Detecting Quinolone-Resistant *Salmonella typhi* in Stool Samples from Selected Abuja Hospitals

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Abstract
Typhoid fever, a disease caused by *Salmonella typhi,* remains a major public health concern in many parts of the world. This study is aimed at molecularly detecting plasmid-mediated Quinolone resistance genes of *Salmonella typhimurium* from *the stool* of patients attending selected hospitals in Karu, Abuja Municipal Area Council, Abuja, Nigeria. A total of 300 stool samples were collected from patients attending ECWA Hospital (EH) and Jikwoyi Medical Centre (JMC), all located within Karu. *Salmonella typhi* was isolated and identified using standard microbiological methods. Antibiotic susceptibility test was carried out using the Clinical Laboratory Standard Institute (CLSI) methods. Molecular detection of the plasmid-mediated Quinolone genes of the isolates was carried out using Polymerase Chain Reaction (PCR). Electrophoresis was performed to detect the presence of plasmids. The overall occurrence of the isolates was 13.6% with JMC having the highest occurrence (17.1%), while EH is (11.8%). The occurrence of the isolates in relation to age was highest at ages ≥50 years in EH (15.0%) and lower at ages 21-30 years (7.5%), while it was higher at age ≥10years in JMC (26.6%) but lower at age 41-50 years (12.5%). The occurrence in relation to gender was higher in males (11.9%) but lower in females (9.8%). Resistance to the Quinolone antibiotics was Ciprofloxacin (7.3%) and Ofloxacin (39.0%) distributed as follows: CIP at EH (8.7%) and OF (43.5%) while at JMC, CIP (5.5%) and OF (33.3%). The most common resistance phenotype was CIP-GEN-CAZ and GEN-TE-CAZ (9.7%), and the general distribution was EH (13.0%) and JMC (11.1%). Most of the isolates were multi-resistant with MAR indices above 0.2, and the commonest MAR index of 1.0 (4.3%) at EH and 0.5 (5.5%) at JMC. The PMQR genes detected had overall frequency in the order: *aac(6')-Ib-cr*(50.0%), *qnrB*(25%), *qnrS*(25%), and *parC* (25%). Susceptibility testing before prescription should be intensified to avoid resistance.

Keywords: Antibiotic resistance; Molecular Detection; Plasmid-mediated Quinolone resistance (PMQR); *Salmonella typhi*; Typhoid fever

Introduction

Salmonella infections pose a significant global public health challenge due to the costs of monitoring, prevention, and treatment (Nguyen & McSorley, 2024). Salmonella, a Gram-negative, rod-shaped, motile bacterium from the Enterobacteriaceae family, includes over 2,579 serovars, with *S. enterica* causing approximately 99% of human infections, primarily through contaminated food, water, or faecal contact (Fierer, 2022). Infections manifest as enteric fever (caused by typhoidal Salmonella, like *S. typhi* and *S. paratyphi*) or gastroenteritis (caused by non-typhoidal Salmonella), with severe risks for vulnerable groups like the elderly, infants, and immunocompromised individuals (Lu *et al.*, 2025; Qamar *et al.*, 2022).

Typhoidal infections, treated with antibiotics like Chloramphenicol and Ciprofloxacin, present symptoms after 8-28 days, while non-typhoidal infections, treated with hydration, show symptoms within 8-72 hours (Nguyen & McSorley, 2024). Rising antimicrobial resistance, particularly in multi-drug resistant (MDR) strains, is a growing concern, driven by mutations and resistance gene transfer (Abimiku *et al.*, 2019; Giorgio & Helaine, 2025). In Nigeria, high Salmonella incidence is exacerbated by co-infections like malaria, inadequate water systems, urbanisation, and poor waste management, with limited research on Salmonella serovars from human stool (Lu *et al.*, 2025; Han*et al.*, 2024). No Salmonella vaccines are included in Nigeria's healthcare policy, and access to safe water remains a challenge (Akinyemi *et al.*, 2017).

The study aims to evaluate the antibiotic susceptibility of Salmonella strains from patient stool in Karu, Abuja, focusing on isolating *S. typhi*, assessing resistance patterns, and detecting plasmid-mediated Quinolone resistance genes to address the critical issue of Antibiotic Resistance.

Materials and Methods

Study Area: This research was carried out in ECWA Hospital and Jikwoyi Medical Centre, both located within Karu town, in Abuja Municipal Area Council, Abuja, Nigeria. Karu is a satellite town in Abuja, the capital city of Nigeria

Sample Size Determination: The sample size used for this study is calculated using (Krejcie & Morgan, 2017) method. The formula is shown below:

$$N=Z^2P\sum/d^2$$

Where N = desired sample size (when the population > 10,000); Z= standard normal deviation, usually set at 1.96, which usually corresponds to 95% confidence level; P= proportion in the target population, set at 50% (0.5), d= tolerated margin of error.
The population was estimated as p< (0.5) for non-infection, confidence estimated used was 95% (≥ 1.5) confidence interval with degree of accuracy of d (0.05). The designed effect of 1 was used. The sample size was obtained as;

$$N= (1.96)^2\times 0.5 \times 0.5 \div (0.05)^2$$
$$= 0.96405 \div 0.025 \approx 384$$

Sampling Method: A convenience sampling method was employed in this study. A total of 300 stool samples were collected from patients presenting with suspected typhoid fever at the selected healthcare facilities (ECWA Hospital and Jikwoyi Medical Centre) during the study period. Patients who met the inclusion criteria and provided informed consent were consecutively enrolled until the predetermined sample size of 300 was reached. This approach allowed for practical data collection within the operational constraints of the hospitals.

