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Plasmid-mediated Quinolone Resistance Genes in *Salmonella typhi* from Patients Attending Selected General Hospitals in Abuja Municipal, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors YBN and RF designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors IHN, RHA and BEB. Author RHA managed the analyses of the study. Authors IY and SKP managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This study investigated the antimicrobial resistance profile and presence of plasmid-mediated quinolone resistance (PMQR) genes in *Salmonella typhi* from patients attending selected general hospitals in Abuja municipal, Nigeria. Four hundred stool samples from patients with suspected typhoid fever were collected from Asokoro General Hospital Abuja (AGH), Garki Hospital Abuja (GHA), Maitama General Hospital Abuja (MGHA) and Wuse General Hospital Abuja (WGHA) and *S. typhi* was isolated and identified using standard microbiological methods. Antimicrobial susceptibility testing of the isolates was carried out using Clinical and Laboratory Standards Institute (CLSI)

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method. Molecular detection of PMQR genes in the ciprofloxacin-resistant isolates was carried out using Polymerase Chain Reaction (PCR) method. The overall occurrence of the isolates was 13.3% (53/400), with the highest hospital-related occurrence at WGHA (18.0%). The occurrence was highest at age 21-30yrs in AGHA (20.0%), GHA (33.3%) and WGHA (45.0%). The occurrence was higher in females at AGHA (12.7%) and GHA (16.0%); but higher in males at MGHA (11.4%) and WGHA (18.2%). Resistance to ciprofloxacin was the least at 30.2%, distributed as follows: AGHA (20.0%), GHA (35.7%), MGHA (36.4%) and WGHA (27.8%). The most common resistance phenotype was: NA-S-XT-AMC-TE-CRO-C-CN with overall occurrence of 9.4% (5/53) observed in AGH (10.0%), GHA (16.7%) and MGHA (18.2%) but not in WGHA. All (100%) isolates were multiple antibiotic resistance (MAR) isolates, with MAR indices above 0.2; and the commonest MAR index of 0.6 (30.0%) in AGHA, 0.8 (35.7%) in GHA; 0.8 (45.6%) in MGHA, and was 0.7 (38.9%) in WGHA. Multidrug resistance (MDR) was the commonest at 96.2% (51/53), with occurrences in the selected hospitals as follows: AGHA (90.0%), GHA (100.0%) and MGHA (100.0%) and WGHA (94.4%).The PMQR genes detected had overall frequency in the order: *aac(6')-Ib-cr* (50.0%) >*qnrB* (37.5%) >*qnrS* (18.8%); *qnrS* was absent in AGHA and WGHA. The genes co-existed with one another with the *qnrB*+ *aac(6')-Ib-cr* combination, present in isolates from all the hospitals, being the most common at (31.3%). Ciprofloxacin was the most effective antibiotic against the isolates; most isolates were MAR with prior exposure to antibiotics; most isolates were MDR and the ciprofloxacin-resistant isolatesharbored *qnrS*, *qnrB* and *aac(6')-Ib-cr* PMQR genes, with *aac(6')-Ib-cr* being the most prevalent.

Keywords: Plasmid; quinolone; genes; Salmonella typhi.

1. INTRODUCTION

Salmonella typhi is a Gram-negative rod shape bacterium that belongs to the family Enterobacteriaceae [1]. This bacterium has been reported to cause Typhoid fever both in developed and developing countries [2]. Typhoid fever caused by *S. typhi* continues to be a major public health problem and have estimated to cause 21.6 million and 216,500 deaths globally [2]. Typhoid is the fourth major killer disease that is most prevalent disease worldwide and responsible for 3 million deaths, 16 million annual typhoid cases and 1.3 million gastroenteritis cases [3]. Typhoid fever is one of the life-threatening illnesses caused by *Salmonella enterica* serovar *typhi* commonly known as *S. typhi* [4]. It is the 4th major disease in the area of higher transmission [4]. The wrong use of antibiotics has led to emergence of multidrug resistance of *S. typhi* in different parts of the world, which eventually appears as a leading cause of long lasting stay in the hospital, additional cost of fitness care and increased morbidity and mortality [5].

Fluroquinolones (FQs) such as ciprofloxacin are widely used in the therapy of typhoid fever because of their high efficacy, lesser side effects and convenient oral dosages [6,7]. However, the extensive use of these agents has led to the development of bacterial resistance to quinolones over time [6,8,9] Fluoroquinolone resistance in now gram-negative bacteria is infact a global issue [10] and is known to be mediated in *S. typhi* by point mutations in quinolone resistance determining regions (QRDR) of the genes encoding DNA gyrase (*gyrA* and *gyrB*) and topoisomerase IV (*parC* and *parE*); and carriage of the plasmid-mediated quinolone resistance (PMQR) genes [7,11,48].

Plasmid-mediated quinolone resistance was first reported in 1998 in the United States in a multi-resistant urinary *Klebsiella pneumoniae*; but it is now being reported in clinical and environmental isolates of other gram-negative as well as and gram-positive bacteria, and appears to be spreading [11,12]. Three mechanisms of PMQR have been discovered. They include: mutations in *qnr* genes (*qnrA*, later *qnrB*, *qnrC*, *qnrD*, *qnrS* and *qnrVC*) [12]; acetylation of quinolones with an appropriate amino nitrogen target (such as ciprofloxacin and norfloxacin) by aminoglycoside acetyltransferase encoded by *aac(6')-Ib-cr* gene; and enhanced efflux through QepA and OqxAB pumps encoded by *qepA* and *oqxAB* genes respectively [11,13].

