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Antibiotic Resistance and Molecular Characterization of *Salmonella Typhi* among Children Attending Selected Primary Health Care Centres in Karu, Nasarawa State

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ABSTRACT

Typhoid fever remains an important public health concern in developing countries, especially among children living in settings characterized by poor sanitation, limited access to safe drinking water, and inappropriate antibiotic use. The emergence of antimicrobial-resistant *Salmonella typhi* further complicates disease management. This study investigated the occurrence, antibiotic resistance patterns, and molecular characteristics of *S. typhi* isolated from children attending selected Primary Health Care Centres in Karu Local Government Area, Nasarawa State, Nigeria. A total of 240 stool samples were collected and examined using standard microbiological procedures. Antimicrobial susceptibility testing was conducted to determine the resistance patterns of the isolates, while Polymerase Chain Reaction (PCR) was employed to detect selected β-lactam resistance genes. *S. typhi* was isolated from 47 samples, giving an overall occurrence rate of 19.6%. The highest occurrence was recorded in Location B (30%), whereas Locations A and E recorded the lowest (15%). The isolates exhibited the highest resistance to Amoxicillin (76.6%), Streptomycin (74.5%), Ofloxacin (57.4%), and Augmentin (51.1%). Resistance was comparatively lower to Gentamicin (44.7%), Perfloxacin (42.6%), Ciprofloxacin (36.2%), Ceftriaxone (29.8%), and Azithromycin (17.0%). Molecular analysis demonstrated the presence of β-lactam resistance genes, with *blaCTX-M* detected in 80.0% of tested isolates, followed by *blaTEM* (60.0%) and *blaSHW* (20.0%). The expected amplicon sizes were 550 bp, 858 bp, and 930 bp, respectively. The findings highlight the continued presence of *S. typhi* and antibiotic-resistant strains among children in Karu. Strengthened surveillance, prudent antibiotic use, and molecular monitoring are recommended.

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1. INTRODUCTION

Typhoid and paratyphoid fever cause an estimated 26 million illnesses globally each year (Buckle *et al.,* 2020). Crump *et al.* (2024) estimated approximately 22 million typhoid cases annually, with at least 200,000 associated deaths. Outbreaks are frequently reported from sub-Saharan Africa and Southeast Asian countries (Muyembe-Tamfum *et al.,* 2019; Baddam *et al.,* 2022). Other regions of Asia and Africa, Latin America, the Caribbean, and Oceania have a medium incidence of 10 to 100 cases per 100,000 person-years (Zakaria *et al.,* 2022).

Salmonella enterica serovar *typhi* and *Salmonella paratyphi* A, B, and C are Gram-negative bacteria which invade the bloodstream and cause typhoid and paratyphoid fever respectively, jointly known as enteric fever (Bhan *et al.,* 2021). Typhoid fever is a symptomatic bacterial infection which affects only humans (Watkins, 2020). Risk factors include eating food prepared outside the home, drinking contaminated water, and eating vegetables grown with human waste as fertilizer (Velema *et al.,* 2021). Symptoms typically include prolonged fever, frontal headache, malaise and marked loss of appetite, sometimes accompanied by abdominal pains, nausea, and in severe cases, intestinal perforation and neurological complications (Luby *et al.,* 2019). Symptoms typically subside in 7-21 days, but mortality occurs in 1-5% of hospitalized patients (Watkins, 2020).

The World Health Organization (2023) reported that typhoid fever is a life-threatening infection caused by *Salmonella typhi*, usually spread through contaminated food or water. Deen *et al.* (2022) stated that typhoid fever is responsible for about 3 million deaths worldwide, with fluoroquinolone resistance increasing globally due to misuse and abuse of these antibiotics. Multidrug-resistant strains of *S. typhi* can spread to high-risk levels due to cross-infections of hospitalized patients (Watkins, 2020).