Demographic Data: Demographic data collected for this study included age and gender of the patients. While additional factors such as socioeconomic status, vaccination history, and travel history could provide valuable context regarding potential risk factors associated with infection and antibiotic resistance, these were beyond the scope of this particular study, given the available resources and time constraints.

Ethical Approval: Ethical approval was sought for and issued by ECWA Community Health Initiative, Garki, Abuja (Find attached). Informed consent was sought from all participants and caregivers (for minors) before sample collection. Participants were assured of confidentiality and the right to withdraw. Data was anonymised to protect the privacy of participants.

Collection of Samples
With the consent of the patients, a total of 300 stool samples were collected from the patients attending the selected healthcare facilities. The patients were provided with sterile plastic screw-top universal bottles to collect their stool, which were immediately transported in ice packs to the laboratory for further examination.

Culture and Isolation of Salmonella Species: Stool samples were cultured in 5ml tetrathionate (TT) broth (Oxoid, UK) and incubated at 37°C for 24 hours. Turbid broths were sub-cultured on xylose-lysine deoxycholate (XLD) Agar and Salmonella-Shigella Agar (SSA) (Oxoid, UK), incubated at 37°C for another 24 hours. Plates showing growth had isolates stored on Nutrient Agar (NA) slants for further analysis (Ghazy Hawal & Talib Bakr, 2023)

Identification of Salmonella isolates: Representative colonies were selected based on colonial and morphological similarities and identified using Gram staining and biochemical tests as per Cheesbrough (2006).

Gram staining: Gram staining followed Cheesbrough (2009). A smear of three pure colonies was prepared on a saline drop, air-dried, heat-fixed, stained with crystal violet for 30 seconds, rinsed, decolourised with acetone, counter-stained with safranin for 60 seconds, rinsed, air-dried, and examined under a x100 oil immersion objective (Cheesbrough, 2009).

Biochemical identification: Biochemical tests, as described by Cheesbrough (2006), included Indole production, Methyl-red, Voges-Proskauer, Citrate utilisation, catalase, nitrate reduction, urease production, and glucose fermentation (TSI) to confirm Salmonella species (Cheesbrough, 2009).

Determination of Antibiotic susceptibility of the isolates: *S. typhi* isolates were tested for antibiotic susceptibility using the Kirby-Bauer disk diffusion method per Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2018). This method was chosen for its widespread acceptance, cost-effectiveness, and reliability in clinical microbiology laboratories for routine susceptibility testing.

The study specifically aimed to assess the resistance patterns against commonly prescribed antibiotics for typhoid fever in the region, including quinolones, which are critical for treatment. Overnight nutrient broth cultures were adjusted to 0.5 McFarland standard, streaked on Mueller-Hinton Agar, and tested with the following antibiotics and their respective disc concentrations: Amoxicillin/Clavulanic acid (AMC, 30µg), Nitrofurantoin (NET, 30µg), Ceftazidime (CAZ, 30µg), Ciprofloxacin (CIP, 5µg), Gentamycin (GEN, 10µg), Ofloxacin (OF, 5µg), Streptomycin (S, 10µg), Sulphamethoxazole/Trimethoprim (COT, 25µg), Tetracycline (TE, 30µg), and Ceftriaxone (CTR, 30µg). Plates were incubated at 37°C for 24 hours, and inhibition zones were measured and interpreted as resistant, intermediate, or susceptible according to the breakpoints provided in the CLSI Performance Standards for Antimicrobial Susceptibility Testing, 28th Edition (Fasih *et al.*, 2023; Ruiz *et al.*, 2022; CLSI, 2018)

Detection of Antibiotic Resistance Genes: Bacterial cells were prepared in Luria Bertani (LB) broth (peptone water, yeast extract, sodium chloride, and sodium hydroxide) per Ruiz *et al.* (2022), autoclaved, inoculated with a colony from xylose deoxycholate plates, and incubated at 37°C for 24 hours. DNA was extracted using Zymo Research Kits, involving centrifugation, lysis buffer incubation, spin column processing, washing, and elution, with DNA stored at -86°C. PCR amplified specific DNA segments using Taq polymerase and gene-specific primers shown in Table 1 (Sarah *et al.*, 2011; Kwak *et al.*, 2013; Xi *et al.*, 2014), with cycles of denaturation (94°C), annealing (primer-dependent temperature), and extension (72°C) for 25-40 cycles in an automated cycler (Uzairue *et al.*, 2023; Inayath *et al.*, 2021).

Table 1: Primers sequences and Quinolone resistance genes used in this study

Target gene	Primer name	Primer sequences	Amplicon size (bp)
gyrA	gyrA-F	5-ATGGCTGAATTACCTCAATC-3	647
	gyrA-R	5-CATCATAGTTATCGATGAAATC-3	
gyrB	gyrB-F	5-TCGGCGACACGGATGACGGC-3	704
	gyrB-R	5-ATCAGGCCTTCACGCGCATC-3	
parC	parC-F	5-ACTTGAAGATGTTTTAGGTGAT-3	459
	parC-R	5-TTAGGAAATCTTGATGGCAA-3	
qnrB	qnrB-F	5-GATCGTGAAAGCCAGAAAGG-3	420
	qnrB-R	5-CGATGCCTGGTAGTTGTCC-3	
qnrS	qnrS-F	5-ACGACATTCGTCAACTGCAA-3	210
	qnrS-R	5-TAAATTGGCACCCTGTAGGC-3	
aac(6')-Ib-cr	aac(6')-F	5-TTGCGATGCTCTATGAGTGGCTA-3	482
	aac(6')-R	5-CTCGAATGCCTGGCGTGTTT-3	

Key: F = Forward strand; R = Reverse strand

Bacterial DNA extraction was also conducted using a heat boiling method, involving incubation in TE buffer and following centrifugation protocols to collect supernatants. The DNA was then analysed for concentration and purity, with storage at -20°C until needed. Electrophoretic analysis of the amplified DNA was done using 1% agarose gel. PCR products were mixed with loading dye, loaded into gel slots, and subjected to electrophoresis. After staining with EtBr and destaining, the DNA bands were observed under UV light, and photographs were taken alongside size estimation using a marker.

