Science World Journal Vol. 21(No 2) 2026 https://dx.doi.org/10.4314/swj.v21i2.32
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

Full Length Research Article

MOLECULAR CHARACTERIZATION AND TETRACYCLINE RESISTANCE DETERMINANTS OF STOOL-DERIVED *SALMONELLA ENTERICA* FROM SELECTED TERTIARY HOSPITALS IN KANO, NIGERIA

Asmau Nna SULAIMAN*

Department of Microbiology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria

*Corresponding Author Email Address: ansulaiman@abu.edu.ng or sulaimanasmau@yahoo.co.uk

ABSTRACT

Enteric bacterial infections, especially those caused by Salmonella species, remain a significant public health concern in sub-Saharan Africa, particularly amid rising antimicrobial resistance. This study investigated enteric bacteria specifically *Salmonella enterica* isolated from 300 stool samples collected from three tertiary hospitals in Kano, Nigeria (AKTH, MAWTH and MMSH), with emphasis on molecular characterisation and tetracycline resistance in *Salmonella enterica*. *Escherichia coli* was the predominant isolate, 27.7% (83/300), followed by *Proteus mirabilis* 5.7 % (17/300), *Citrobacter* species 5.3% (16/300), *Enterobacter* species 4.0% (12/300), *Yersinia* species 1.7% (5/300), and *Salmonella enterica* 1.3% (4/300), reflecting pathogen distributions commonly reported across African settings. Four *Salmonella* isolates (1.3%) were confirmed as *Salmonella enterica* by 16S rRNA gene sequencing, with sequence similarity ranging from 96.05–97.64% to reference strains. Phylogenetic analysis demonstrated clustering with internationally reported serovars from South Africa, China, and India, indicating global lineage relatedness. The sequences were deposited in GenBank (PP857911.1–PP857914.1), contributing to global surveillance datasets. PCR screening of tetracycline-resistant isolates revealed universal detection of the *tetD* gene (4/4) and *tetB* in one isolate, while *tetA*, *tetC*, *tetO,* and *tetS* were not detected. The predominance of *tetD* contrasts with many global reports in which tetA and tetB are more common, suggesting a distinct local resistance profile, potentially driven by regional antibiotic selection pressures and mobile genetic elements. The presence of efflux-mediated resistance determinants highlights the risk of horizontal gene transfer and the possible emergence of multidrug-resistant strains. These findings underscore the need for strengthened antimicrobial stewardship, expanded molecular surveillance, and broader profiling of resistance genes to mitigate the spread of resistant *Salmonella enterica* in northern Nigeria.

Keywords: *Salmonella enterica*, tetracycline resistance, antibiotic resistance, molecular detection, polymerase chain reaction.

INTRODUCTION

Salmonella enterica is a significant cause of foodborne and invasive infections worldwide, posing a major public health challenge due to its capacity for rapid dissemination and acquisition of antimicrobial resistance. The global rise in multidrug-resistant (MDR) *Salmonella* strains, particularly those resistant to commonly used antibiotics such as tetracycline, has complicated treatment strategies and increased morbidity and mortality, especially in low- and middle-income countries (Uzairue *et al.,* 2023; Borah *et al.,* 2022; Nguyen *et al.,* 2023). Tetracycline resistance in *S. enterica* is frequently mediated by the acquisition of specific resistance genes, including *tetA*, *tetB*, and tetG, which are often associated with mobile genetic elements that facilitate horizontal gene transfer and the spread of resistance within and between bacterial populations (Uzairue *et al.,* 2023; Long *et al.,* 2022; Vázquez *et al.,* 2023; Borah *et al.,* 2022; Petrin *et al.,* 2023).

In Nigeria, the burden of *Salmonella* infections remains high, with recent studies reporting a substantial prevalence of MDR *S. enterica* isolates from both clinical and environmental sources (Uzairue *et al.,* 2023; Adamu *et al.,* 2024; Ajayi *et al.,* 2019; Monsi *et al.,* 2025). Notably, resistance to tetracycline among *Salmonella* isolates from Nigerian patients has been documented, with molecular analyses revealing key resistance determinants, including *tetA* and *tetB* (Uzairue *et al.,* 2023; Ajayi *et al.,* 2019; Monsi *et al.,* 2025). The detection and characterization of these resistance genes are critical for understanding the epidemiology of antimicrobial resistance, guiding effective treatment, and informing public health interventions.