Children are particularly vulnerable due to their immature immune systems, poor sanitation, contaminated water supplies, and limited access to health care. The increasing emergence of antibiotic-resistant strains of *Salmonella typhi* poses a significant threat to effective treatment. Despite the burden of typhoid fever, there is limited data on the antibiotic resistance patterns and molecular characteristics of *Salmonella typhi* among children attending selected primary health care Centres in Karu LGA. Molecular characterization is essential for identifying circulating resistant strains, understanding genetic mechanisms of resistance, tracking transmission patterns and supporting effective infection control strategies (WHO, 2023). Empirical treatment is often used in primary health care settings without prior susceptibility testing, which may lead to treatment failure. Therefore, generating local data on resistance profiles will guide clinicians in selecting appropriate antibiotics for children.

The aim of this study was to investigate antibiotic resistance and molecular characterization of *Salmonella typhi* among children attending selected Primary Health Care Centres in Karu, Nasarawa State, Nigeria. The objectives were to determine the prevalence of *Salmonella typhi*, determine the antibiotic resistance profile, detect the presence of beta-lactam resistance genes, determine the resistance genes in *Salmonella typhi* isolates, and determine if the resistance is transferable.

3. MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Karu Local Government Area (LGA) of Nasarawa State, Nigeria. Karu LGA is located in the western part of Nasarawa State and shares a boundary with the Federal Capital Territory (FCT), Abuja. The study involved selected Primary Health Care Centres including Masaka, New Nyanya, Ado, Aso-Pada/Adehi, and Mararaba. Karu LGA covers an estimated land area of approximately 2,938 km² and lies within the Guinea Savannah ecological zone (Akwa *et al.,* 2007).

3.2 Sample Collection

A total of 240 stool samples were collected from selected Primary Health Care Centres in Karu in sterile universal containers and transported in an ice box to the Microbiology Laboratory, Nasarawa State University, Keffi, within an hour for analysis.

3.3 Isolation and Identification of *Salmonella typhi*

Salmonella typhi was isolated from stool samples as described by Cheesbrough (2021). A loopful of stool samples was inoculated in 5ml of Selenite F broth and incubated at 37°C for 24h. The broth was streaked on XLD agar plates and incubated at 37°C for 24h. Colourless colonies with black coloration were further streaked on Bismuth Sulphate agar and incubated at 37°C for 24h. Black metallic sheen colonies were selected as presumptive *Salmonella typhi*.

3.4 Gram Staining and Microscopy

Air-dried smears were fixed and stained with crystal violet for 60 seconds, rinsed, flooded with iodine for 60 seconds, decolorized with acetone, and counterstained with safranin for 20-30 seconds. Slides were examined under the microscope at ×100 objective lens (Cheesbrough, 2021).

3.5 Biochemical Screening Tests

The following biochemical tests were carried out: Triple Sugar Iron (TSI), Urease, Indole, Citrate, Voges Proskauer, and Methyl Red (IMViC) (Cheesbrough, 2021).

3.6 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility was performed as described by CLSI (2022) using the Kirby-Bauer disk diffusion method. Commercially prepared antibiotic discs included levofloxacin (20µg), amoxicillin (30µg), cefotaxime (10µg), sparfloxacin (10µg), ciprofloxacin (30µg), gentamicin (30µg), tarivid (10µg), augmentin (10µg), azithromycin (12µg) and perfloxacin (30µg). Mueller Hinton agar was used for sensitivity testing. Inoculum was standardized to 0.5 McFarland standards. Plates were incubated at 37°C for 18-24 hours and zones of inhibition measured and interpreted using CLSI (2022) guidelines.

3.7 Determination of Multiple Antibiotic Resistance Index (MARI)

MAR index was determined for all isolates resistant to at least two antibiotics as follows (Ngwai *et al.,* 2014):
MAR Index = No. of antibiotics isolate is resistant to / No. of antibiotics isolate is exposed to

3.8 Phenotypic Detection of Extended Spectrum Beta-Lactamase

ESBL production was detected by the Double Disc Synergy Test (DDST) using levofloxacin (20 µg) and cefotaxime (10 µg) discs with amoxicillin/clavulanic acid (30/10 µg) disc as recommended by CLSI (2022). An increase of ≥5 mm in inhibition zones of cephalosporin in combination with clavulanic acid compared to cephalosporin alone was interpreted as ESBL positive.