Plasmid-mediated quinolone resistances in *S. typhi* isolated from clinical and environmental sources have been reported globally [9,14-17]. In Nigeria, although there are few documented evidences [18-22] and none in the area under study. Hence, more extended studies on molecular characterization of quinolone resistance in the country still needed to be done.

This study investigated the antimicrobial resistance profile and carriage of PMQR genes in *S. typhi* isolated from stool of suspected typhoid fever patients attending some selected public general hospitals in Abuja municipal, Nigeria, to provide a possible basis for the presence of the observed ciprofloxacin resistance. This study has far reaching implication as fluoroquinolones are drugs of choice for the treatment of typhoid fever caused by *S. typhi*.

2. MATERIALS 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 antibiotic discs and potency that was used in this study include: Amoxicillin/Clavulanic acid (AMC: 30 µg), Ceftazidime (CAZ: 30 µg),Ceftriaxone (CRO: 30 µg), Ciprofloxacin (CIP: 5 µg), Sulphamethoxazole/Trimethoprim (SXT: 25µg), Gentamicin (CN: 10 µg),Chloramphenicol (CH: 30µg), streptomycin (S: 30 µg), Nalidixic acid (NA: 30 µg) and Tetracycline (TE: 30 µg). All the discs were sourced from Oxoid Ltd, U.K.

2.3 Chemicals and Reagents

The chemicals and reagents that was used in this study include: Acridine orange, Carbol fuschin, 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.

2.5 Study Location

The study was carried out at selected general hospitals in Abuja municipal, Nigeria, namely: Asokoro General Hospital Abuja (AGHA), Garki Hospital (GHA), Wuse General Hospital Abuja (WGHA) and Maitama General Hospital Abuja (MGHA). Asokoro General Hospital is a 250-bed public hospital located at No.31 Julius Ngerere Crescent, Off Yakubu Gowon Crescent, Asokoro, Abuja, Nigeria. Garki Hospital is a 120-bed private-public hospital located at Tafewa Balewa Way, Area 8, Garki, Abuja, Nigeria. Wuse General Hospital is 200-bed public hospital located at Conakry Street, Off Herbert Macaulay Way Zone 3, Wuse, Abuja, Nigeria. Maitama General Hospital is a public hospital located at No. 61 Aguyi Ironsi Street, Maitama, Abuja, Nigeria.

Table 1. Primers used, their sequences and amplicon sizes

S/N	Target genes	Sequence	Amplicon size (bp)	References
1	*qnrA* forward *qnrA* reverse	5'-CAGCAAGAGGATTTCTCACG-3' 5'-AATCCGGCAGCACTATTACTA-3'	630	[23]
2	*qnrB* forward *qnrB* reverse	5'-CCTGAGCGGCACTGAATTTAT-3' 5'-GTTTGCTGCTCGCCAGTCGA-3'	488	[23]
3	*qnrS* forward *qnrS* reverse	5'-GCAAGTTCATTCAACAGGT-3' 5'-TCTAAACCGTCGAGTTCGGCG-3'	428	[23]
4	*OqxA* forward *OqxA* reverse	5'-CCGCACCGAATAAATTAGTCC-3' 5'-GGCGAGGTTTTGATAGTGGA-3'	313	[23]
5	*OqxB* forward *OqxB* reverse	5'-CCGCACCGAATAAATTAGTCC-3' 5'-GGCGAGGTTTTGATAGTGGA-3'	313	[23]
6	*aac-(6')-Ib-cr*	5'-TTGGAAGCGGGGACGGAM-3' 5'-ACACGGCTGGACCATA-3'	266	[23]
7	*qepA*Forward Reverse	5'-CTGCAGGTACTGCGTCATG-3' 5'-CGTGTTGCTGGAGTTCTTC-3'	403	[23]

2.6 Inclusion and Exclusion Criteria

Patients included in the study were only those with suspected cases of typhoid fever on visible clinical symptoms namely: Malaise, headache, fatigue signs, diarrhea and cough in the suspected hospitals. Patients without cases of typhoid fever and those with cases of typhoid fever who are on antibiotics were excluded from this study.

2.7 Sample Size Determination

The sample size was calculated manually, using the formula below as described [24].

$$N = \frac{z^2 p \Sigma}{d^2}$$

Where,

N= Desired sample size (when the population >10,000);
Z= Standard normal deviate, usually set at 1.96, which usually correspond to
95% confidence level;
P= proportion in the target population, set at 50% = 0.05)
∑= prevalence for non-infection confidence estimated at 95% (≥1.5)
Confidence interval = 0.05
d= tolerated margin of error or degree of accuracy = 0.05
The sample size (N) was calculated as follows:

$$N = \frac{(1.96)^2 \times 0.5 \times 0.5}{(0.05)^2}$$

$$N = \frac{0.9604}{0.0025}$$

$$N = 348$$

2.8 Sample Collection

A total of 400 stool samples of patients with suspected cases of typhoid fever were collected using sterile container and transported using ice pack to the Microbiology Laboratory, Nasarawa State University, Keffi for analysis.

2.9 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 [25] and further identified using KB003HI25^{TM} identification kits following manufacturer's instruction.