Despite the recognized threat of antibiotic-resistant *Salmonella*, there is limited molecular surveillance data on the distribution of tetracycline resistance genes among clinical isolates in northern Nigeria. This study aims to address this gap by employing molecular techniques to detect and characterize tetracycline resistance genes in *S. enterica* isolates from patients in selected hospitals in Kano, Nigeria. The findings will contribute to the growing body of knowledge on antimicrobial resistance in the region and support the development of targeted strategies for controlling and managing *Salmonella* infections.

MATERIALS AND METHODS

Study Area

The study was conducted in Kano State, located in the North-Western region of Nigeria. Kano City serves as the state capital and principal urban centre. The state shares boundaries with Katsina and Jigawa States to the North, Kaduna State to the South, and Bauchi State to the South-East. Kano State was established on 27 May 1967 and presently comprises 44 Local Government Areas (LGAs), covering a total land area of approximately 20,131 km². Geographically, it lies at latitude 11.7574°N and longitude 8.6601°E. The Kano metropolitan area consists of six core urban LGAs—Tarauni, Nassarawa, Kano Municipal, Gwale, Fagge, and

Dala—and two peri-urban LGAs, Kumbotso and Ungogo. Based on the 2006 National Population Census, the state had a population of 9,401,288, with an estimated annual growth rate of 3.5% (National Population Commission [NPC], 2006).
This study was carried out in three tertiary healthcare facilities within Kano metropolis: Aminu Kano Teaching Hospital, Muhammad Abdullahi Wase Teaching Hospital, and Murtala Muhammad Specialist Hospital. These institutions serve as major referral centers, providing specialized medical services to residents of Kano State and neighbouring states.

Study Design
The research was a cross-sectional study

Sample Size
This was calculated using a prevalence rate of 6.1% obtained from a study conducted in Niger State, Nigeria (Olusola *et al.,* 2025), and the formula described by Aroye (2004).
$N = z^2 pq / l^2$
Where, Z = 1.96 for 95% confidence interval
P = prevalence rate (6.1%)
q = 1 – P
l = allowable error (5%)
n = the number of samples

$= \frac{(1.96)2 \times 0.061 \times 0.939}{(0.05)2}$

= 88.0172
Approximately 88.
For this study, 300 samples were collected from the selected hospitals, i.e., 100 samples from each hospital.

Sample Population
Clinical isolates from study participants were obtained from three major hospitals in Kano: Muhammad Abdullahi Wase Teaching Hospital (MAWTH), Aminu Kano Teaching Hospital (AKTH), and Murtala Muhammad Specialist Hospital (MMSH).

Ethical Approval
Ethical approval was obtained from the ethical committee of Aminu Kano Teaching Hospital (AKTH), with ethical approval number: NHREC/21/08/2008/AKTH/EC/2726, and the Ministry of Health, with ethical approval number: MOH/Off/797/T.I/1657. This was after the submission of an introductory letter retrieved from the Department of Microbiology, Faculty of Life Sciences, College of Natural and Pharmaceutical Sciences, Bayero University, Kano, Nigeria.

Collection and Reconfirmation of Clinical Bacterial Isolates
Isolates were obtained from the laboratory of the selected hospitals and transported to the laboratory on ice blocks. The isolates were screened by subculturing colourless isolates with or without black centres on *Salmonella Shigella* agar. These were incubated for 24 hours at 37 °C to screen for species (Sulaiman *et al.,* 2024).

Identification of Bacterial Isolates

Gram Staining and Biochemical Characterization
The isolates were subjected to Gram Staining to determine their morphology and Gram reaction. Suspected *Salmonella* and *Shigella* species, characterized by colourless colonies with or without black centers on *Salmonella Shigella* agar, were further investigated using some biochemical tests: IMViC (Indole, Methyl Red, Vogues Proskauer, and Citrate Utilisation), Triple Sugar Iron (TSI), and Motility tests, as described by Sulaiman *et al.* (2024).

16S rRNA Sequencing and Tetracycline Resistant Genotypic Detection

Bacterial Cell Preparation
Bacterial cells resistant to tetracycline were prepared as described by Dubey (2009). Pure colonies of the isolates were picked and inoculated into 5mL of Luria-Bertani (LB) broth and incubated at 37°C for 18 hours.