Statistical analysis
Statistical analysis for antimicrobial resistance was based on Clinical and Laboratory Standards Institute guidelines, comparing proportions of hospital data, gender, and age groups using the chi-square test at a significance level of p=0.05. Results were presented in Mean ± SD.

Results and Discussion
Isolation and identification of *Salmonella enterica:* The colourless colonies on SSA with a black centre, which

Table 2: Cultural, morphological, and biochemical characteristics of Salmonella typhi isolated from stool of patients attending the selected hospitals.

Cultural Characteristics	Morphological Characteristics	Biochemical Characteristics												Inference
	Morphology	Gram	OXD	MOT	UR	TDA	CUT	LYS	H_2S	ONPG	NIT	LAC	MAL	IN	MR	
Colonies that were colorless on MCA, black on SSA.	Rod shape	-	-	+	-	-	-	+	+	-	+	-	+	-	+	*Salmonella typhi*

Key: OXD=Oxidase; MOT=Motility test; UR=Urease test; TDA= Tryptophan deaminase; LYS=Lysine; 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

were gram-negative, rod-shaped, TSI positive and other *Salmonella typhi.*

Occurrence of *Salmonella typhi:* Out of the 300 stool samples obtained from patients attending the selected hospitals, the overall occurrence of *Salmonella typhi* was 41(13.6%). Distribution in relation to the hospital was EH 23(11.8%) and JMC 18(17.1%), indicating that the percentage occurrence of *S. typhi* was high in JMC (17.1%) but low in EH 23(11.8%), as shown in Table 3.

Occurrence of isolates in the selected hospitals in relation to age of Patients: The occurrence of the isolates in relation to the age of patients is shown in Table 4. The occurrence of the isolates was high in ages ≥ 50(17.1%) and ≤ 10yrs (16.4%) but low in ages 21-30yrs (11.1%) and 31-40yrs (11.8%) while the percentage occurrence in relation to age in EH was high at age ≥ 50yrs (15%) and 41-50yrs (13.3%) but low in ages 21-30yrs (7.5%) JMC has high occurrence in ages ≤ 10yrs (26.6%) and ≥50yrs (20.0%) but low ages 41-50yrs (12.5%). The occurrence of *S. typhi* in relation to the age of patients with suspected typhoid fever was statistically insignificant (P>0.05).

Occurrence of isolates in the selected Hospitals in relation to gender of patients: The overall occurrence of the isolates in relation to gender was high in females (14.7%) but less in males (12.7%), as shown in Table 5. The percentage occurrence of the isolates at EH was high in females (13.3%) but low in males (10.5%), while at JMC, it was higher in males (17.7%) but lower in females (16.7%). The occurrence of the isolates in patients with suspected typhoid fever in relation to gender was also statistically insignificant

Antibiotic Resistance of *S. typhi:* The Antimicrobial resistance profile of the isolates is given in Table 6. Resistance to ciprofloxacin was the least at (7.3%) but was highly resistant to Tetracycline at (87.7%), and Amoxicillin/Clavulanic acid also at (87.7%), and its distribution is as follows: EH had a high resistance to Streptomycin (91.3%) and Amoxicillin/Clavulanic acid (86.9%) but less resistant to Ciprofloxacin (8.7%) and Ofloxacin (43.5%) while in JMC the isolate were more resistant to Amoxicillin/Clavulanic acid (88.9%) and Tetracycline (88.3%) but less resistant to Ciprofloxacin (5.5%).

Table 3: Occurrence of Salmonella typhi from the stool of patients attending the selected hospitals.

Hospital	No. of samples	No. *S. typhi positive samples* (%)
JMC	105	18(17.1)
EH	195	23(11.8)
TOTAL	300	41(13.6)

Key: EH=ECWA Hospital; JMC=Jikwoyi Medical Centre

Table 4: Occurrence of Salmonella typhi isolated from the selected Hospitals in relation to the Age of Patients.

AGE	EH		JMC		Total	
(Years)	No. Examined	*S. typhi* +ve No. (%)	No. Examined	*S. typhi* +ve No. (%)	No. Examined	*S. typhi* +ve No. (%)
≤10	40	5(12.5)	15	4(26.6)	55	9(16.4)
11-20	40	5(12.5)	20	3(15.0)	60	8(13.3)
21-30	30	3(7.5)	15	2(13.3)	45	5(11.1)
31-40	35	3(8.6)	16	3(18.8)	51	6(11.8)
41-50	30	4(13.3)	24	3(12.5)	54	7(12.9)
≥50	20	3(15.0)	15	3(20.0)	35	6(17.1)
Total	195	23(11.8)	105	18(17.3)	300	41(13.2)

Key: EH=ECWA Hospital; JMC=Jikwoyi Medical Centre

Table 5: Occurrence of Salmonella typhi isolates in the selected Hospitals in relation to the gender of patients.