2.10 Antimicrobial Susceptibility Testing

The antimicrobial susceptibility testing of the isolates was carried out as described by Clinical and Laboratory Standards Institute [26]. Briefly, one pure colony 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 Standard was prepared as follows: 0.5 ml of 1.172% (w/v) $BaCl_2.2H_2O$ was added into 99.5 ml of 1% (w/v) H_2SO_4 [26].

A sterile swab stick was soaked in the standardized bacteria suspension and streaked on Mueller-Hinton agar plates and the antibiotic disc were aseptically placed at the centre of the plates and allowed to stand for 1 h for pre-diffusion. The plates were incubated at 37ºC 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 [26].

2.10.1 Determination of multiple antibiotic resistance index

Multiple Antibiotic Resistance (MAR) was defined here as resistance to 2 or more antibiotics; and the MAR index of the isolates was determined as described previously [8] using the formula:

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

2.11 Classification of Antimicrobial Resistance

Antibiotic resistance in the isolates were classified into: multidrug resistance (MDR: non-susceptibility to at least 1 agent in at least 3 antimicrobial categories); extensive drug resistance (XDR: non-susceptible to at least 1 agent in all but at most 2 antimicrobial categories); pan drug resistance (PDR: non-susceptibility to all antimicrobials listed) [27].

2.12 Molecular Detection of Plasmid-mediated Quinolone Resistance Genes

2.12.1 DNA extraction

The DNA of ciprofloxacin resistant isolates was extracted using boiling method as described [28]. Briefly, following the purification, one pure colony

Table 2. Cultural, morphological and biochemical characteristics of *Salmonella typhi* isolated from patients with suspected cases of typhoid fever attending selected general hospitals in Abuja municipal, Nigeria

Cultural characteristics	Morphological characteristics		Biochemical characteristics													Inference
	Gram	Morphology	OXD	MOT	UR	TDA	CUT	LYS	H_2S	ONPG	NIT	LAC	MAL	IN	MR	
Colonies that were colorless on MCA, black on SSA and black metallic sheen on BSA	-	Rod shape	-	+	-	-	-	+	+	-	+	-	+	-	+	*S. Typhi*

OXD=Oxidase MOT= Motility test; UR: Urease test= TDA; LYS= Lysine utilization; H_2S= Hydrogen sulphide; ONPG= Onpg-Galactosidase test; NIT= Nitrate test; LAC= Lactose fermentation test; MAL=Maltonate test; IN= Indole fermentation test; MR= Methyl red; MCA= MacConkey agar; SSA= Salmonella-Shigella agar; BSA: Bismuth Sulphite agar

of ciprofloxacin isolates, was inoculated into 2 ml of LB broth and incubated at 37ºC for 8 h. Exactly 200 µl of the LB culture was then transferred into Eppendorf tube and centrifuged in a micro centrifuge at 3200 revolutions per minute (rpm) for 2 min at room temperature. The supernatant was discarded leaving the cells in the tube. The cells were washed twice with washing buffer. About 0.5 ml of sterile phosphate buffer was added to the pellet and vortexed for 5sec. It was then heated at 90ºC for 10 min. It was then cooled down rapidly by freezing for 10 min. It was then centrifuged at 3200 rpm for 1 min to separate the DNA and the cells debris. 300 µl of the supernatant containing the DNA was then transferred into 2 ml Eppendorf tube and stored at -10ºC for further use [28].

2.12.2 Amplification of target genes

The DNA amplification of target PMQR genes in ciprofloxacin-resistant isolates was carried out using single plex method by modification of the method described [28]. 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 $MgCl_2$, 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 cycler was closed and the door was closed. Then *qnrA*, *qnrB* and *qnrS* genes were amplified under the following conditions: initial denaturation at 94ºC for 5 min followed by 32 cycles of amplification at 94ºC for 45 sec each, annealing at 53ºC for 45sec, with final extension at 72ºC for 5min [28].

The amplification condition for detection of *aac(6')-Ib-cr* was carried out as follows: initial denaturation at 95ºC for 20 min, annealing at 59ºC for 40 sec and initial extension at 70ºC for 30 sec and with final extension at 72ºC for 5 min [28].

2.12.3 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 [28].

3. RESULTS AND DISCUSSION

3.1 Isolation and Identification of *Salmonella typhi*

The cultural, morphological and biochemical characteristics of the *S. typhi* isolated from the patients is as presented in Table 2. The organism which grew with colourless colonies on *Salmonella*-Shigella (SSA) Agar, black metallic sheen on Bismuth Sulphite Agar, Gram negative, rod shape, lysine-positive, nitrate-positive, Hydrogen sulphide-positive, Maltose-positive and Methyl red-positive was identified as *S. typhi*.

3.2 Occurrence of *Salmonella typhi*

The occurrence of *S. typhi* isolated from the patients is as given in Table 3. The isolation rate for *S. typhi* was13.3 % (53/400). As shown in Fig. 1, the occurrence in relation to the hospitals was most prevalent at WGHA (18.0%) and the least at AGHA (10.0%).

Table 3. Occurrence of *Salmonella typhi* from stool of patients with suspected typhoid fever in some selected general hospital in Abuja municipal, Nigeria in relation to Hospital

Number of samples	No. (%) *S. typhi*
400	53(13.3)

The occurrence of *S. typhi* in relation to age of patients is as shown in Fig. 2. The occurrence was highest at age 21-30yrs in AGHA (20.0%), GHA (33.3%) and WGHA (45.0%); and ≤10 yrs in MGHA (30.0%); but least at 11-20 yrs. (AGHA, 4.3%), 31-40 yrs (GHA, 3.0%), 21-30 yrs (MGHA, 5.0%) and 31-40 yrs (WGHA, 4.8%). The occurrences of *S. typhi* in relation to age of patients with suspected typhoid fever was statistically insignificant ($p > 0.05$).

