

Original Research Article

Molecular Characterization of Plasmid-mediated Quinolone Resistant *Salmonella typhi* From Patients Attending Federal Medical Center, Jabi, Abuja, Nigeria

ABSTRACT

Aims: This study investigates and reports the detection of *qnr* genes (plasmid-mediated quinolone resistance PMQR) in *S. typhi* isolated from stool of patients with suspected typhoid fever, in Federal Medical Center, Jabi, Abuja, Nigeria.

Study Design: Cross sectional study

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

Methodology: *Salmonella typhi* was isolated from stool of patients with suspected typhoid fever using standard cultural and microbiological methods. Antibiotic susceptibility testing were performed using the disc diffusion method to investigate the ability of *S. typhi* to resist some antibiotics. *Qnr* genes (*qnrC, qnrD, qnrS*) were detected by PCR and amplification.

Results: 17 out of 150 (11.3%) of the samples collected had *S. typhi*. Antibiotic resistance in the isolates in decreasing order were as follows: cefotaxime (100.0%), amoxicillin/ clavulanic acid (94.1%), nalidixic acid (94.1%), cefuroxime (94.1%), imipenems (88.2%), ceftriaxone/sulbactam (82.4%), ciprofloxacin (82.4%), gentamicin (58.8%), levofloxacin (47.1%) and ofloxacin (29.4%). The commonest antibiotic resistant phenotype was AUG-CTX-IMP-OFX-CN-NA-CXM-CRO-CIP-LBC at 29.4%. Multiple antibiotic resistance (MAR) was observed in 100% (17/17) of the isolates with the common MAR indices being 1.0 (29.4%), 0.7 (23.5%), 0.8 (17.6%) and 0.5 (17.6%). 33.3% of the isolates in FMCJ were positive for *qnrC, qnrD, qnrS, qnrA+qnrC* and *qnrS+ aac(6)-Ib-cras* all of *qnrC, qnrD, and qnrS* genes were all expressed in the isolates.

Conclusion: The *S. typhi* isolates showed lower resistances to ofloxacin, levofloxacin, and gentamicin, and all isolates were MAR, with resistance to 10 antibiotics being the most predominant. In addition, *qnrC, qnrD, and qnrS* resistance genes were all expressed in the isolates.

Keywords: *Salmonella, Typhoid fever, Antibiotics, qnrB*

1. INTRODUCTION

Salmonella typhi infections in humans are a major public health challenge globally, especially in developing countries, and particularly Africa as a continent, where there is inadequate sanitation due to a large number of low-and middle-income homes (1, 2). This organism is the causative agent of typhoid fever, a serious and potentially fatal bloodstream infection. This disease/infection has contributed significantly to mortality rates in many parts of developing nations and the world (3). More than 21 million people are said to be infected with typhoid fever every year, and regrettably, over 90% of them might die (4).

Reports and studies have suggested that typhoid infections are becoming increasingly resistant to many antimicrobials, 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 (5, 6). Recent studies have shown that *S. typhi* has shown resistance to antibiotics such as chloramphenicol, ciprofloxacin, and levofloxacin, and can therefore be referred to as multidrug-resistant (MDR) (7). However, quinolone antibiotics seem to be used as an alternative treatment due to the resistance to these first-line antimicrobial agents (8).

New reports of resistance to quinolone antibiotics has led the World Health Organization (WHO) to designate *Salmonella spp.* resistant to quinolone as a pathogen for which new treatments are urgently required (9). Studies have shown that people infected with quinolone-resistant *S. typhi* have prolonged fever experiences and more treatment complications or failures (10, 11). Quinolone resistance is caused by chromosomal mutations in the genes of resistant bacteria that code for targeted enzymes, such as DNA gyrase and topoisomerase IV (12). Also, plasmid-encoded genes known to be another route to acquiring quinolone resistance. Groups of genes known as PMQR include the *qnr* families (*qnrA, qnrB, qnrC, qnrD, qnrE, qnrS, and qnrVC*) amongst others [13, 14]. PMQR makes the spread for quinolone resistance easier, leading to high levels of resistance and treatment failures [15].

In Abuja, Nigeria, there have been few researches to characterize *S. typhi* isolates or look at the prevalence of quinolone resistance among *S. typhi* isolated from patients. This study aimed 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 Federal Medical Center, Jabi, Abuja, Nigeria.