DNA Extraction
Genomic DNA was isolated using a ZR Fungal/Bacterial DNA purification kit (Zymo Research Corporation) according to the manufacturer's instructions. Bacterial cells from an overnight growth culture were harvested by centrifugation at 4°C, 800 rpm (6800 × g) in a microcentrifuge for 2 minutes in an Eppendorf tube. The step was repeated to achieve a higher cell yield. The supernatant was discarded, and the cells were harvested. The harvested cell pellets were dislodged, and 200µL of deionized water was added and mixed thoroughly by vortexing. Exactly 400µL of the lysis solution was transferred to the Zymo-Spin™ IV Spin Filter in a collection tube and centrifuged at 7,000 rpm for 1 minute. One thousand two hundred micro litres of DNA Binding Buffers were added to the filtrate in the collection tube from the preceding Step. Next, 800 µL of the mixture from the step above was transferred to the Zymo spin IIC column in a new collection tube and centrifuged at 10000 × g for 1 minute. The flow-through from the previous step in the collection tube was discarded, and the step was repeated. Exactly 200 µL DNA Pre-Wash Buffer was added to the Zymo-Spin Column in a new collection tube and centrifuged at 10,000 x *g* for 1 minute, and then washed with 500 µL Bacterial DNA Wash Buffer. The column was transferred to a clean 1.5 mL microcentrifuge tube, and 100 µL of DNA Elution Buffer was added directly to the column matrix, then centrifuged at 10,000 × *g* for 1 minute to elute the DNA (Oko *et al*., 2020).

Primer Design
The oligonucleotide primers used for Polymerase Chain Reaction (PCR) were obtained from Zymo Research Corporation through Inqaba Biotech West Africa Limited, and the manufacturer's instructions were followed. The primers are as shown in Table 1..

Table 1: Primers for 16srRNA and Tetracycline-Resistant Genes Used in this Study

Primer	Class Targeted	Sequence 5'- 3'	Amplicon Size (bp)	Annealing Temperature (^{O}C)	Reference
F27 R5	Bacteria 16s rRNA gene	AGA GTT TGA TCI TGG CTC AG ACG GIT ACC TTG TTA CGA CTT	1500	55	Kullin *et al.*, 2015
TET A- FW TET A- RV	TET A	GCGCGATCTGGTTCACTCG AGTCGACAGYRGCGCCGG C	164	61	Aminov *et al.,* 2004
TET B- FW TET B- RV	TET B	TACGTGAATTTATTGCTTCGG ATACAGCATCCAAAGCGCAC	206	61	Aminov *et al.,* 2004
TET C- FW TET C- RV	TET C	GCGGGATATCGTCCATTCCG GCGTAGAGGATCCACAGGACG	207	68	Aminov *et al.,* 2004
TET D- FW TET D- RV	TET D	GGAATATCTCCCGGAAGCGG CACATTGGACAGTGCCAGCAG	187	68	Aminov *et al.,* 2004
TET O- FW TET O- RV	TET O	ACGGARAGTTTATTGTATACC TGGCGTATCTATAATGTTGAC	171	60	Aminov *et al.,* 2004
TET S- FW TET S- RV	TET S	GAAAGCTTACTATACAGTAGC AGGAGTATCTACAATATTTAC	169	50	Aminov *et al.,* 2004

PCR Amplification of 16S rRNA and Tetracycline Resistant Genes

Amplification of 16S rRNA and tetracycline-resistant genes was carried out using the Zymo Research PCR protocol, following extensive optimization of the reaction to improve amplification. The following procedures were carried out. The thin–walled PCR tubes were marked, and the following components were added to each isolate in a single reaction of 50μL in the PCR tube: 25μL of Dream Taq PCR master mix, 1.0μL of forward primer, 1.0μL of reverse primer, 7.0μL of template DNA (genomic DNA), and 16μL nuclease-free water to make up a total volume of 50μL. The sample was centrifuged, and PCR was performed under the conditions specified by Zymo Research Corporation (Oko *et al*., 2020).

PCR Conditions for 16S rRNA Genes

Denaturation at 95°C was carried out for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 7 minutes. No template was used as a negative control (Kullin *et al*., 2015).