3.9 Molecular Detection of Antibiotic Resistant Genes

3.9.1 DNA Extraction

DNA was extracted using the boiling method as described by Patel *et al.* (2023). Briefly, 1 pure colony was inoculated into 2ml LB broth and incubated at 37°C for 8 hours. 200µl was centrifuged at 3200 rpm for 2 min, supernatant discarded, cells washed twice, 0.5 ml sterile phosphate buffer added, vortexed, heated at 90°C for 10 min, cooled rapidly, and centrifuged at 3200 rpm for 1 min. 300µl supernatant containing DNA was stored at -10°C.

3.9.2 DNA Quantification

Extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer (Amuta *et al.,* 2021).

3.9.3 Amplification of Target Genes by Multiplex PCR

DNA amplification was carried out using multiplex PCR in 25µl total volume using ABI9700 Applied Biosystems thermal cycler. Each reaction consisted of 12.5µl DreamTaq Green PCR Master Mix, 5.5µl nuclease-free water, 0.5µl of each primer (10µM), and 2µl DNA lysate. Running conditions: 1 cycle denaturation at 94°C for 15 min, 25 cycles denaturation at 94°C for 30 sec, annealing at 58°C for 90 sec, elongation at 72°C for 60 sec, and final elongation at 72°C for 10 min.

3.9.4 Agarose Gel Electrophoresis

One percent agarose gel was used to resolve DNA fragments. The gel was prepared by combining 1g agarose in 10ml 10X TBE buffer and 90ml sterile distilled water, heated in a microwave for 2 minutes. 0.7µl ethidium bromide was added, gel poured onto a mini horizontal gel electrophoresis tank, and allowed to set for 30 minutes. Samples were loaded and electrophoresis carried out at 100-200mA for one hour. The gel was visualized under UV light and documented.

3.10 Detection of Transferable Resistance Genes

Conjugation assay was performed to determine if antibiotic resistance genes are transferable. Donor strain (resistant *S. typhi* isolate) and recipient strain (antibiotic-sensitive *E. coli* K-12) were grown separately in nutrient broth overnight, mixed at 1:1 ratio, and incubated at 37°C for 4-24 hours. Filter mating method was also used where the mixture was filtered through a sterile membrane filter (0.22µm), placed on nutrient agar plate, and incubated at 37°C for 18-24 hours.

4. RESULTS

4.1 Isolation and Identification of *Salmonella typhi*

The cultural, morphological and biochemical characteristies of the isolate as given in Table 4.1.

4.2 Occurrence of *Salmonella* typhi

Out of 240 stool samples from children attending selected Primary Health care centers Karu, Nasarawa State, Nigeria, occurrence of *Salmonella* enteric serovar typhi isolated was 47 (18.75). Where location B had the highest percentage occurrence of 15 (30%), followed by location D and C with percentage occurrence of (20%) in the same order, while location E and A had the least percentage occurrence of (15%) respectively as shown in Table 4.2.

4.3 Antibiotic Resistance Profile of *Salmonella typhi*

The resistance of the isolates to commonly used antibiotics is as shown in Table 4.3. The isolates were more resistant to: Amoxiciline 36 (76.6%), Streptomycin 35(74.5%), Ofloxacine 27 (57.4%), and Augmentin 24 (51.1) but lower resistance to Gentamycin 21 (44.7%), perfloxacin 20 (42.6%), Ciprofloxacin 17 (36.2%), Cetriaxone 14 (29.8%) and Azithromycin 8 (17.0%).

4.4: Antibiotics Resistance Phenotypes of *Salmonella typhi* Isolates

Antibiotics resistance phenotypes of the isolates are as given in Table 4.4. Many resistance phenotypes were observed, with the most common phenotype OFX, LEV, CIP, PEF, CRO, CN, S, AMX, AZM 4(8.5%).