2. MATERIAL AND METHODS

2.1 Bacteria Isolates

One hundred and fifty stool samples, were collected from patients in Federal Medical Center, Jabi, Abuja (FMCJ). Federal Medical Center, Jabi, is a secondary healthcare facility located in Jabi, an area right in the Federal Capital Territory Each stool sample was picked using a sterile spoon as described by Abimiku et al. [16] with some modifications. The stool were scooped and dropped into a sterile stool container before being transported to the Microbiology Laboratory at the Nasarawa State University, Keffi, for same-day analysis or stored in a refrigerator (Model PRN 1313 HCA, BEKO, Germany) at 5°C for latter-day analysis.

Salmonella typhi was isolated from stool samples as earlier described by [17]. 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 was streaked on XLD agar plates and the plates were incubated at 37°C for 24 h. A colourless colony with black colouration were further streaked on Bismuth sulphate agar plates and incubated at 37°C for 24 h. Black metallic sheen colonies on Bismuth sulphate agar were selected as presumptive *S. typhi*.

Identification of *E. coli* was done by morphological, cultural and biochemical characteristics using Gram staining, indole test, methyl red test, Voges-Proskauer test, citrate test and oxidase test as described in the Bacteriological Analytical Manual [18] and Cheesbrough [17]. Further identification was done using KB003HI25 TM identification kits following manufacturer's instruction. The bacterium was stored in the refrigerator on nutrient agar (Oxoid Ltd, UK) slants and reactivated by sub-culturing on MCA for use in further research.

2.2 Antibiotic Susceptibility Testing

The antibiotic susceptibility test of the isolates was carried out as earlier described by Clinical and Laboratory Standards Institute [19]. 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 [19]. 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 antibiotics used 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). 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 [20].

2.2.1 Determination of Multiple Antibiotic Resistance (MAR) Index

The MAR index of the isolates was determined as described previously [21] using the formula:

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

2.3 Molecular detection of Quinolone resistance genes

2.3.1 DNA Extraction

The DNA was extracted by a method as earlier described by [16] 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.

2.3.2 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 [22]. 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 [23].

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 [23]. The primers used are listed in Table 1.

2.3.3 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 [24]

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 11.3 % (17/150). In relation to age of patients, the occurrence of *S. typhi* was highest at age 21-30 yrs (25.0%), but lowest in age 41-50 (7.0%) as shown in Table 2.

Table 1: Primers and target genes with amplicon sizes for PMQR genes in *Salmonella typhi*

Target gene	Primer Sequence	Annealing Temperature (°C)	Amplicon size (bp)	Reference
<i>qnrA</i>	5'- CCGCTTTTATCAGTGTGACT-5' 3'-ACTCTATGCCAAAGCAGTTG -3'	55	188	[25]
<i>qnrB</i>	5'- GATCGTGAAAGCCAGAAAGG -5'3'- ACGATGCCTGGTAGTTGTCC -3'	54	469	[25]
<i>qnrC</i>	5'-GGGTTGTACATTTATTGAATCG -5'3'- CACCTACCCATTTATTTTCA -3'	54	308	[25]

<i>qnrD</i>	5'-CGAGATCAATTTACGGGGAATA-5' 3'-AACCAAGCTGAAGCGCCTG - 3'	57	582	[25]
<i>QnrS</i>	5'- ACGACATTCGTCAACTGCAA- 5' 3'-TAAATTGGCACCCCTGTAGGC- 3'	55	417	[25]
<i>aac(6')</i> - <i>lb</i>	5'- TTGCGATGCTCTATGAGTGGCTA-5' 3'-CTCGAATGCCTGGCGTGTTT- 3'	57	482	[25]
Class1In tegron	5'-TCCACGCATCGTCAGGC -5' 3'-CCTCCC GCACGATGATC -3'	55	280	[25]

F (5')= Forward; R (3')= Reverse; bp = Base pair

Table 2. Occurrence of *Salmonella typhi* from stool of patients with suspected typhoid fever in Federal Medical Centre, Jabi, Abuja Nigeria in relation to age

Age	No of Samples	Number (%) <i>Salmonella typhi</i>
≤ 10	7	0 (0)
11- 20	20	2(10.0)
21- 30	28	7(25.0)
31- 40	32	3 (9.4)
41- 50	43	3(7.0)
> 50	20	2 (10. 0)
Total	150	17 (2.3)

3.2 Antimicrobial Resistance Profile

The Antibiotic Resistance of *S.typhi* isolates from stool of patients from Federal Medical Centre, Jabi, Abuja, Nigeria is as given in Table 3. The isolates from FMCJ were more resistant to Cefotaxime (100.0%), Amoxicillin/Clavulanate, Nalidixic acid and Cefuroxime (94.0%) but less resistance to ofloxacin (47.1%).