	No. Samples		No. (%) *S. enteric* +ve		
GENDER	EH	JMC	EH	JMC	Total
Male	10545		11(10.5)	8(17.7)	19(12.7)
Female	90	60	12(13.3)	10(16.6)	22(14.7)
Total	195	105	23(11.8)	18(17.1)	41(13.6)

Key: EH=ECWA Hospital; JMC=Jikwoyi Medical Centre

Table 6: Antibiotic Resistance of Salmonella typhi isolated from Stool of Patients attending the selected Hospitals

Antibiotics	Disc content (µg)	EH(%) n=23	JMC(%) n=18	Total n=41
GEN	10	13(56.5)	9(50)	22(53.7)
CAZ	30	15(65.2)	12(66.7)	27(65.9)
CIP	5	2(8.7)	1(5.5)	3(7.3)
NET	30	12(52)	6(33.3)	18(43.9)
TE	30	21(9.3)	15(83.3)	36(87.7)
AMC	30	20(86.9)	16(88.9)	36(87.7)
OF	5	10(43.5)	6(33.3)	16(39.0)
CTR	30	14(60.9)	7(38.9)	21(51.2)
S	30	21(91.3)	13(72.2)	34(82.9)
COT	25	20(86.9)	10(55.5)	30(73.2)

Key: AMC=Amoxicillin/Clavulanic acid; CAZ=Ceftazidime; CTR=Ceftriaxone; CIP=Ciprofloxacin; GEN=Gentamicin; COT=Sulphamethoxazole/Trimethoprim; TE=Tetracycline; NET=Nitrofurantioin; S=Streptomycin; OF=Oflaxacin

Antibiotic Resistance Phenotype of *S. typhi:* Antimicrobial resistance of the *S. typhi* isolates was distributed into different phenotypes as given in Table 7. The most common phenotype was CIP-GEN-CAZ and GEN-TE-CAZ, with an overall occurrence of 9.7% (4/41) observed in EH (13.0%) and JMC (11.1%).

Multiple Antibiotic Resistance Index: All (100%) *S. typhi* isolates exhibited multiple antibiotic resistance, which means showing resistance to at least one agent in three or more classes of antibiotics used. The highest and the lowest occurrences of the MAR ratio were 0.6 (26.8%) and 1.0 (4.8%), respectively. The highest MAR index in EH was 3.0 (30.4%) and the lowest was 1.0 (5.5%), while that of JMC was 0.6 (27.8) and 1.0(5.5), respectively, as shown in Table 8.

Agarose gel electrophoresis of the amplified genes: The agarose gel electrophoretic separation of amplified Quinolone genes was carried out as described earlier, and the amplified DNA bands for *qnrS*, *gyrA*, *parC*, *qnrB* and *aac(6')-Ib-cr* genes and their respective amplicon sizes is as shown in Figure 1.

Occurrence of Quinolone resistance genes: The occurrence of Quinolone resistance genes of the isolates, as shown in Table 9, indicates that the occurrence of the gene *qnrS* (50.0%) was high, while the occurrence of *gyrA*, *parC*, *qnrB* and *aac(6')-Ib-cr* genes was low, with a percentage occurrence of 25.0%.

Salmonella species spread mainly through stool, with transmission occurring via contaminated food and water or direct faecal-oral contact (Han *et al.*, 2024; Qamar *et al.*, 2022). In a study of *Salmonella typhi* prevalence in Karu, Abuja, Nigeria, the occurrence was 13.6%, slightly higher than a previous study in 2020 but lower than findings from 2018 and 2020 by other researchers (Ekundayo and Enya, 2018; Adamu *et al.*, 2020; Fasema *et al.*, 2020). This suggests *Salmonella typhi* causes typhoid fever in the area.

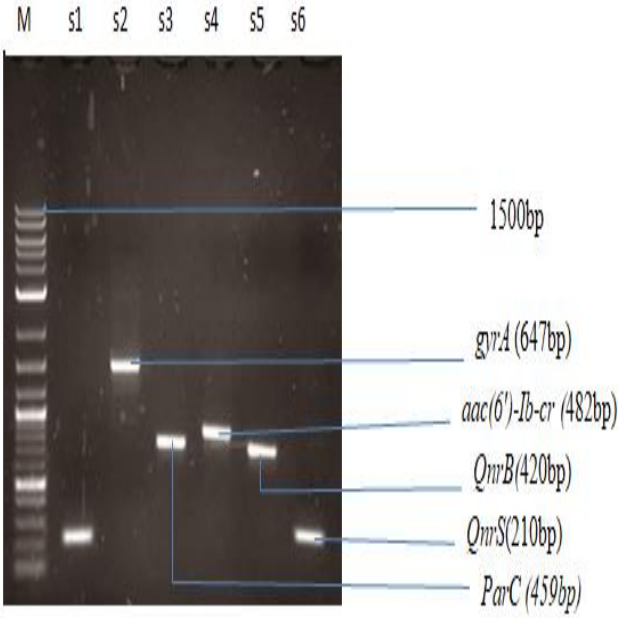

Figure 1: Agarose gel electrophoresis of the amplified quinolone-resistant genes (Captured in Table 1) from *Salmonella typhi* isolates. Lanes S1 and S6 represent the Q*nr*S (210p) gene band, lane S2 represents the *gyrAI* (647bp) gene band. Lane S3 represents the *ParC* (459bp) gene band, Lane S4 represents the *aac(6')-Ib-cr* (482bp), and Lane M represents the 1500 DNA ladder.