4.5 Multiple Antibiotic Resistance (MAR) Index of *Salmonella* typhi isolates

Multiple antibiotics resistance (MAR), define here as resistance to at least two antibiotics, was observed in all isolates 47 (100%) of resistance isolates. The MAR indices of the isolates are shown in Table 4.4. All the MAR isolates have MAR index ≥ 0.3 the commonest was 0.8 in sample A and E with occurrence of 36.4% and 44.4% respectively.

4.6 Detection of Beta-lactam Resistance Genes

Table 4.6 presents the molecular detection of three major beta-lactam resistance genes among the bacterial isolates. The genes investigated were bla_{TEM}, bla_{SHV}, and bla_{CTX-M}.

The molecular detection of three ESBL ressitance genes (bla_{TEM}, bla_{SHV} and bla_{CTX-M}) among the bacteria isolates. The bla_{CTX-M} gene was the most prevalent, detected in 4 isolates (80.0%), followed by bla_{TEM} in 3 isolates (60.0%). The blaSHV gene was the least prevalent, occurring in 1 isolate (20.0%). The expected PCRamplicon sizes were 550bp for bla_{CTX-M}, 858 bp for bla_{TEM}, and 930bp for bla_{SHV}. Overall, the findings indicate that bla_{CTX-M} is the predominant ESBL gene among the isolates, suggesting it is the major genetic determinant of β-lactam antibiotic ressitance in the study population as shown in Table 4.6.

Table 4.1: Cultural, Morphological and biochemical characteristics of *Salmonella* enteric serovar *typhi*

Cultural Characteristics	Morphological Characteristics		Biochemical Characteristics						Inference
	Gram Staining	Morphology	IND	MR	VP	CIT	MOT	MAL	
Colonies that were Colourless on MCA, Black on SSA	-	Rod-shape	-	+	-	-	+	-	*S. typhi*

MCA = MaConkey agar, SSA = Salmonella Shigella Agar, IND = Indole, MR = Methyl red, VP= Voges Proskauer, CT Citrate, - = negative, + = Positive

Table 4.2: Occurrence of *Salmonella* enteric serovar *typhi* isolated with respct to locations.

Location	No. of Samples	No (%) of Isolates	No of Negative
A	70	11 (15.7)	59
B	50	15 (30.0)	35
C	20	4 (20.0)	16
D	40	8 (20.0)	32
E	60	9 (15.0)	51
Total	240	47 (19.6)	193

$X^2 = 4.91$; P-value =0.30, DF= 4

Key: A Masaka primary health care; B New Nyanya primary health care, C-Ado primary health care, D- Adehi primary health care, E-Mararaba primary health care.

Table 4.3: Antibiotic Resistance profile of *Salmonella* enteric serovar *typhi*

Antibiotics	Disc Content µg	No. (%) resistance					Total
		A = 11	B= 15	C = 4	D= 8	E = 9	N = 47
Levofloxacin (LEV)	20	7 (63.6)	12 (80)	3 (75)	6 (75)	8 (88.9)	36 (76.6)
Ciprofloxacin (CIP)	10	4 (36.4)	4 (26.7)	1 (25.0)	2 (25.0)	6 (66.7)	17 (36.2)
Perfloxacin (PEF)	30	5 (45.5)	5 (33.3)	0 (0.0)	6 (75.0)	4 (44.4)	20 (42.6)
Ofloxacin (OFX)	30	3 (27.3)	12(80.0)	3 (75.0)	4 (50.0)	5 (55.6)	27 (57.4)
Ceftriaxone (CRO)	5	4 (36.4)	3 (20.0)	1 (25.0)	2 (25.0)	4 (44.4)	14 (29.8)
Azithromycin (AZM)	12	3 (27.3)	2 (13.3)	0 (0.0)	0 (0.0)	3 (33.3)	8 (17.0)
Gentamicin (CN)	10	5 (45.5)	8 (53.3)	0 (0.0)	3 (37.5)	5 (55.6)	21 (44.7)
Augmentin (AUG)	10	3 (27.3)	9 (60.0)	2 (50.0)	2 (25.0)	8 (88.9)	24 (51.1)
Amoxicillin (AMX)	30	8 (72.7)	14(93.3)	1 (25.0)	5 (62.5)	8 (88.9)	36 (76.6)
Streptomycin (S)	30	7 (77.0)	13(86.7)	3 (75.0)	5 (62.5)	7 (77.8)	35 (74.5)