Table 3. Antimicrobial resistance profile of *S.typhi* from stool of patients with suspected typhoid fever in Federal Medical Centre, Jabi, Abuja Nigeria

Antibiotics	Disc Content (µg)	No. (%) resistance in <i>S.typhi</i> (n=17)
Amoxicillin/Clavulanate (AMC)	30	16(94.1)
Cefotaxime (CTX)	25	17 (100.0)
Imipenem/Cilastatin (IMP)	10	15(88.2)
Ofloxacin (OFX)	5	5(29.4)
Gentamicin (CN)	10	10(58.8)
Nalidixic acid (NA)	30	16(94.1)
Cefuroxime (CXM)	30	16(94.1)
Ceftriaxone/Sulbactam (CRO)	30	14(82.4)

Ciprofloxacin (CIP)	5	14(82.4)
Levofloxacin (LBC)	5	8(47.1)

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 (100.0%) of the 17 isolates. This suggests the possibility that most of the isolates originated from an environment where abuse of antibiotics was regular [26]. The commonest indices were 1.0 (29.4%), 0.7 (23.5%), 0.8 (17.6%) and 0.5 (17.6%).

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. 33.3% of the isolates in were positive for *qnrC*, *qnrD*, *qnrS*, *qnrA+qnrC* and *qnrS+ aac(6)-Ib-cr*

Table 4. Multiple Antibiotics Resistance (MAR) Index of *Salmonella typhi* isolates from stool of patients from Federal Medical Center, Abuja, Nigeria

No. of Antibiotic Resistance (a)	No Antibiotics Tested (b)	of MAR Index (a/b)	No (%) of MAR Isolates (n =17)
10	10	1.0	5(29.4)
9	10	0.9	0(0.0)
8	10	0.8	3(17.6)
7	10	0.7	4(23.5)
6	10	0.6	2(11.8)
5	10	0.5	3(17.6)
4	10	0.4	0(0.0)

Table 5. Molecular Detection of plasmid mediated Quinolone Resistance genes in Quinolone resistant *Salmonella typhi* from Federal Medical Center, Abuja, Nigeria.

Quinolone resistance Genes	No. (%) of <i>S. typhi</i> (n = 3)
<i>QnrA</i>	0 (0.0)
<i>QnrC</i>	1 (33.3)
<i>QnrD</i>	1 (33.3)
<i>QnrS</i>	1 (33.3)
<i>aac(6)-Ib-cr</i>	0 (0.0)
<i>qnrA+qnrC</i>	1 (33.3)
<i>qnrC + qnrD</i>	2(66.6)
<i>qnrD + qnrS</i>	2 (66.6)
<i>qnrS + aac(6)-Ib-cr</i>	1 (33.3)
<i>qnrA + qnrC + qnrD +qnrS + aac(6)-Ib-cr</i>	3 (99.9)