The occurrence of the *S. typhi* isolated from the patients in relation to gender is as shown in Fig. 3. The occurrence was higher in females at AGHA (12.7%) and GHA (16.0%) than males (AGHA, 6.7%; GHA, 8.0%); but higher in males at MGHA (11.4%) and WGHA (18.2%) than females (MGHA, 10.0%; WGHA, 17.9%).The occurrences of *S. typhi* in relation to gender were statistically insignificant ($p > 0.05$).

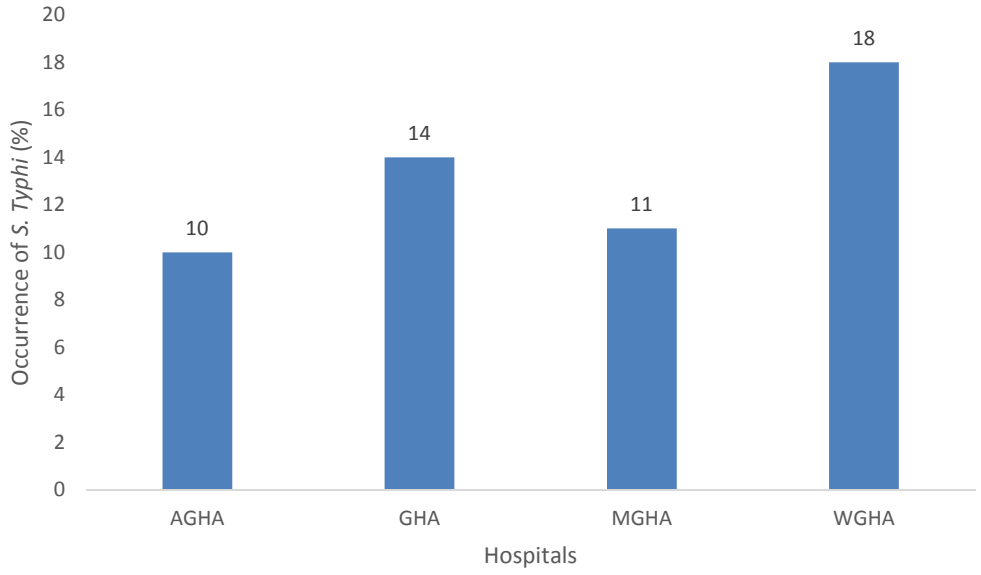

Fig. 1. Occurrence of *Salmonella typhi* in stool of patients with suspected typhoid fever attending selected hospitals in Abuja municipal, Nigeria, in relation to hospital (AGHA= Asokoro General Hospital, Abuja; GHA= Garki Hospital, Abuja; MGHA = Maitama General Hospital, Abuja; WGHA= Wuse General Hospital, Abuja)

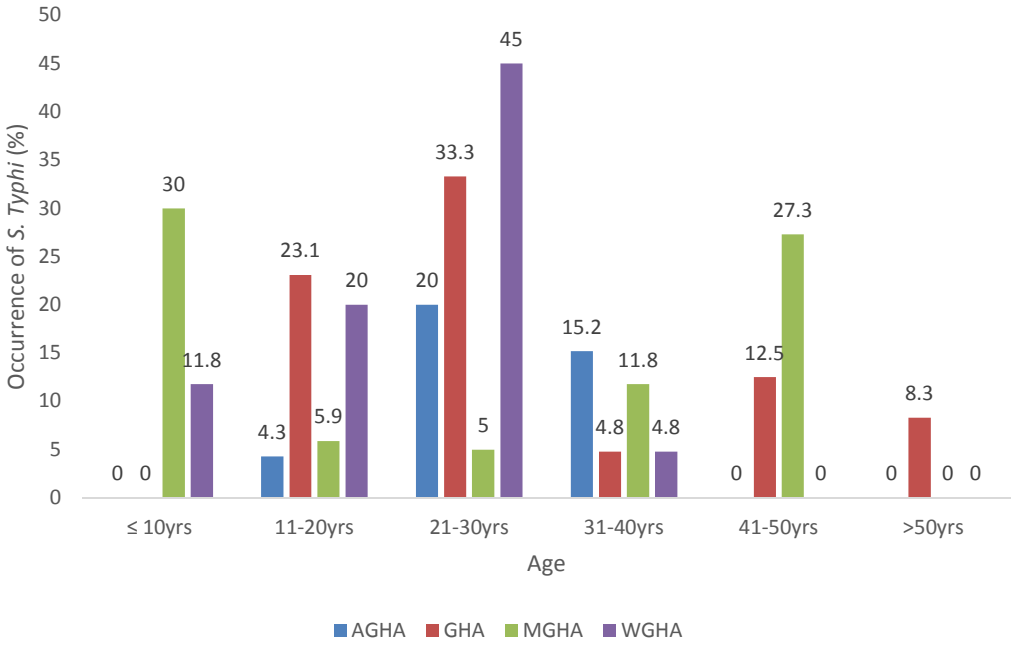

Fig. 2. Occurrence of *Salmonella typhi* in stool of patients with suspected typhoid fever attending selected hospitals in Abuja municipal, Nigeria, in relation to age (AGHA= Asokoro General Hospital, Abuja; GHA= Garki Hospital Abuja; MGHA= Maitama General Hospital, Abuja; WGH= Wuse General Hospital, Abuja)

