics, with all isolates demonstrating multiple antibiotic resistance (MAR), primarily resisting 9 and 8 antibiotics. Additionally, the qnrA resistance gene was the most frequently detected gene among them.


*Keywords: Salmonella typhi; stool; antibiotics; resistance; qnrA.*

## 1. INTRODUCTION

*"Salmonella Typhi (S. Typhi),* the causative agent of typhoid fever, a prevalent and resilient pathogen specific to humans, has long been acknowledged as a leading cause of mortality due to enteric infections" [1,2]. "This Gram-negative, rod-shaped, flagellated, aerobic bacterium poses significant health risks, particularly in low- and middle-income countries" [3]. "Every year, there are more than 20 million reported cases of typhoid fever, resulting in over 200,000 fatalities globally" [3]. The medication for Salmonellosis infection, known over time, were Penicillin, Chloramphenicol and trimethoprim. An increase in resistance to such antibiotics and many more drugs led to the emergence of newer antibiotics, known as the Fluoroquinolone groups [4]. The unresponsiveness of fluoroquinolones is attributed to two major mechanisms: mutations that are chromosome-mediated in the quinolone resistance-determining regions (QRDR) of topoisomerase, and plasmid-mediated resistance genes belonging to the qnr groups, both of which contribute to fluoroquinolone resistance [5]. Fluoroquinolone resistance arises from both chromosomal mutations and plasmid-encoded genes [6]. The qnr determinants have been identified in numerous enterobacterial species across various regions, including Asia, America, Europe, and Africa [7,8]. To date, six qnrA variants (*qnrA1* to *qnrA6*) have been discovered. Additionally, other plasmid-mediated resistance determinants, such as qnrB (*qnrB1* to *qnrB5*) and qnrS (qnrS1 and qnrS2), have been found in enterobacterial species [9]. This current study investigated the presence of *qnrA*, *qnrB* and *qnrS* genes in quinolone-resistant *Salmonella typhi* (*S. typhi*) isolated from stool samples of patients with suspected typhoid fever in National Hospital, Abuja, Nigeria.

## 2. MATERIALS AND METHODS

### 2.1 Materials

#### 2.1.1 Media

Bacteriological media used for this study include: Nutrient agar (NA); Triple Sugar Iron agar (TSI); Mueller-Hinton agar (MHA); Xylose Lysine Deoxychocolate agar (XLD); Mueller-Hinton

broth (MHB); Salmonella-Shigella (SSA) Agar; Bismuth sulfite agar (BSA); Simmons Citrate agar (SCA); Peptone water (PW), and Selenite F- Broth (SFB).

#### 2.1.2 Equipment

The equipment used in this study include: Oven, Incubator, Refrigerator/Freezer, Thermocycler, Autoclave, Gel electrophoresis machine, Laminar air flow cabinet, Microscope, Spectrophotometer, UV illuminator, Centrifuge, Touch plate Super Mixer, Microwave oven, Electronic weighing balance, Vortex machine, and Gel Doc system.

#### 2.1.3 Chemicals and reagents

The chemicals and reagents utilized in this study include: Carbol fuschin, Pottasium hydroxide, Crystal violet, Creatinine, Ethanol, Xylene solution, Acridine orange, Kovac's reagents, Iodine solution Ethydium bromide, EDTA and Glycerol.

#### 2.1.4 Bacteria Isolates

Confirmed *S. typhi* isolates from the stool of patients were obtained and used for this study.

#### 2.1.5 Study location

The study was carried out at the National Hospital, Central Business District, Abuja (NHA). NHA is a 400 - bed hospital (Secondary health facility) that renders specialist services in all areas of medicine, located in Garki, within the Federal Capital Territory of Nigeria.

### 2.2 Methods

#### 2.2.1 Sample collection

A total of 150 stool samples from patients with suspected typhoid fever were collected using sterile containers and transported on ice packs to the Microbiology Laboratory at Nasarawa State University, Keffi, for analysis.