Table 4.4: Antibiotics Resistance Phenotypes of *Salmonella typhi* Isolates

Antibiotics Resistance Phenotypes	Frequency %					Total
	A = 11	B= 15	C = 4	D= 8	E = 9	N = 47
OFX, LEV, CIP, CRO	0(0.0)	2(13.3)	0(0.0)	0(0.0)	0(0.0)	2(4.3)
OFX, LEV, CIP, PEF, CRO	0(0.0)	0(0.0)	1(25)	0(0.0)	0(0.0)	1(2.1)
OFX, LEV, CIP, PEF, CRO, CN	1(9.1)	1(6.7)	2(50)	0(0.0)	0(0.0)	1(2.1)
OFX, LEV, CIP, PEF, CRO, CN, S	1(9.1)	0(0.0)	0(0.0)	0(0.0)	1(11.1)	1(2.1)
OFX, LEV, CIP, PEF, CRO, CN, AZM	0(0.0)	0(0.0)	1 (25)	0(0.0)	0(0.0)	1(2.1)
OFX, LEV, CIP, PEF, CRO, CN, AMX	1(9.1)	0(0.0)	1 (25)	0(0.0)	0(0.0)	2 (4.3)
OFX, LEV, CIP, PEF, CRO, CN, S, AZM	0(0.0)	0(0.0)	0(0.0)	1(12.5)	0(0.0)	1(2.1)
OFX, LEV, CIP, PEF, CRO, CN, S, AMX	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(11.1)	1(2.1)
OFX, LEV, CIP, PEF, CRO, CN, AZM, AUG	0(0.0)	0(0.0)	2 (50)	0(0.0)	0(0.0)	2(4.3)
OFX, LEV, CIP, PEF, CRO, CN, S, AMX, AZM	1(9.1)	1(6.7)	0(0.0)	1(12.5)	1(11.1)	4(8.5)
OFX, LEV, CIP, PEF, CRO, CN, S, AMX, AZM, AUG	0(0.0)	1 (6.7)	0(0.0)	0(0.0)	0(0.0)	1(2.1)

Key: OFX= Ofloxacin , LEV= Levofloxacin, CIP= Ciprofloxacin, CRO= Ceftriaxone, S= Streptomycin, AMX= Amoxiciline, AZM= Azithromycin, CN= Gentamycin, AUG= Augmentin, PEF= Perfloxacin

Table 4.5: Multiple Antibiotic Resistance (MAR) index of *Salmonella* enteric serovar *typhi*

No. of Antibiotic Resistance (a)	No. of Antibiotic Tested (b)	MAR Index =47 (a/b)	No (%) of MAR Isolates					Total
			A = 11	B= 15	C = 4	D= 8	E = 9	N = 47
10	10	1.0	0 (00)	2 (13.3)	1 (25.0)	0 (00)	0 (00)	3 (6.4)
9	10	0.9	0 (00)	0 (00)	1 (25.0)	0 (00)	1 (11.1)	2 (4.3)
8	10	0.8	2 (18.2)	3 (20.0)	2 (50.0)	1 (12.5)	4 (44.4)	12(25.5)
7	10	0.7	2 (18.2)	2 (13.3)	3 (75.0)	2 (25.0)	2 (22.2)	11(23.4)
6	10	0.6	4(36.4)	1 (6.7)	3 (75.0)	1 (12.5)	1 (11.1)	10(21.3)
5	10	0.5	0 (00)	3 (20.0)	1 (25.0)	1 (12.5)	1 (11.1)	6 (12.8)
4	10	0.4	1 (9.1)	2 (13.3)	0 (00)	1 (12.5)	2 (22.2)	6 (12.8)
3	10	0.3	0 (00)	0 (00)	0 (00)	0 (00)	0 (00)	0 (00)

Key: A Masaka primary health care; B New Nyanya primary health care, C-Ado primary health care, D- Adehi primary health care, E-Mararaba primary health care.