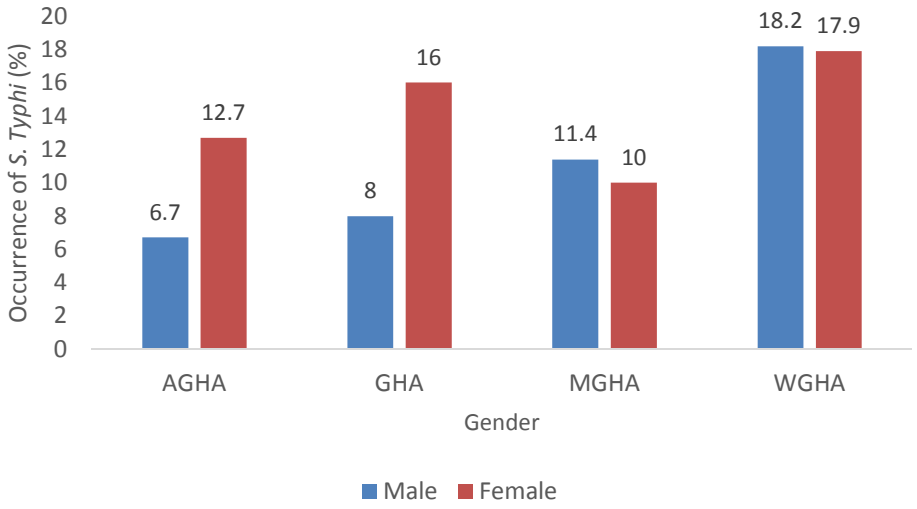

Fig. 3. Occurrence of *Salmonella typhi* from stool of patients with suspected typhoid fever attending selected hospitals in Abuja municipal, Nigeria, in relation to gender (AGHA= Asokoro General Hospital Abuja; GHA= Garki Hospital Abuja; MGHA = Maitama General Hospital, Abuja; WGH= Wuse General Hospital, Abuja)

3.3 Antimicrobial Resistance Profile

The antimicrobial resistance profile of the isolates is as given in Table 4. Resistance to ciprofloxacin was the least at 30.2%, distributed as follows: AGHA (20.0%), GHA (35.7%), MGHA (36.4%) and WGHA (27.8%). For isolates from AGHA, resistance was high to amoxicillin/clavulanic acid, sulphamethoxazole/ trimethoprim, nalidixic acid, tetracycline and streptomycin; but low to gentamicin and ciprofloxacin. For GHA, resistance was high to chloramphenicol, tetracycline and nalidixic acid, amoxicillin/clavulanic acid and sulphamethoxazole/ trimethoprim; but low to ceftazidine and ciprofloxacin. For MGHA, resistance was high to amoxicillin/clavulanic acid, streptomycin and tetracycline; but low to ciprofloxacin. For isolates from WGHA, resistance was high to tetracycline and Nalidixic acid; but low to ciprofloxacin and gentamicin. The differences in the resistances of *S. typhi* isolates were statistically insignificant (P> 0.05).

3.4 Antimicrobial Resistance Phenotypes

Antimicrobial resistances in the *S. typhi* isolates were distributed into different phenotypes as given in Table 5. The most common phenotype was: NA-S-XT-AMC-TE-CRO-C-CN with overall occurrence of 9.4% (5/53) observed in AGH (10.0%), GHA (16.7%) and MGHA (18.2%) and (0%) in WGHA.

3.5 Multiple Antibiotic Resistance (MAR) Index

All (100%) of isolates were MAR isolates; and the MAR indices are as presented in Table 6. The highest and lowest MAR ratios were 1.0 and 0.4 at overall occurrences of 5.7% and 3.8% respectively. The overall most common MAR index was 0.8 at a frequency of 26.4%. The commonest MAR index in AGHA was 0.6 (30.0%); GHA was 0.8 (35.7%); MGHA was 0.8 (45.6%) and WGHA was 0.7 (38.9%).

3.6 Classes of Antimicrobial Resistance

The various classes of antimicrobial resistances in the *S. typhi* isolates are as distributed in Table 7. Multidrug resistance (MDR) was the commonest at 96.2% (51/53), with occurrences in the selected hospitals as follows: AGHA (90.0%), GHA (100.0%) and MGHA (100.0%) and WGHA (94.4%).

3.7 Plasmid-mediated Quinolone Resistance Genes

The detection of PMQR genes is as shown in Table 8. The PMQR genes detected had overall frequency in the order: *aac(6')-Ib-cr* (50.0%) >*qnrB* (37.5%) >*qnrS* (18.8%); *qnrS* was absent in AGHA and WGHA.