#### 2.2.2 Isolation and Identification of *Salmonella typhi*

*Salmonella typhi* was isolated and identified through a series of tests including, Voges-Proskauer test, Gram staining, citrate test, indole test, oxidase test and methyl red test as described by [10]. Additionally, it was further identified using KB003HI25 TM identification kits according to the manufacturer's instructions [11].

#### 2.2.3 Antibiotic susceptibility testing

"The antibiotic susceptibility test of the isolates was conducted using the Kirby-Bauer disc diffusion method, as modified by the Clinical and Laboratory Standards Institute (CLSI)" [10]. "Briefly, three pure colonies from the stool samples of patients in the selected hospital were inoculated into 5 ml of sterile 0.85% (w/v) NaCl (normal saline). The turbidity of the bacterial suspension was adjusted to match the 0.5 McFarland standard. This standard was prepared by mixing 0.5 ml of 1.172% (w/v) BaCl2·2H2O with 99.5 ml of 1% (w/v) H2SO4" [12]. "A sterile swab was then soaked in the standardized bacterial suspension and streaked onto Mueller-Hinton agar plates. Antibiotic discs were aseptically placed at the center of the plates, incubated at room temperature for 1 hour, and then re-incubated at 37ºC for 17 hours. The discs used included Amoxicillin/Clavulanate (AMC: 30 µg), Imipenem/Cilastatin (IMP: 10 µg), Gentamicin (CN: 10 µg), Levofloxacin (LBC: 5 µg). Cefotaxime (CTX: 25 µg), Ofloxacin (OFX: 5 µg), Cefuroxime (CXM: 30 µg), Nalidixic acid (NA: 30 µg), Ciprofloxacin (CIP: 5 µg), and Ceftriaxone/Sulbactam (CRO: 30 µg). After incubation, the diameters of the zones of inhibition were measured to the nearest millimeter (mm) using a ruler, and the susceptibility test results were interpreted using CLSI susceptibility breakpoints" [10,11].

#### 2.2.4 Determination of multiple antibiotic resistance (MAR) index

The MAR index of the isolates was calculated as previously described [13] using the following formula:

MAR Index = No antibiotics isolate is resistant to/ No. of antibiotics tested.

### 2.3 Molecular detection of Quinolone resistance genes

#### 2.3.1 DNA extraction

The modified method described by Abimiku et al [14] was used to extract the bacterial DNA. Initially, ten milliliters of an overnight broth culture of the bacterial isolate in 1 ml of Luria-Bertani (LB) were centrifuged at 14,000 rpm for 3

minutes. “After discarding the supernatant, the cell pellet was re-suspended in 1 ml of sterile distilled water and transferred to a 1.5 ml centrifuge tube. The tube was then centrifuged at 14,000 rpm for 10 minutes, and the supernatant was carefully removed. Next, the pellet was re-suspended in 100 µl of sterile distilled water by vortexing” [11]. The tube underwent another centrifugation at 14,000 g for 10 minutes, and the supernatant was again discarded. The cells were then re-suspended in 500 µl of normal saline and heated at 95°C for 20 minutes. After cooling on ice for 10 minutes, the heated bacterial suspension was centrifuged for 3 minutes at 14,000 rpm. Finally, the supernatant containing the DNA was transferred to a 1.5 ml microcentrifuge tube and stored at -20°C for future use.

### 2.3.2 Amplification of target genes

To amplify the plasmid-mediated quinolone-resistant genes in ciprofloxacin-resistant *S. typhi* isolates, a modified single-plex method was employed, as previously described by [15]. “The amplification reaction was performed in artificial tubes with a total volume of 25 µl. The reaction mixture consisted of 5 µl master mix, 2.4 µl of primers (0.4 µl each of forward and reverse primers), 0.5 µl MgCl2, 1.5 µl DNA template, and 15.6 µl nuclease-free water. These tubes were then placed in a thermal cycler and sealed” [11].

For the amplification of qnrA, qnrB, and qnrS genes, the following conditions were applied: an initial denaturation step at 94ºC for 5 minutes, followed by 32 cycles of denaturation at 94ºC for 45 seconds, annealing at 53ºC for 45 seconds, and a final extension at 72ºC for 5 minutes [15,11].