Table 4.6: Detection of Beta-lactam Resistance Genes

Genes	No. of Positive	Size of genes (bp)	Percentage %
bla TEM	3	858	60.0%
blaSHW	1	930	20.0%
blaCTX-M	4	550	80.0%

4.6.1 Overall Interpretation of PCR Result

The PCR results demonstrate that beta-lactam resistance genes are present among the isolates, with blaCTX-M being the most dominant gene, followed by blaTEM, while blaSHW was the least detected. The absence of bands in the negative control confirms that there was no contamination during the PCR process.

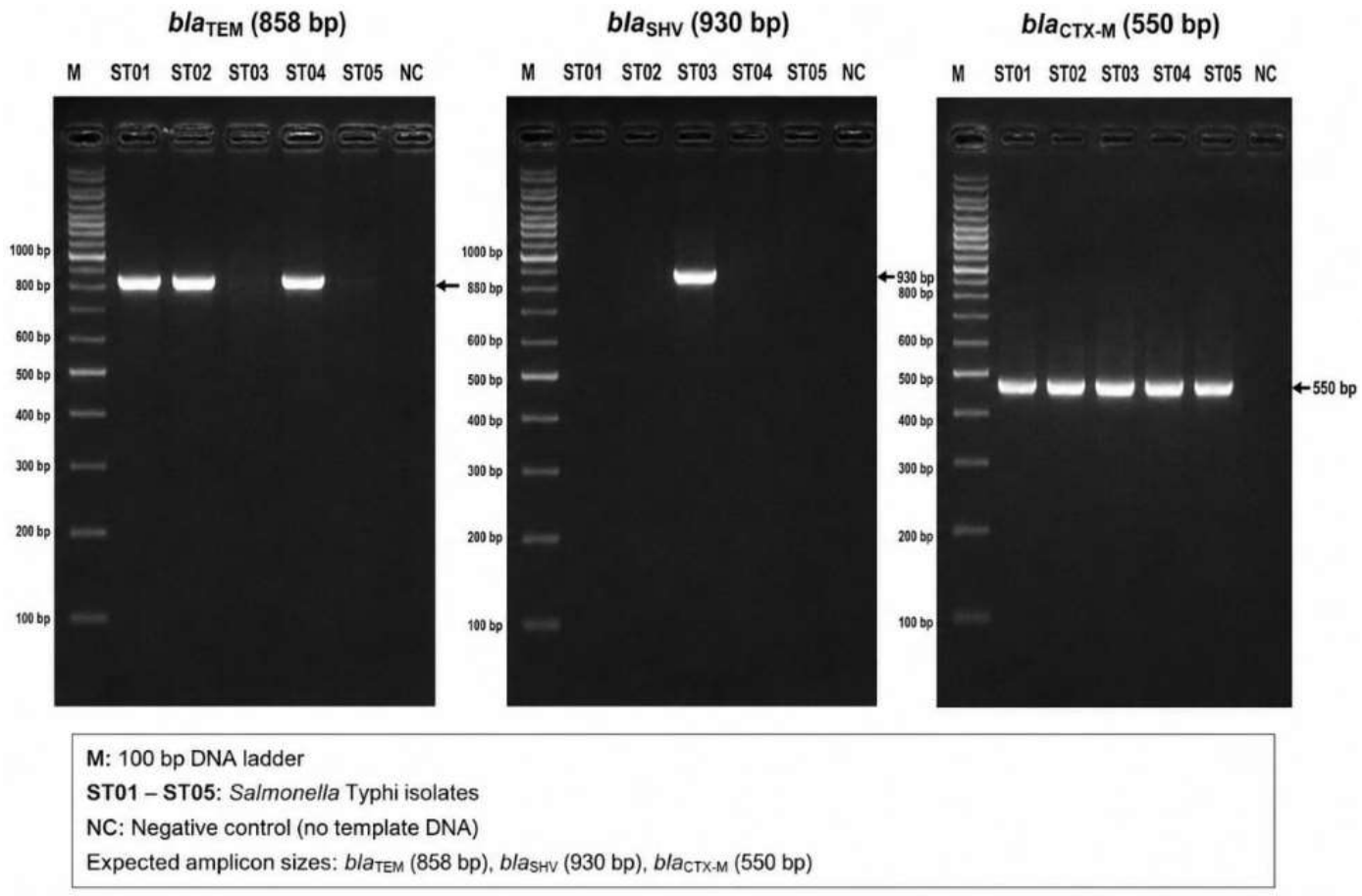


Figure 4.1-3: PCR Detection of bla$_{TEM}$ (858 bp); bla$_{SHW}$ (930bp); bla$_{CTX-M}$ (550 bp).
Key: M= Molecular ladder, ST01=Stool sample 1, ST02= Stool sample 2, ST03= Stool sample 3, ST04= Stool sample 4, ST05=Stool sample 5, NC= Negative Control.

4. DISCUSSION OF FINDINGS

The present study recorded an occurrence rate of 19.6% for Salmonella enterica serovar Typhi among stool samples collected from children attending five selected Primary Health Care Centres in Karu, Nasarawa State. This prevalence is lower than the 32.2% reported by Raseala *et al.* (2020) in Enugu State. However, the findings are consistent with previous studies conducted by Patel *et al.* (2023) and Otoikhian and Okoror (2022), which identified S. typhi as a leading cause of typhoid fever, especially in developing nations.

Variations in the prevalence of *S. typhi* were observed among the different Primary Health Care Centres. These differences were not statistically significant (P = 0.30), suggesting that factors such as variations in antibiotic usage patterns, sanitation practices, and healthcare-seeking behaviour may influence the distribution of the pathogen.

The susceptibility testing revealed that *S. typhi* isolates were highly sensitive to azithromycin, β-lactam antibiotics, and certain fluoroquinolones. Conversely, reduced susceptibility was observed against streptomycin, amoxicillin, ofloxacin, and levofloxacin. These findings are in agreement with those reported by