PCR Conditions for Tetracycline Efflux Pump Genes (Tet A, Tet B, Tet C, and Tet D)

For Tet A and Tet B, denaturation at 94°C was carried out for 5 minutes, followed by 25 cycles of denaturation at 94°C for 5 seconds, with 30 seconds of annealing/extension at 61°C, and a final extension at 61°C for 7 minutes. Negative controls (e.g., a non-complementary template) were included for each primer pair used (Aminov *et al.,* 2004).

For Tet C and Tet D, denaturation at 94°C was carried out for 5 minutes, followed by 25 cycles of denaturation at 94°C for 5 seconds, with 10 seconds of annealing/extension at 68°C, and a final extension at 68°C for 7 minutes. Negative controls (e.g., a non-complementary template) were included for each primer pair used (Aminov *et al.,* 2004).

PCR Conditions for Ribosomal Protection Protein Genes (Tet O and Tet S)

For Tet O and Tet S, denaturation at 94°C was carried out for 5 minutes, followed by 25 cycles of denaturing at 94°C for 30 seconds, 30 seconds of annealing (Tet O, 60°C; Tet S, 50°C), 30 seconds of extension at 72°C, with final extension at 72°C for 7 minutes. Negative (e.g., a non-complementary template) controls were included for each primer pair used (Aminov *et al.,* 2004).

Agarose Gel Electrophoresis

The DNA extracted was subjected to agarose gel electrophoresis. A 1.5% agarose gel was prepared by adding 1.5g of agarose to a 10x Tris-acetate-ethylenediamine tetraacetic acid (TAE) buffer (2mL 10x TAE) and 98 mL of distilled water in a 250mL beaker, then heating until the agarose dissolved. Then, 5 μL of ethidium bromide was added to the dissolved agarose solution as a dye, and the mixture was mixed. The gel was poured into a mini horizontal gel electrophoresis tank, with the casting combs labelled red at the base to ensure easy visibility of the wells during PCR product loading. It was allowed to set and solidify. The comb was carefully removed after the gel had completely solidified. One-time concentration (1x) electrophoresis buffer was added to the reservoir until the buffer just covered the agarose gel. Exactly 5 μL of gel tracking dye (bromophenol blue) was added to 15 μL of each sample with gentle mixing, then loaded into the wells of the gel. The mini horizontal electrophoresis gel set up was covered, and the electrodes were connected running from cathode (-) to anode (+). Electrophoresis was carried out at 75 mV for 30 minutes to allow easy separation of the sample based on molecular weight. Upon completion of the electrophoresis, the gel was removed and viewed under a Trans-illuminator UV light at a wavelength of 302nm to visualize the DNA bands. The band's pattern of the target gene was photographed with a Polaroid camera (Oko *et al*., 2020).

https://dx.doi.org/10.4314/swj.v21i2.32

Polymerase Chain Reaction (PCR) Product Purification
The PCR product was purified with the following protocol: 20 µL of absolute ethanol was added to the PCR product and incubated at room temperature for 15 minutes, after which it was centrifuged at 10000rpm for 15 minutes. The supernatant was decanted, then further spun at 10000rpm for 15 minutes; this was followed by the addition of 40 µL of 70% ethanol, and the supernatant was decanted. The product was air-dried, after which 10 µL of ultrapure water was added, and the amplicons were visualized on a 1.5% agarose gel. The product from the purification process was loaded onto the 3130xl genetic analyzer (Applied Biosystems, HITACHI) to obtain nucleotide sequences (Oko *et al*., 2020).

Nucleotide Sequence Analysis
The sequences were analyzed with the BIOEDIT (version 7.2.5.0) software, after which the Basic Local Alignment Search Tool (BLAST) was carried out on the National Centre for Biotechnology Information (NCBI) website (*www.ncbi.nlm.nih.gov*) to determine the hits of the subject's sequences deposited in the NCBI Genebank to identify the organism (Oko *et al*., 2020).

Phylogenetic Analysis
Phylogenetic analysis of bacterial sequences was performed in MEGA 7 after aligning 16S rRNA sequences using Clustal W.