“To detect the aac(6)-1b-cr gene, the amplification conditions were as follows: an initial denaturation step at 95ºC for 20 minutes, annealing at 59ºC for 40 seconds, initial extension at 70ºC for 30 seconds, and a final extension at 72ºC for 5 minutes” [15]. The specific primers used can be found in Table 1.

### 2.3.3 Agarose gel electrophoresis

“The PCR products (10 µl) were subjected to analysis on a 1.5% (w/v) agarose gel (Gibco Life Technologies, Paisley, United Kingdom) at 100 mV for 60 minutes using the BIO-RAD Power Pac 3000. To establish a reference, a molecular weight marker (1-kb DNA Ladder) was utilized. After staining the gel with ethidium bromide, the DNA bands were visualized and captured under UV light using a UVitec and Video copy processor”, as described by [16].

**Table 1. Primers and their sequences**

| Target gene | Primer Sequence | Annealing Temperature (°C) | Amplicon size (bp) | Reference |
|---|---|---|---|---|
| *qnrA* | 5'- CCGCTTTTATCAGTGTGACT-5'<br>3'-ACTCTATGCCAAAGCAGTTG -3' | 55 | 188 | [17] |
| *qnrB* | 5'- GATCGTGAAAGCCAGAAAGG -5'3'-ACGATGCCTGGTAGTTGTCC -3' | 54 | 469 | [17] |
| *qnrC* | 5'-GGGTTGTACATTTATTGAATCG -5'3'-CACCTACCCATTTATTTTCA -3' | 54 | 308 | [17] |
| *qnrD* | 5'-CGAGATCAATTTACGGGGAATA-5'<br>3'-AACAAGCTGAAGCGCCTG - 3' | 57 | 582 | [17] |
| *QnrS* | 5'- ACGACATTCGTCAACTGCAA- 5'<br>3'-TAAATTGGCACCCTGTAGGC- 3' | 55 | 417 | [17] |
| *aac(6')-Ib* | 5'- TTGCGATGCTCTATGAGTGGCTA-5'<br>3'-CTCGAATGCCTGGCGTGTTT- 3' | 57 | 482 | [17] |
| Class1 Integron | 5'-TCCACGCATCGTCAGGC -5'<br>3'-CCTCCCGCACGATGATC -3' | 55 | 280 | [17] |

## 3. RESULTS AND DISCUSSION

### 3.1 Isolation and Identification of *Salmonella typhi*

The microorganism, which displayed colonies without color on Salmonella-Shigella (SSA) Agar, a distinct black metallic sheen on Bismuth Sulphite Agar, and possessed the characteristics of being Gram-negative, rod-shaped, nitrate-positive, hydrogen sulfide-positive, and methyl red-positive, was determined to be *S. typhi.*

### 3.2 Occurrence of *Salmonella typhi*

The occurrence of *S. typhi* was 6.7% (10/150). As regards patient's age,, the occurrence of *S. typhi* was highest at age >50yrs (17.6%), but lowest in age ≤ 10 (0.0%) as shown in Table 2.

### 3.3 Antimicrobial Resistance Profile

The Antibiotic Resistance of S.*typhi* isolates from of patients with suspected typhoid fever in University of National Hospital, Abuja, is shown in Table 3. The isolates from NHA were totally resistant to Cefotaxime (100.0%), imipenem (100.0%), and cefuroxime (100.0%), with all other antibiotics having a resistance percentage not less than 60%.

### 3.4 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 the isolates. The commonest indices were 0.9 (30.0%), 0.8 (30.0%), and 1.0 (20.0%) as shown in Table 4.

### 3.5 Molecular Detection of Plasmid Mediated Quinolone resistant genes

Table 5 presents the plasmid-mediated quinolone-resistant genes that were found in the isolates resistant to quinolones. Among these genes, the only ones detected were *qnrA* and aac(6′)-Ib-cr.