Table 4. Antimicrobial resistance profile of *Salmonella typhi* from stool of patients with suspected typhoid fever attending selected general hospitals in Abuja municipal, Nigeria

Antibiotics	Disc content (µg)	No. (%) Resistance (n= 53)				Total (%) resistance (n= 53)
		AGHA (n= 10)	GHA (n= 14)	MGHA (n= 11)	WGHA (n= 18)	
Amoxicillin/Clavulanic acid (AMC)	30	10(100.0)	13(92.9)	11(100.0)	16(88.9)	50(94.3)
Ceftazidime (CAZ)	30	4 (40.0)	5 (35.7)	5 (45.5)	8(44.4)	22(41.5)
Ceftriaxone (CRO)	30	8 (80.0)	10(57.1)	9(81.8)	13(72.2)	40(75.5)
Chloramphenicol (C)	30	7(70.0)	14(100.0)	8(72.7)	10(55.6)	39(73.6)
Ciprofloxacin (CIP)	5	2 (20.0)	5 (35.7)	4(36.4)	5(27.8)	16(30.2)
Gentamicin (CN)	10	2(20.0)	6 (42.9)	8 (72.7)	6(33.3)	22(41.5)
Streptomycin (S)	30	9 (90.0)	10 (71.4)	11(100.0)	14(77.8)	44(83.0)
Sulphamethoxazole/ Trimethoprim (SXT)	25	10(100.0)	13 (92.9)	10(90.9)	17(94.4)	50(94.3)
Tetracycline (TE)	30	9 (90.0)	14(100.0)	11(100.0)	18(100.0)	52(98.1)
Nalidixic acid (NA)	30	10(100.0)	14(100.0)	10 (90.9)	18(100.0)	52(98.1)

AGHA= Asokoro General Hospital, Abuja, GHA= Garki Hospital Abuja, MGHA= Maitama General Hospital, Abuja. WGHA= Wuse General Hospital Abuja

Table 5. Antimicrobial resistance phenotypes of *Salmonella typhi* isolated from stool of patients with suspected typhoid fever in selected general hospitals in Abuja municipal, Nigeria

Antibiotics resistance phenotype	Frequency (%)				Total (%) Isolates (n= 53)
	AGHA (n= 10)	GHA (n=14)	MGHA (n= 11)	WGHA (n= 18)	
AMC, TE, CAZ, CRO	1(10.0)	0 (0.0)	0(0 .0)	0(0.0)	1(1.9)
NA, S, TE, CRO	0(0.0)	1(7.1)	0(0.0)	0(0.0)	1(1.9)
NA, S, SXT, TE, C	0(0.0)	0(0.0)	0(0.0)	1(5.6)	1(1.9)
NA, SXT, AMC, CAZ, C	0(0.0)	0(0)	0(0.0)	1(5.6)	1(1.9)
NA, SXT, AMC, TE, CRO	0(0.0)	0(0.0)	1(9.1)	0(0.0)	1(1.9)
S, AMC, TE, CAZ, CRO	0(0.0)	1(7.1)	0(0.0)	0(0.0)	1(1.9)
NA, S, SXT, AMC, TE, CRO	1(10.0)	0(0.0)	1(9.1)	1(5.6)	3(5.7)
NA, S, SXT, AMC, TE, C	0(0.0)	0(0.0)	0(0.0)	2(11.1)	2(3.8)
NA, SXT, AMC, TE, CAZ, C	0(0.0)	0(0.0)	0(0.0)	1(5.6)	1(1.9)
NA, SXT, AMC, TE, CRO, CIP	0(0.0)	0(0.0)	1(9.1)	2(11.1)	3(5.7)
NA, SXT, AMC, TE, CRO, C	1(10.0)	2(16.7)	0(0.0)	0(0.0)	3(5.7)
NA, S, SXT, AMC, TE, CAZ	1(10.0)	0(0.0)	0(0.0)	0(0.0)	1(1.9)
NA, S, SXT, TE,C, CN	0(0.0)	0(0.0)	1(9.1)	0(0.0)	1(1.9)

Antibiotics resistance phenotype	Frequency (%)				Total (%) Isolates (n= 53)
	AGHA (n= 10)	GHA (n=14)	MGHA (n= 11)	WGHA (n= 18)	
NA, S, SXT,AMC, TE, C, CN	1(10.0)	0(0.0)	0(0.0)	0(0.0)	1(1.9)
NA, S, AMC, TE, CAZ, CRO, C	0(0.0)	1(7.1)	0(0.0)	0(0.0)	1(1.9)
NA, S, SXT, AMC, TE, CAZ, CRO	0(0)	0(0.0)	0(0.0)	1(5.6)	1(1.9)
NA, S, SXT, AMC, TE, CRO, C	0(0)	0(0.0)	0 (0)	3(16.7)	3(5.7)
NA, S, SXT, AMC, TE, C, CN	0(0.0)	0(0.0)	2 18.2)	0(0.0)	2(3.8)
NA, S, SXT, AMC, TE, CRO, CN	1(10.0)	1(7.1)	0(0.0)	1(5.6)	3(5.7)
NA, , SXT, AMC, TE,CAZ, C, CN	0(0.0)	1(7.1)	0(0.0)	0(0.0)	1(1.9)
S, SXT, AMC, TE, CRO, C, CN	0(0.0)	1(7.1)	0(0.0)	0(0.0)	1(1.9)
NA, S, SXT, AMC, TE, CAZ, CRO, CIP	1(10.0)	0(0.0)	0(0.0)	2(11.1)	3(5.7)
NA, S, SXT, AMC, TE, CRO, C, CN	1(10.0)	2(16.7)	2(18.2)	0(0.0)	5(9.4)
NA, S, SXT, AMC, TE, CAZ, CRO, C	0(0.0)	1(7.1)	0(0.0)	0(0.0)	1(1.9)
NA, S, SXT, AMC, TE, CAZ, CRO, CN	0(0.0)	1(7.1)	0(0.0)	0(0.0)	1(1.9)
NA, S, SXT, AMC, TE, CRO, CIP, C	0(0.0)	1(7.1)	0(0.0)	2(11.1)	3(5.7)
NA, S, SXT, AMC, TE, CAZ, CRO, CIP, C	0(0.0)	0(0.0)	1(9.1)	1(5.6)	2(3.8)
NA, S, SXT, AMC, TE, CAZ, CIP, C, CN	0(0.0)	1(7.1)	1(9.1)	0(0.0)	2(3.8)
NA, S, SXT, AMC, TE, CAZ, CRO, CIP, C, CN	1(10.0)	1(7.1)	1(9.1)	0(0.0)	3(5.7)