RESULTS
The biochemical characteristics and Gram reaction of the isolates suspected to be *Salmonella* species, based on cultural morphology on Salmonella Shigella agar, showed clear differentiation among the organisms, as shown in Table 2.

Table 2: Biochemical characteristics of the isolates from the stool of patients attending selected hospitals in Kano State

Gram Reaction	Indole test	Methyl red test	Voges Proskauer test	Citrate utilisation test	Triple sugar iron test	Motility test	Inference
Negative rods	-	+	-	+	K/A+ H_2S	+	***Proteus mirabilis***
Negative rods	+	+	-	-	A/A+ gas	+	***Escherichia coli***
Negative rods	-	-	+	+	A/A+gas	+	***Enterobacter* species**
Negative rods	-	+	-	+	K/A+H_2S+gas	+	***Salmonella* species**
Negative rods	-	+	-	+	A/A or K/A +H_2S	+	***Citrobacter freundi***
Negative rods	+	+	-	-	A/A(no gas, no H_2S)	-	***Yersinia* species**

Key: - negative, + positive, A/A- acid/acid, K/A-alkaline/acid

Frequency Distribution of Bacterial Isolates from the Stool of Patients among Selected Hospitals in Kano State
Of the 300 bacterial isolates collected from the selected hospitals, 137 (45.7%) were identified using conventional biochemical tests, while 163 (54.3%) showed no growth. The occurrence of bacterial isolates collected from Aminu-Kano Teaching Hospital (AKTH), Murtala Muhammad Specialist Hospital (MMSH), and Muhammad Abdullahi Wase Teaching Hospital (MAWTH) is presented in Table 3. Among these were: *Proteus mirabilis* 17/300 (5.7 %), *Escherichia coli* 83/300 (27.7%), *Enterobacter* species 12/300 (4.0 %), *Salmonella* species 4/300 (1.3%), *Citrobacter* species 16/300 (5.3%), and *Yersinia* species 5/300 (1.7%).

Table 3: Frequency Distribution of Bacterial Isolates from the Stool of Patients among the Selected Hospitals in Kano State

Bacteria	Hospital			Frequency (%) N=300	χ^2	p-value
	AKTH N=100	MAWTH N=100	MMSH N=100			
Proteus mirabilis	10 (3.3)	4(1.3)	3 (1.1)	17 (5.7)	29.4113	0.0011
Escherichia coli	38 (12.67)	12 (4.0)	33 (11.0)	83 (27.7)		
Enterobacter species	4 (1.3)	3 (1.0)	5 (1.7)	12 (4.0)		
***Salmonella* species**	**0 (0.0)**	**4 (1.3)**	**0 (0.0)**	**4 (1.3)**		
Citrobacter species	5 (1.7)	9 (3.0)	2 (0.7)	16 (5.3)		
Yersinia species	2 (0.7)	1 (0.3)	2 (0.7)	5 (1.7)		
TOTAL				137 (45.7)		

Key: χ^2= Chi square
N= Total number of isolates
AKTH= Aminu Kano Teaching Hospital
MMSH= Murtala Muhammad Specialist Hospital
MAWTH= Muhammad Abdullahi Wase Teaching Hospital

Science World Journal Vol. 21(No 2) 2026
https://dx.doi.org/10.4314/swj.v21i2.32
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

Polymerase Chain Reaction (PCR) Targeting 16S rRNA in *Salmonella* species, Basic Local Alignment Search Tool (BLAST), and Phylogenetic Analysis

The agarose gel electrophoresis image of the PCR amplicons, resolved on a 1.5% agarose gel in 1x TAE buffer, was run at 75 mV for 30 minutes. Lanes 1-4 represent the amplified 16S rRNA gene fragments from the *Salmonella* species. The amplicons migrated as distinct bands of 1500 bp. The negative control (Lane NC) showed no amplification. The DNA ladder (Lane ML) indicates the molecular weight standards, ranging from 100-1500bp. This is shown in Plate I.