### 3.6 Discussion

*Salmonella typhi,* the bacterium responsible for Typhoid fever, is known for its resistance to multiple antibiotics. It is a major health worry as it causes infections and death in many countries, especially in developing nations [18], as well as illnesses in Africa, particularly Nigeria. In our study, 10(6.7%) identified as *S. typhi.* This percentage is lower than 13% reported in a study by Jubair et al in Iraq [19], 47% reported by Jabeen et al in Pakistan [20], and 80% in Middle Eastern countries reported in a study by Rahman et al. [21].

**Table 2. Age distribution of the study population**

| Age | No of Samples | Number (%) *S. typhi* |
|---|---|---|
| ≤ 10 | 15 | 0(0.0) |
| 11- 20 | 18 | 1(5.6) |
| 21- 30 | 35 | 3(8.6) |
| 31- 40 | 35 | 2(5.7) |
| 41- 50 | 30 | 1(3.3) |
| > 50 | 17 | 3(17.6) |
| **Total** | **150** | **10(6.7)** |

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

| Antibiotics | Disc Content (µg) | No. (%) resistance in S.*typhi* (n=10) |
|---|---|---|
| Amoxicillin/Clavulanate (AMC) | 30 | 9(90.0) |
| Cefotaxime (CTX) | 25 | 10(100.0) |
| Imipenem/Cilastatin (IMP) | 10 | 10(100.0 |
| Ofloxacin (OFX) | 5 | 6(60.0) |
| Gentamicin (CN) | 10 | 7(70.0) |
| Nalidixic acid (NA) | 30 | 9(90.0) |
| Cefuroxime (CXM) | 30 | 10(100.0) |
| Ceftriaxone/Sulbactam (CRO) | 30 | 7(70.0) |
| Ciprofloxacin (CIP) | 5 | 7(70.0) |
| Levofloxacin (LBC) | 5 | 8(80.0) |

**Table 4. Multiple Antibiotics Resistance (MAR) Index of *Salmonella typhi* isolates from stool of patients from National 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 | 2(20.0) |
| 9 | 10 | 0.9 | 3(30.0) |
| 8 | 10 | 0.8 | 3(30.0) |
| 7 | 10 | 0.7 | 1(10.0) |
| 6 | 10 | 0.6 | 0(0.0) |
| 5 | 10 | 0.5 | 1(10.0) |
| 4 | 10 | 0.4 | 0(0.0) |

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

| Quinolone resistance Genes | No. (%) of *S. typhi* ) (n = 1) |
|---|---|
| *Qnr*A | 1(100.0) |
| *Qnr*C | 0 (0.0) |
| *QnrD* | 0 (0.0) |
| *Qnr*S | 0 (0.0) |
| *aac(6')-Ib-cr* | 0 (0.0) |
| *qnrA + qnrC* | 1 (100.0) |
| *qnrC + qnrD* | 0 (0.0) |
| *qnrD + qnrS* | 0 (0.0)) |
| *qnrS + aac(6')-Ib-cr* | 0 (0.0) |
| *qnrA + qnrC + qnrD +qnrS + aac(6')-Ib-cr* | 1 (100.0) |