Table 7: Antibiotic Resistance phenotype of Salmonella typhi isolated from the Stool of patients attending the selected Hospitals.

Antibiotics	No. (%) Resistance EH. n=23	No. (%) Resistance JMC n=18	Total No.=41
CIP, GEN, CAZ	3(13.0)	1(5.5)	4(9.7)
GEN, COT, CIP	1(4.3)	0(0)	1(2.4)
GEN, NET,TE	2(8.7)	2(11.1)	4(9.7)
GEN,CAZ,CTR	1(4.3)	0(0)	1(2.4)
CAZ,AMC,TE,CIP	1(4.3)	1(5.5)	2(4.9)
GEN,CAZ,AMC,CTR	0(0)	1(5.5)	1(2.4)
CAZ,NET,AMC,TE,OFX	1(4.3)	1(5.5)	2(4.9)
CAZ,CIP,AMC,NET,OFX	1(4.3)	0(0)	1(2.4)
CAZ,CIP,AMC,TE,S,COT	2(8.7)	1(5.5)	3(7.3)
CAZ,NET,TE,AMC,OFX,CTR	0(0)	1(5.5)	1(2.4)
CAZ,NET,AMC,OFX,CTR,S	1(4.3)	2(11.1)	3(7.3)
CAZ,NET,S,COT,CTR ,TE	2(8.7)	1(5.5)	3(7.3)
GEN,CAZ,NET,S,COT,TE,CTR	2(8.7)	1(5.5)	3(7.3)
GEN,NET,S,SXT,CTR,OFX,AMC	0(0)	1(5.5)	1(2.4)
GEN,,CAZ,S,COT,CTR,OFX.TE,AMC,	0(0)	1(5.5)	1(2.4)
GEN,CAZ,S,COT,OFX,TE,AMC,NET	1(4.3)	0(0)	1(2.4)
CAZ,S,COT,OFX,AMC,TE,NET,CIP	2(8.7)	1(5.5)	3(7.3)
CAZ,S,COT,CIP,OFX,AMC,TE,NET	1(4.3)	1(5.5)	2(4.9)
CAZ,CIP,S,COT,TE,AMC,NET,OFX,CTR	0(0)	1(5.5)	1(2.4)
GEN,CIP,S,COT,TE,AMC,CAZ,OFX,NET	1(4.3)	0(0)	1(2.4)
GEN.CIP.S.COT.TE.AMC.NET.OFX.CTR.CAZ	1(4.3)	1(5.5)	2(4.9)

Key: AMC=Amoxicillin/Clavulanic acid; CAZ=Ceftazidime; CTR=Ceftriaxone; CIP=Ciprofloxacin; GEN=Gentamicin; COT=Sulphamethoxazole/Trimethoprim; TE=Tetracycline; NA=Nalidixic acid; S=Streptomycin; OFX=Oflaxacin

Table 8: Multiple Antibiotic Resistance (MAR) index of Salmonella typhi isolated from the stool of patients with suspected typhoid fever attending the selected Hospitals.

No. of antibiotics tested (a)	No. of antibiotics resistance (b)	MAR index (a/b)	No. (%) MAR isolates (n =41)		Total (%) MAR isolates (n= 41)
			EH (n=23)	JMC (n=18)	
10	10	1.0	1(4.34)	1(5.55)	2(4.8)
10	9	0.9	1(4.34)	1(5.55)	2(4.8)
10	8	0.8	4(17.4)	3(16.7)	7(17.1)
10	7	0.7	2(8.7)	1(11.1)	3(7.3)
10	6	0.6	6(26.1)	5(27.8)	11(26.8)
10	5	0.5	2(8.7)	1(5.55)	3(7.3)
10	4	0.4	1(4.34)	2(11.1)	3(7.3)
10	3	0.3	7(30.4)	3(16.7)	10(24.4)

Key: MAR= Multiple Antibiotic Resistance; EH=ECWA Hospital; JMC=Jikwoyi Medical Centre

Table 9: Occurrence of Quinolone resistance genes of Salmonella typhi from patients attending the selected hospitals.

Quinolone Resistance Genes	No. (%) *S. typhi* (n=4)
GyrA	1(25.0)
ParC	1(25.0)
QnrS	2(50.0)
QnrB	1(25.0)
aac(6')-Ib-cr	1(25.0)

The study found the highest occurrence of infections in individuals aged 50 and above (16.4%) and the lowest in those aged 21-30 (11.1%). This differs from previous research that indicated higher rates in younger patients, potentially due to economic conditions and hygiene practices among the elderly (Abdullahi *et al.*, 2017). Age and gender showed no statistically significant impact on typhoid fever occurrence (Fasema *et al.*, 2020; Ekundayo and Enya, 2018). However, infections were more common in females (14.7%) compared to males (12.7%), which aligns with some previous studies but contradicts others (Martins *et al.*, 2019).

In agreement with Akinyemi *et al.* (2017), the isolates showed high resistance to common antibiotics like Amoxicillin (87.7%), Tetracycline (87.7%), and Streptomycin (82.9%), likely due to misuse of these drugs. In contrast, resistance to Ciprofloxacin (7.3%) and Ofloxacin (39.0%) was low, suggesting these drugs are still effective (Cosby *et al.*, 2015). Many isolates were multi-resistant, with 26.8% showing multi-drug resistance (MDR), indicating treatment challenges. The presence of Quinolone resistance genes linked to both chromosomal and plasmid-mediated resistance highlights potential public health concerns, as these genes can spread to other bacteria (Eleazar *et al.*, 2024; Fasema *et al.*, 2020).