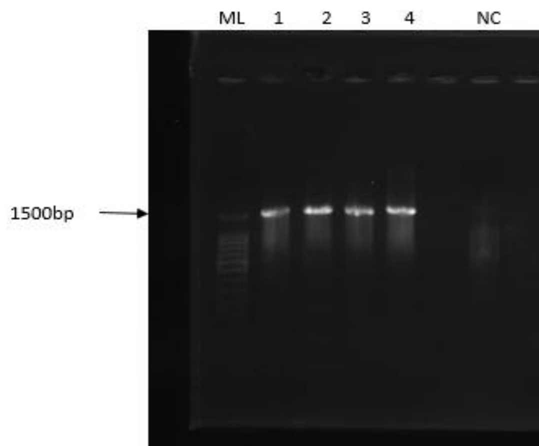

Plate I: 1.5% Agarose Gel Electrophoresis Showing 16S rRNA genes PCR amplicons of 1500bp from *Salmonella* species (Lanes 1-4) with 1500bp ladder (Lane ML)

The identities of the sequenced isolates after BLAST on the National Centre for Biotechnology Information (NCBI) website are indicated in Table 4. Four *Salmonella* species 16S rRNA sequences were subjected to BLAST on the National Centre for Biotechnology Information (NCBI) website, and the closest relatives are shown in the table, together with their accession numbers. Their percentage identity score ranged from 96% to 97%. The expected values (E-values) for all the sequences that were subjected to BLAST were 0.0. They have been deposited in NCBI GenBank and assigned accession numbers, as shown in Table 5. The phylogenetic tree (cladogram) shows the relationship between 16S rRNA sequences of *Salmonella* species. This was indicated in Figure 1. It shows the relationships among the 16S rRNA sequences of *Salmonella enterica* strains. The tree was unrooted. The sequences of reference strains obtained from the Genbank database are indicated with their respective accession numbers. The scale bar represents the nucleotide difference between the species; bootstrap tests were carried out with 500 replicates, and the values at the nodes indicate the bootstrap percentages.

Table 4: Identification of Sequenced Organisms Using Basic Local Alignment Search Tool (BLAST)

Isolate Code	Similar Sequences of *Salmonella enterica* from NCBI Gene Bank (Country)	Identity Score	E-value	NCBI Accession Number
AK33	*Salmonella enterica* subsp. *enterica* serovar Heidelberg strain CP019176.1 (South Africa)	97.64	0.0	MH356683.1
	Salmonella enterica subsp. *enterica* serovar Paratyphi B strain JQ694526.1 (South Africa)	97.64	0.0	MH352212.1
	Salmonella enterica subsp. *enterica* serovar Heidelberg strain CP016504.1 (South Africa)	97.36	0.0	MH356708.1
AK34	*Salmonella enterica* subsp. *enterica* serovar Rissen strain SeqrSC0091 (China)	96.92	0.0	MH548513.1
	Salmonella enterica subsp. *enterica* serovar Rissen strain SeqrSC0087 (China)	96.92	0.0	MH548509.1
	Salmonella enterica subsp. *enterica* serovar Rissen strain SeqrSC0097 (China)	96.83	0.0	MH548519.1
MA86	*Salmonella enterica* subsp. *enterica* serovar Shubra strain RM_AST_SM097 (China)	96.55	0.0	MK809202.1
	Salmonella enterica subsp. *enterica* serovar Shubra strain RM_AST_SM095 (China)	96.55	0.0	MK809201.1
	Salmonella enterica strain E140 (India)	96.16	0.0	MG725952.1
MA123	*Salmonella enterica* subsp. *enterica* serovar Heidelberg strain CP019176.1 (South Africa)	96.23	0.0	MH356683.1
	Salmonella enterica subsp. *enterica* serovar Paratyphi B strain JQ694526.1 (South Africa)	96.23	0.0	MH352212.1
	Salmonella enterica subsp. *enterica* serovar Heidelberg strain CP016504.1 (South Africa)	96.05	0.0	MH356708.1