**MAR isolates are those with resistance to at least two antibiotics [16]*

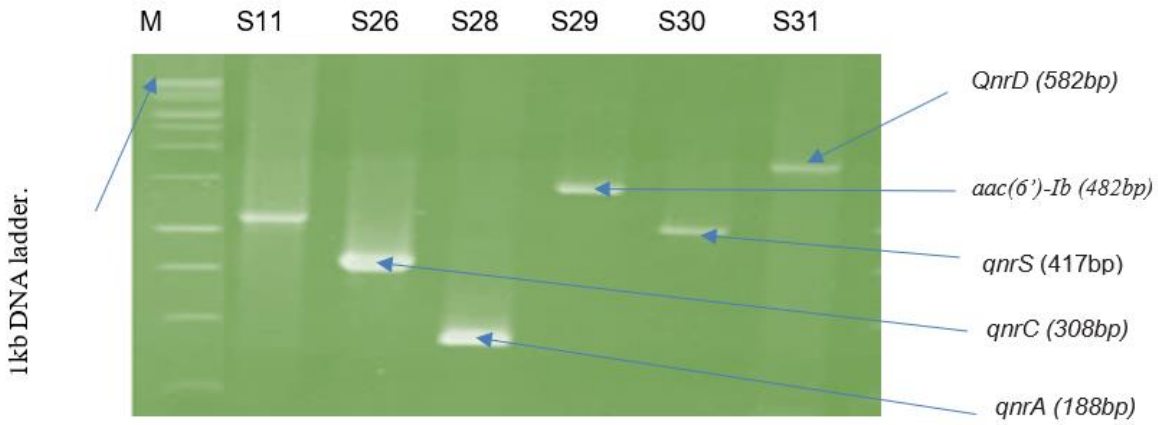


**Fig. 1. Agarose gel electrophoresis of amplified quinolone resistance genes in *Salmonella typhi*. Lane S11 and Lane S30 show the qnrS gene (417 bp); Lane S26 shows the qnrC gene (308 bp); Lane S28 shows the qnrA gene (188 bp); Lane S29 shows the aac(6')-Ib gene (482 bp); Lane S30 shows the qnrS gene (417 bp); and Lane S31 shows the qnrD gene (582 bp). Lane M represents the 1 kb DNA molecular ladder**

The prevalence of *S. typhi* in stool samples from patients increased notably among those aged over 50 years (17.6%), closely followed by age 21-30 years (8.6%). These figures differ from reports in a studies by Jubair et al in Iraq [19], Aljanaby et al in Iraq [22] and Safdar et al in Pakistan [23], where age 21-30 years had a higher occurrence 15.8%, 16.2% and 16.7% respectively. The higher rates of occurrence among adults in these studies might be due to

exposure and lack of good hygiene especially with food.

The significant resistance observed among the isolates to antibiotics like cefuroxime, cefotaxime, imipenem, amoxicillin/clavulanate, and nalidixic acid (all exceeding 90%) in this study is likely attributed to antibiotic abuse and misuse. The isolates were highly resistant to cefuroxime, cefotaxime and imipenem (all 100%). This was in contrast with findings from a study in Iraq by Njum et al [24], where isolates were sensitive (100%) for ceftriaxone, cefepime, cefazolin and chloramphenicol. Furthermore, similar resistance to antibiotics including Ofloxacin (60.0%), gentamicin (70.0%), and ciprofloxacin (70.0%) was observed in a study by Njum et al. [24] and Namratha et al. [25] In Iraq and India respectively. The antibiotic resistance of these isolates suggests they could be the most effective option for treating infections caused by S. typhi in this region.

Multiple antibiotic resistance (MAR), characterized by resistance to at least two antibiotics, was detected in all 15 isolates (100.0%). This study focused on the three predominant PMQR genes (qnrA, qnrB, and qnrS), known for their role in promoting the dissemination of quinolone-resistant *S. typhi*. However, only qnrA and aac(6′)-Ib-cr genes were detected, each with a 100.0% occurrence rate. In South Korea, the qnrB gene was found in 70.0% of S. typhi strains isolated [26]. In Iran, *qnrA* was present in 30.4% of the isolates, *qnrS* in over 50.0%, and *qnrB* in 1.1%. A study on qnr genes in *S. typhi* isolates from India found qnrB to be the most common gene [11].

## 4. CONCLUSION

This study found that cephalosporins (ceftriaxone, cefuroxime, and cefotaxime) along with carbapenems (imipenem) effectively treat *S. typhi* clinical isolates from typhoid fever patients. The study also identified plasmid-mediated quinolone-resistant genes in *S. typhi* clinical isolates from suspected typhoid fever patients in Abuja, Nigeria, with *qnrA* being prominently detected. These resistance genes likely contribute to quinolone resistance in *S. typhi*. Given the emergence of plasmid-mediated quinolone-resistant genes in S. typhi, it is crucial to restrict the use of quinolones and implement rigorous infection control measures to prevent and control the spread of resistant strains.

## CONSENT

All authors declare that ‘written informed consent was obtained from the patient approved parties for publication of this study.

## ETHICAL APPROVAL

As per international standards or university standards written ethical approval has been collected and preserved by the author(s).

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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