In chromosomal mutations, resistance to quinolones often develops through mutations in the genes encoding DNA gyrase (*gyrA* and *gyrB*) and topoisomerase IV (*parC* and *parE*), which are the primary targets of these antibiotics. These mutations alter the drug's binding site, reducing its effectiveness (Adetunji *et al.*, 2025; Ipinlaye and Oluyege, 2023). The detection of *gyrA* and *parC* mutations in 25% of the isolates indicates the presence of such chromosomally-mediated resistance. The plasmid-mediated Quinolone Resistance (PMQR) genes are typically found on mobile genetic elements (plasmids) and can be easily transferred between bacteria, contributing significantly to the rapid spread of resistance. *qnr* genes (*qnrB*, *qnrS*) encode pentapeptide repeat proteins that protect DNA gyrase and topoisomerase IV from quinolone binding, thereby reducing the antibiotic's efficacy (Fasema *et al.*, 2024; Ipinlaye and Oluyege, 2023). The presence of *qnrB* and *qnrS* in 25% and 50% of the isolates, respectively, is particularly alarming as it indicates the potential for horizontal gene transfer, disseminating quinolone resistance to other bacterial species (Fasema *et al.*, 2020; Ipinlaye *et al.*, 2024).

The *aac(6')-Ib-cr* gene encodes an aminoglycoside acetyltransferase that can also modify Ciprofloxacin and Norfloxacin, reducing their activity. Its detection in 25% of the isolates is a worrying finding, as it contributes to reduced susceptibility to quinolones and often co-occurs with other resistance genes, further complicating treatment options (Abdullahi *et al.*, 2017). The co-existence of both chromosomal mutations and PMQR genes in *S. typhi* isolates from this region suggests a complex and evolving resistance landscape. While Ciprofloxacin currently shows low resistance, the presence of these resistance genes indicates a potential for future therapeutic failures if their prevalence increases. This highlights the urgent need for continuous surveillance of antimicrobial resistance patterns and mechanisms.

However, this study has several limitations that may affect the generalizability of the findings. The sample was restricted to a specific area within Abuja, which may not reflect the broader context of Nigeria. Also, data on participants' socioeconomic status, vaccination history, and travel history were not collected, potentially influencing antibiotic susceptibility outcomes. While the antibiotic susceptibility testing was thorough, it focused only on quinolone resistance genes and may have missed other important resistance mechanisms.

Furthermore, the absence of data on clinical outcomes makes it challenging to relate susceptibility patterns to real-world health implications. This gap emphasises the necessity for further research. Expanding the focus to include a broader range of data—both geographical and clinical—will enhance our understanding of antibiotic resistance and its impacts. These limitations highlight the need for further research to expand on the findings of this study.

Conclusion

The study found that the occurrence of *Salmonella typhi* was 13.6%. Isolates were more common in JMC (17.1%) than in EH (11.8%), and were higher in females (14.7%) than in males (12.7%). Infection rates were highest in those aged 50 and older (12.9%) and lowest in those aged 21-30 (11.1%). The isolates showed significant resistance to Tetracycline (87.7%), Streptomycin (82.9%), and Co-trimoxazole (73.2%). Effective antibiotics were Ciprofloxacin and Ofloxacin, while resistance genes were detected.

Recommendations

Based on these findings, the following specific intervention strategies are proposed to combat antibiotic resistance effectively and improve public health outcomes:

1. Strengthen Water, Sanitation, and Hygiene (WASH) Activities: Due to the risks of faecal-oral transmission of Salmonella, the public health WASH initiatives must provide and promote access to clean and safe drinking water alongside improved sanitation facilities as well as proper handwashing (before food preparation and after defecation). This approach mitigates a major infection source and the consequent transmission of resistant strain infections.
2. Mandatory Susceptibility Testing: Advocate for and enforce the implementation of mandatory antibody susceptibility testing for all patients prior to starting treatment for suspected case of typhoid fever. This approach enhances effective treatment for patients and mitigates empirical treatment with useless drugs, thus controlling the resistance problem.
3. Clinical Guidelines and Education: Create and share defined clinical instructions for the irrational use of antibiotics with special attention to the use of Ciprofloxacin and Ofloxacin which are currently effective as the first line for uncomplicated typhoid fever in this zone. At the same time, inform health care workers about the risks of irrational prescribing and the need to comply with treatment protocols.
4. Public Awareness Campaigns: Launch public awareness campaigns to educate communities about the dangers of antibiotic misuse, including self-medication, incomplete courses, and the use of antibiotics for viral infections.
5. Strengthen Surveillance and Monitoring: Establish a robust and continuous surveillance system for antimicrobial resistance in *Salmonella typhi* and other enteric pathogens across health facilities in Abuja and beyond. This should include both phenotypic susceptibility testing and molecular detection of resistance genes to track emerging resistance patterns and inform public health interventions in real-time.

6. Vaccination Programs: Advocate for the inclusion of typhoid vaccines in national healthcare policies and implement targeted vaccination programs for high-risk groups, especially children and individuals in endemic areas. Vaccination can significantly reduce the incidence of typhoid fever, thereby decreasing the overall need for antibiotics and reducing selective pressure for resistance.
7. One Health Approach: Implement a "One Health" approach that recognizes the interconnectedness of human, animal, and environmental health in the context of antimicrobial resistance. This involves monitoring antibiotic use in agriculture, regulating access to veterinary antibiotics, and addressing environmental contamination from resistant bacteria.