Raseala *et al.* (2020) but differ from the observations of Sodagari *et al.* (2019). The reduced effectiveness of these antibiotics may be linked to their excessive and inappropriate use, which promotes the development of resistant bacterial strains (Zwe, 2021).

The molecular characterization of the isolates revealed the presence of beta-lactam resistance genes, with blaCTX-M being the most prevalent (80.0%), followed by blaTEM (60.0%) and blaSHW (20.0%). These findings are consistent with those of Akinyemi *et al.* (2018), who reported the presence of blaCTX-M in *S. typhi* isolates from Lagos, Nigeria. The high prevalence of blaCTX-M is particularly concerning, as this gene confers resistance to extended-spectrum cephalosporins, which are among the last-line antibiotics for treating severe typhoid fever. The presence of these resistance genes in *S. typhi* isolates from children highlights the urgent need for molecular surveillance and antimicrobial stewardship programs in the study area.

5. CONCLUSION

This study established the presence of *Salmonella typhi* among children attending selected Primary Health Care Centres in Karu, Nasarawa State, with an occurrence rate of 19.6%. The findings revealed that some of the isolates exhibited resistance to commonly used antibiotics, indicating the emergence and spread of antimicrobial-resistant strains within the study population. Molecular characterization further confirmed the presence of beta-lactam resistance genes among the isolates, demonstrating the genetic basis of resistance. The study concludes that antibiotic-resistant *Salmonella typhi* is circulating among children in Karu and poses a significant public health challenge. Strengthening surveillance, promoting rational antibiotic use, improving sanitation, and integrating molecular diagnostic methods into routine healthcare services are essential for effective control and management of typhoid fever in the study area.

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Conflicts of Interest

The author declared that the research was conducted in the absence of any commercial or financial relationship that could appeared as potential influence to the work reported in this paper.

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