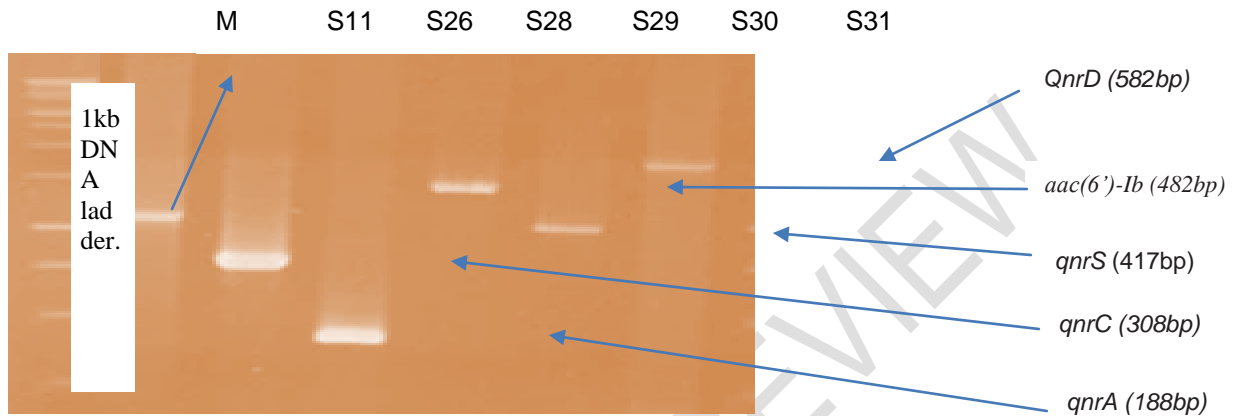


Figure 1: Agarose gel electrophoresis of the amplified quinolones resistance genes of *Salmonella typhi*. Lane S11 and Lane S30 represent the expression of the *qnrS* (417bp) gene; Lane S26 represent the expression of the *qnrC* (308bp) gene; Lane S28 represent the expression of the *qnrA* (188bp) gene; Lane S29 represent the expression of the *aac(6)-Ib* (482bp) gene; Lane S30 represent the expression of the *qnrS* (417bp) gene and Lane S31 represent the expression of the *QnrD* (582bp) gene; while Lane M represents 1kb DNA molecular ladder.

In the current study, the occurrence of *S. typhi* from stool of patients with suspected typhoid fever was 17(11.3%). This is less 26.3% and 46.5% reported by [27] and [28]. The prevalence of *S. typhi* is consistent with data stating that most of *S.typhi* –infected patients originate from Africa and Latin America(29). Typhoid fever is common in these countries, including Nigeria due to a lack of good sanitation or public health administration(30).

The high resistance of the isolates from selected Tertiary hospitals to antibiotics such as Amoxicillin/Clavulanate, Cefotaxime, Imipenem, nalidixic acid and Cefuroxime as observed in the study was not surprising and may be due to misuse and abuse of the antibiotics. The high resistance of the isolates to Amoxicillin/Clavulanate and nalidixic acid was lesser than (100.0%) and (100.0%) reported by (31). The resistance of *S. typhi* isolates to Ofloxacin, Gentamicin, Ciprofloxacin and Levofloxacin observed was low and was less than 91% as reported by [32] but was greater than 1.2% as reported by [33]. The low resistance to Ofloxacin, Gentamicin, Levofloxacin and ciprofloxacin by the isolates could be that such antibiotics may not have been misused or abused in the study location. This further justifies their uses as common drugs of choice for the treatment of typhoidal *Salmonella*[34]. The low resistance of the mentioned isolates to antibiotics mentioned is an indication that such antibiotics may not have been abused in the study location. The result of our findings on the categories of antibiotic resistance in *S. typhi* shows that most of the isolates were multidrug resistance and this finding is also in agreement with the study earlier described by [35]. The occurrence of MDR resistance isolates observed in this study is an indication that such isolates may cause infection. Thus is that difficult to treat using conventional antibiotics since, outbreaks of typhoid fever caused by *S. typhi* have been reported worldwide [36].

The occurrence of plasmid mediated quinolone resistance genes in *S. typhi* isolated observed in this study was an indicator that such genes may be responsible for quinolone resistance. Our findings in this study shows that commonest PMQR genes was *qnrS* with percentage occurrence of 33.3% and this is different from the study earlier reported [36, 37]. The occurrence of *qnrS* in resistant isolates was an indication that the resistance may be due to acetylation of the quinolone isolates observed in this study was lower than 64% reported by [38].

4. CONCLUSION

S. typhi isolates from stool of patients suspected of having typhoid fever in FMCJ were more resistant to cefotaxime, imipenem, naladixic acid and amoxicillin acid/clavulanate, but less resistant to ofloxacin, and gentamicin. This implies that the antibiotics are useful in the treatment of infections by *S. typhi*. The presence of plasmid-mediated quinolones-resistant genes is quite significant. The *qnrS* gene was more prevalent than the *qnrC* and *qnrA*. These genes may contribute to the quinolone resistance of *S. typhi*. The appearance of plasmid-mediated quinolones-resistant genes in *S. typhi* means limiting the use of quinolones and good infection control is important to check 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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