Future Research

Based on the results and limitations of this study, several avenues for future research are encouraged:

1. Long-term, longitudinal studies should be carried out to monitor the evolution of antibiotic resistance patterns and the prevalence of specific resistance genes in *S. typhi* over time in Abuja and other Nigerian cities.
2. The investigation of molecular mechanisms of resistance to other commonly used antibiotics, particularly those showing high resistance rates in this study (e.g., Tetracycline, Amoxicillin/Clavulanic acid), to understand the full scope of MDR.
3. Carrying out epidemiological studies that incorporate a broader range of demographic, socioeconomic, and behavioural risk factors (e.g., water sources, food handling practices, previous antibiotic use, travel history, vaccination status) to identify key drivers of *S. typhi* infection and antibiotic resistance.
4. Future studies should integrate clinical outcome data (e.g., duration of fever, hospitalisation rates, incidence of complications, treatment failure rates) with microbiological findings to quantify the impact of antibiotic resistance on patient health.
5. The effectiveness of specific public health interventions (e.g., WASH programs, antibiotic stewardship initiatives, vaccination campaigns) on reducing the incidence of typhoid fever and the prevalence of antibiotic-resistant *S. typhi* strains in the community should be evaluated.
6. Besides human and animal samples, the presence of *S. typhi* and its resistance genes in environmental samples (water sources, food products) to identify potential reservoirs and transmission pathways should also be explored.

Declaration of interest

The authors declare no conflict of interest.

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References

1. Abdullahi, M., Olonitola, S. O., Umoh, V. J., and Inabo, I. H. (2017). Antibacterial resistance profile and PCR detection of antibiotic resistance genes in *Salmonella serovars* isolated from blood samples of hospitalised subjects in Kano, North-West, Nigeria. *British Microbiology Research Journal*, 5(3), 245-256.
2. Abimiku, R. H., Ngwai, Y. B., Nkene, I. H., Bassey, B. E., Tsaku, P. A., Ibrahim, T., Tama, S. C., Ishaleku, D., and Pennap, G. R. I. (2019). Molecular diversity and extended-spectrum beta-lactamase resistance of diarrheagenic *Escherichia coli* from patients attending selected health care facilities in Nasarawa State, Nigeria. *International Journal of Pathogen Research*, 3(1), 1-18.
3. Adamu, U., Yusha, U. M., Usman, A. D., and Abdulhadi, S. K. (2020). Antibiotic resistance pattern of Salmonella species from typhoid patients in Jigawa State, Nigeria. *Nevel Research in Microbiology Journal*, 4(2), 696-703.
4. Adetunji, V. O., Odetoyin, B. W., Salami, F. A., and Afolabi, O. J. (2025). Genomic diversity and antibiotic resistance of *Escherichia coli* and Salmonella from poultry farms in Oyo State, Nigeria. *Microorganisms*, 13(6), 1174. https://doi.org/10.3390/microorganisms13061174
5. Akinyemi, K. O., Bamidele, A. I., Akeeb, O. B. O., and Fakorede, C. O. (2017). Occurrence of extended-spectrum and AmpC beta-lactamases in multiple drug-resistant Salmonella isolates from clinical samples in Lagos, Nigeria. *Infection and Drug Resistance*, 10, 19-25.
6. Clinical and Laboratory Standards Institute. (2018). Performance standards for antimicrobial susceptibility testing, 28th Edition (CLSI supplement M100). Clinical and Laboratory Standards Institute.
7. Cheesbrough, M. (2006). District laboratory practice in tropical countries Part 2 (2nd ed.). Cambridge University Press.
8. Cheesbrough, M. (2009). District Laboratory Practice in Tropical Countries Part 1 (2nd ed.) Cambridge University Press.
9. Cosby, D. E., Cox, N. A., Harrison, M. A., Wilson, J. L., Buhr, R. J., and Fedorka-Cray, P. J. (2015). Salmonella and antimicrobial resistance in broilers: A review. *Journal of Applied Poultry Research*, 24, 408-426.
10. Eleazar, C. I., Chinemerem, A. D., and Nweke, R. N. (2024). Antibiotic susceptibility patterns of enteric fever Salmonella isolates from infected patients in Enugu, Nigeria. *International Journal of Pathogen Research*, 13(4), 64-71. https://doi.org/10.9734/ijpr/2024/v13i43015
11. Ekundayo, E. O., and Enya, E. (2018). *In-vitro* antibiotic susceptibility profile of Salmonella enterica serovar typhi isolated from faecal specimens of humans in Umuahia Metropolis, Abia State, Nigeria. *African Journal of Microbiology Research*, 12(20), 470-475.
12. Fasema, R., Bassey, B. E., Ngwai, Y. B., Nkene, I. H., Abimiku, R. H., Paron, S. K., and Yahaya, I. (2020). Plasmid-mediated Quinolone resistance genes in *S. typhi* from patients attending selected general hospitals in Abuja Municipal, Abuja, Nigeria. *Asian Journal of Biochemistry, Genetics and Molecular Biology*, 4(2), 45-59.
13. Fasema, R., Ngwai, Y. B., Ishaleku, D., Nkene, I. H., Abimiku, R. H., Tama, S. C., and Igbawua, I. N. (2024). Molecular characterisation of plasmid-mediated quinolone-resistant *Salmonella typhi* from patients attending Federal Medical Centre, Jabi, Abuja, Nigeria. *International Journal of Pathogen Research*, 13(2), 9-19.
14. Fasih, F., Fatima, A., Baig, S., Naseem, S., Tauheed, M. M., and Gohar, H. (2023). Antimicrobial susceptibility of bacteraemic isolates of *Salmonella enterica serovar typhi* and *paratyphi* infection in Pakistan from 2017-2020. JPMA. *The Journal of the Pakistan Medical Association*, 73(3), 505-510. https://doi.org/10.47391/JPMA.6083