AMC= Amoxicillin/Clavulanic acid; CAZ= Ceftazidime; CRO= Cefriaxone; C= Chloramphenicol;CIP= Ciprofloxacin; CN= Gentamicin; S= Streptomycin; SXT= Sulphamethoxazole/trimethoprim;TE= Tetracycline; NA= Nalidixic acid; AGHA= Asokoro General Hospital, Abuja; GHA= Garki Hospital, Abuja; MGHA= Maitama General Hospital, Abuja; WGHA= Wuse General Hospital, Abuja

Table 6. Multiple Antibiotic Resistance (MAR) index of *Salmonella typhi* isolated from stool of patients with suspected typhoid fever attending selected general hospitals in Abuja municipal, Nigeria

No. of antibiotic resistance(a)	No. of antibiotics tested (b)	MAR index (a/b)	No. (%) MAR isolates (n =53)				Total (%) MAR isolates (n= 53)
			AGHA (n= 10)	GHA (n= 14)	MGHA (n =11)	WGHA (n= 18)	
10	10	1.0	1(10.0)	0(0.0)	0(0.0)	2(11.1)	3(5.7)
9	10	0.9	1(10.0)	2 (14.3)	0(0.0)	1(5.6)	4(7.5)
8	10	0.8	2(20.0)	5(35.7)	5(45.6)	2(11.1)	14(26.4)
7	10	0.7	1(10.0)	4(28.6)	2(18.2)	7(38.9)	12(22.6)
6	10	0.6	3(30.0)	1(7.1)	3(27.3)	5(27.8)	12(22.6)
5	10	0.5	1(10.0)	1(7.1)	1(9.1)	1(5.6)	4(7.5)
4	10	0.4	1(10.0)	1(7.1)	0(0.0)	0(0.0)	2(3.8)

AGHA= Asokoro General Hospital, Abuja; GHA= Garki Hospital, Abuja, MGHA = Maitama General Hospital, Abuja; WGHA= Wuse General Hospital, Abuja

Table 7. Classes of antimicrobial resistance in the *Salmonella typhi* isolates from stool of patients with suspected cases of typhoid fever attending selected general hospitals in Abuja municipal, Nigeria

Classes of antimicrobial resistance	No. (%) isolates				Total (%) isolates (n= 53)
	AGHA (n = 10)	GHA (n = 14)	MGHA (n = 11)	WGHA (n = 18)	
MDR	9 (90.0)	14 (100.0)	11(100.0)	17 (94.4)	51(96.2)
XDR	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0(0.0)
PDR	1(10.0)	0 (0.0)	0 (0.0)	1 (5.6)	2(3.8)

AGHA= Asokoro General Hospital, Abuja; GHA= Garki Hospital, Abuja; MGHA= Maitama General Hospital, Abuja; WGHA= Wuse General Hospital, Abuja; MDR= Multi-drug resistance (non-susceptible to ≥1 agent in ≥3 antimicrobial categories); XDR = Extensive drug resistance (non-susceptible to ≥1 agent in all but ≤2 antimicrobial categories); PDR=Pan drug resistance (non-susceptible to all antimicrobial listed) [7]

Table 8. Molecular detection of plasmid-mediated quinolone resistance genes in ciprofloxacin-resistant *Salmonella typhi* from stool of patients with suspected cases of typhoid fever in selected general hospitals in Abuja municipal, Nigeria

Plasmid quinolone resistance genes	No. (%) of *S. typhi*				Total (%) isolates (n= 16)
	AGHA (n = 2)	GHA (n =5)	MGHA (n = 4)	WGHA (n = 5)	
qnrB	1 (50.0)	2 (40.0)	2(50.0)	1 (20.0)	6(37.5)
qnrS	0 (0.0)	2 (40.0)	1(25.0)	0 (0.0)	3(18.8)
aac(6')-Ib-cr	1(50.0)	3(60.0)	2 (50.0)	2 (40.0)	8(50.0)

AGHA= Asokoro Genenral Hospital Abuja; GHA= Garki Hospital Abuja; MGHA= Maitama General Hospital Abuja; WGHA= Wuse General Hospital Abuja

Table 9. Co-existence of plasmid-mediated quinolone resistance genes in ciprofloxacin-resistant *Salmonella typhi* from stool of patients with suspected cases of typhoid fever in selected general hospitals in Abuja municipal, Nigeria

Plasmid quinolone resistance genes	No. (%) of *S. typhi*				Total (%) isolates (n= 16)
	AGHA (n = 2)	GHA (n =5)	MGHA (n = 4)	WGHA (n = 5)	
qnrB+ qnrS	0 (0.0)	2 (40 .0)	0 (0.0)	1 (20.0)	3(18.8)
qnrB + aac(6')-Ib-cr	1 (50.0)	2 (40.0)	1(25.0)	1 (20.0)	5(31.3)
qnrS + aac(6')-Ib-cr	0 (0.0)	3 (60.0)	0 (0.0)	0 (0.0)	3(18.8)
qnrB +qnrS + aac(6')-Ib-cr	1 (50.0)	1 (20.0)	0(0.0)	1 (20.0)	3(18.8)