15. Fierer J. (2022). Invasive Non-typhoidal Salmonella (iNTS) Infections. *Clinical Infectious Diseases*: an official publication of the Infectious Diseases Society of America, 75(4), 732–738. https://doi.org/10.1093/cid/ciac035
16. Ghazy Hawal, R., and Talib Bakr, M. (2023). Isolation and Molecular Identification of *Salmonella pullorum* from Broiler Chicken in Iraqi Fields. *Archives of Razi Institute*, 78(2), 587–592. https://doi.org/10.22092/ARI.2022.359468.2424
17. Giorgio, R. T., and Helaine, S. (2025). Antibiotic-recalcitrant Salmonella during infection. *Nature Reviews*. Microbiology, 23(5), 276–287. https://doi.org/10.1038/s41579-024-01124-z
18. Han, J., Aljahdali, N., Zhao, S., Tang, H., Harbottle, H., Hoffmann, M., Frye, J. G., and Foley, S. L. (2024). Infection biology of *Salmonella enterica*. *EcoSal Plus*, 12(1), eesp00012023. https://doi.org/10.1128/ecosalplus.esp-0001-2023
19. Inayath, S. B., Broor, S., Gupta, R., Agarwal, P., Majumder, S., Anveshi, A. K., and Gaind, R. (2021). Validation of Pefloxacin for detection of fluoroquinolone (FQ) resistance among *Salmonella typhi* with special reference to GyrB mutations. *Journal of Medical Microbiology*, 70(8), 10.1099/jmm.0.001398. https://doi.org/10.1099/jmm.0.001398
20. Ipinlaye, J. O., and Oluyege, A. O. (2023). Molecular epidemiology of fluoroquinolone-resistance genes among non-typhoidal Salmonella species from humans and poultry in Ido Ekiti, Nigeria. *Microbiology Research Journal International*, 33(12), 59–69. https://doi.org/10.9734/mrji/2023/v33i12771
21. Ipinlaye, J. O., Oluyege, A. O., and Adewunmi, F. A. (2024). Antibiotic susceptibility profile of non-typhoidal Salmonella from human and poultry faeces in Southwest Nigeria. *Journal of Advances in Microbiology*, 24(3), 17-22. https://doi.org/10.9734/jamb/2024/v24i3646
22. Krejcie, R. V., and Morgan, D. W. (2017). Determining sample size for research activities. *Journal of the National Education Association Bulletin*, 38, 99.
23. Kwak, Y.G., Truong-Bolduc, Q.C., Bin-Kim, H., Song, K.H., Kim, E.S., and Hooper, D.C. (2013). Association of norB overexpression and fluoroquinolone resistance in clinical isolates of *Staphylococcus aureus* from Korea. *Journal of Antimicrobial Agents and Chemotherapy*, 68(12),2766-72.
24. Lu, J., Wu, H., Wu, S., Wang, S., Fan, H., Ruan, H., Qiao, J., Caiyin, Q., and Wen, M. (2025). Salmonella: Infection mechanism and control strategies. *Microbiological research*, 292, 128013. https://doi.org/10.1016/j.micres.2024.128013
25. Martins, E. O., Michael, O. I., Uzoamaka, M., Obinna, D. O., and Harish, C. G. (2019). Typhoid fever among febrile Nigerian patients: Prevalence, diagnostic performance of the Widal test and antibiotic multi-drug resistance. *Malawi Medical Journal*, 31(3), 184-192.
26. Nguyen, A. T., and McSorley, S. J. (2024). Fighting the enemy within: Systemic immune defence against mucosal Salmonella infection. *Immunology Letters*, 270, 106930. https://doi.org/10.1016/j.imlet.2024.106930
27. Qamar, F. N., Hussain, W., and Qureshi, S. (2022). Salmonellosis Including Enteric Fever. *Paediatric Clinics of North America*, 69(1), 65–77. https://doi.org/10.1016/j.pcl.2021.09.007
28. Ruiz, J., Flores-Paredes, W., Luque, N., Albornoz, R., Rojas, N., Espinoza, M., and Pons, M. J. (2022). Retrospective analysis of the emergence of antibiotic-resistant *Salmonella enterica* infections in a level IV hospital from Lima, Peru. Tropical Doctor, 52(1), 68–73. https://doi.org/10.1177/00494755211060990
29. Sarah, M.S., Timothy, J., Richard, I. and Michael, M. (2011). Phylogenetic and Genomic Characterisation of Porcine Enterotoxigenic *Escherichia coli,* University of Minnesota. Shepard_Sara_August 2011.pdf.txt
30. Uzairue, L. I., Shittu, O. B., Ojo, O. E., Obuotor, T. M., Olanipekun, G., Ajose, T., Arogbonlo, R., Medugu, N., Ebruke, B., and Obaro, S. K. (2023). Antimicrobial resistance and virulence genes of invasive *Salmonella enterica* from children with bacteremia in north-central Nigeria. *SAGE Open Medicine*, 11, 20503121231175322. https://doi.org/10.1177/20503121231175322
31. Xie, R., Huo, S., Li, Y., Chen, L., Zhang, F., and Wu, X. (2014). Molecular epidemiological survey on quinolone resistance genotype and phenotype of *Escherichia coli* in Septicemic broilers in Hebei, China. *Poultry Science*, 93(2):335-339.