AGHA= Asokoro General Hospital Abuja; GHA= Garki Hospital Abuja; MGHA= Maitama General Hospital Abuja; WGHA= Wuse General Hospital Abuja

The distribution of the genes in relation to their co-existence with one another is given in Table 9. The genes co-existed with one another with the *qnrB + aac(6')-Ib-cr* combination, present in isolates from all the hospitals, being the most common at (31.3%). The *qnrB + qnrS* combination was absent in AGHA and WGHA; *qnrS + aac(6')-Ib-cr* combination was absent in AGHA, MGHA and WGHA; and *qnrB + qnrS + aac(6')-Ib-cr* combination was absent in MGHA.

Typhoid fever is a disease common in developing countries as a result of poor sanitation, crowding and social abyss [2]. Antimicrobial agents, including fluoroquinolones, have been the mainstay control of the disease. However, resistance has limited the application of these agents to manage the disease. This study investigated the antimicrobial resistance profile and carriage of PMQR genes in *S. typhi* from stool of suspected typhoid fever patients attending selected general hospitals in Abuja municipal, Nigeria.

The overall occurrence of 13.3% for *S. typhi* in stool of the patients found in this study was high, although less than 26.3% and 46.5% reported previously [29,30]. Similarly, the occurrence of *S. typhi* isolates observed in this study was also similar with another study earlier reported [31],

[32]. The isolation of *S. typhi* from stool of patients with suspected typhoid fever in the selected General hospitals was an indication that such organism may be responsible for typhoid fever since *S. typhi has* widely been reported by researchers as the most common cause of typhoid fever [33]. The occurrence of *S. typhi* from stool of patients in relationship to their age was higher at age 21-30 yrs at Asokoro General Hospital Abuja, Garki Hospital Abuja and Wuse General Hospital Abuja in agreement with a study earlier reported which reported high occurrence of *S. typhi* in patients of age > 10-41 yrs [31] and 21-31 yrs [34]. Though our findings also show that the differences in the occurrence of *S. typhi* in relation to age in selected hospitals were statistically insignificant which implies that age may not necessarily be a factor for the occurrence of *S. typhi*.

The occurrence of *S. typhi* in female than male in selected hospitals namely Asokoro General Hospital and Garki Hospital Abuja was not in agreement with the study earlier described [13, 35]. In addition, the high occurrence of the isolates is more in male than female in Maitama General Hospital Abuja and Wuse General Hospital Abuja was in agreement with the study earlier described [35]. Though the occurrence of the isolates in related to gender was statistically insignificant which suggest that the gender of individual may not be necessary factor for the occurrence of *S. typhi*.

The high resistance of the isolated for selected hospitals to antibiotics such as amoxicillin/ clavulanic acid, ceftriaxone, chloramphenicol, streptomycin; sulphamethoxazole/trimethoprim, tetracycline and nalidixic acid as observed in this study was not surprising and may be due to misuse and abuse of the antibiotics. The high resistance of the isolated to tetracycline, ceftriaxone and sulphamethoxazole/trimethoprim was higher than 85.71 %, 68.51% and 97.15% reported [36].

The low resistance of the isolates to ciprofloxacin and gentamicin justify their use as common drugs of choice for the treatment of typhoidal *Salmonella* [37]. The low resistance of this isolates to antibiotics mention was 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 [38,39]. The percentage occurrence of MDR isolates in the selected hospital observed in this study was higher than 15% reported [38], [39]. 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 [40].

The occurrence of plasmid mediated quinolones resistance genes in *S. typhi* isolated observed in this study was an indicator that such genes may be responsible for Ciprofloxacin resistance. Our findings in this study shows that commonest PMQR genes was *aac-(6')-Ib-cr* and *qnrB* and this is not different from the study earlier reported [40,41]. The occurrence of *aac-(6')-Ib- cr* genes in resistant isolates was an indication that the resistance may be due to acetylation of the Ciprofloxacin while *qnrB* gene may be due to the percentage detection of *aac-(6')-Ib- cr, qnrB* and *qnrS* in ciprofloxacin isolates observed in this study was lower than 64% reported [42].

The resistance of *S. typhi* isolates to ciprofloxacin observed was low and was less than 91% and 41% as reported [43,44], but was greater than 1.2% as reported [45]. The low resistance to ciprofloxacin by the isolates could be that such antibiotics may not have been misused or abused in the study location [46]. Also, the resistance to ciprofloxacin justifies the use of it as the most common antibiotic prescribed for treatment of typhoid fever. *Salmonella typhi is* one the most common organism associated with both hospital and community acquired infection [47,48].

4. CONCLUSION

The overall occurrence of *S. typhi* from stool of patients attending selected general hospitals in Abuja municipal, Nigeria was 13.3%, with the lowest (10.0%) hospital-based occurrence in Asokoro General Hospital compared and the highest (18.0%) in Wuse General Hospital. Patients aged 21-30 years harbored more bacteria. No gender influence on occurrence. The antibiotics ceftazidime, gentamicin, and ciprofloxacin were the most effective against the isolates. Most of the isolates were MDR and *aac-(6')-Ib-cr* genes were the most common genes detected in ciprofloxacin- resistant isolates in the selected hospitals.

CONSENT

All authors declare that 'written informed consent was obtained from the patient.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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