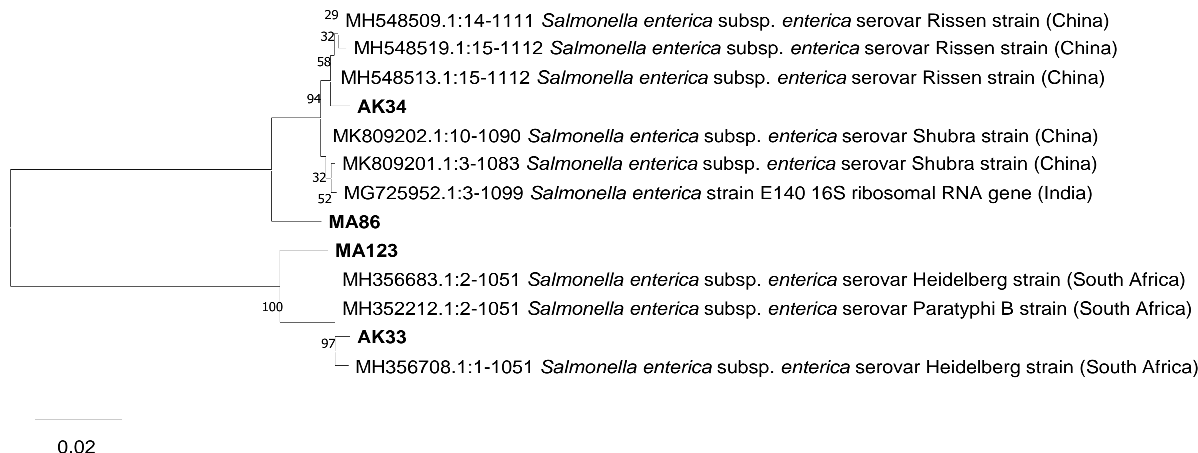

Table 5: Accession Number Assigned to *Salmonella enterica* 16S rRNA sequences from this Study in the GenBank of the National Centre for Biotechnology Information (NCBI)

ISOLATE CODE	ORGANISMS	ACCESSION NUMBER	BASE PAIR
AK33	*Salmonella enterica* strain Dala 16S ribosomal RNA gene, partial sequence	PP857911.1	1109
AK34	*Salmonella enterica* sub sp. *enterica* strain Fagge 16S ribosomal RNA gene, partial sequence	PP857912.1	880
MA86	*Salmonella enterica* sub sp. *enterica* strain Kumbotso 16S ribosomal RNA gene, partial sequence	PP857913.1	1140
MA123	*Salmonella enterica* strain Nassarawa 16S ribosomal RNA gene, partial sequence	PP857914.1	879

Polymerase Chain Reaction (PCR) Targeting Some Tetracycline- Resistant Genes in *Salmonella* species.

The agarose gel electrophoresis image of the PCR amplicons, resolved on a 1.5% agarose gel in 1x TAE buffer, was run at 75 mV for 30 minutes. Lanes 1-4 represent the unamplified Tet A gene fragments from *Salmonella enterica*. There was no amplicon migration at 164bp. The negative control (Lane NC) showed no amplification. The DNA ladder (Lane ML) indicates the molecular weight standards, ranging from 100-1500bp. There was background noise in all lanes due to nonspecific binding/dimer artifacts, visible as faint bands below 100 bp. This is shown in Plate II.

Plate II: 1.5% Agarose Gel Electrophoresis Showing Absence of Tet A genes PCR amplicons of 164bp from *Salmonella* species with 1500bp Ladder (Lane ML)

The agarose gel electrophoresis image of the PCR amplicons, resolved on a 1.5% agarose gel in 1x TAE buffer, was run at 75 mV for 30 minutes. Lane 1 represents the amplified Tet B gene fragment from *Salmonella enterica*. The amplicon migrated at 206bp. The negative control (Lane NC) showed no amplification. The DNA ladder (Lane ML) indicates the molecular weight standards, ranging from 100-1500bp. There was background noise in all lanes due to nonspecific binding/dimer artifacts, visible as faint bands below 100 bp. This is shown in Plate III.

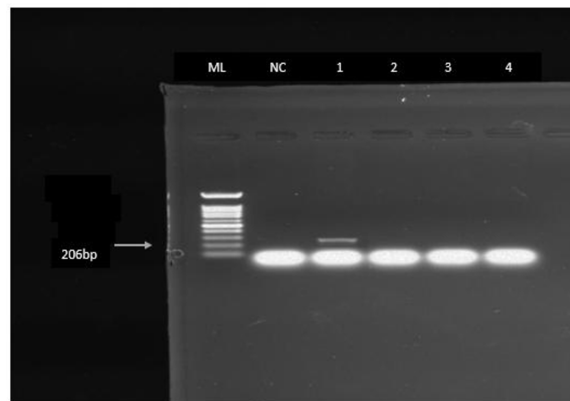

Plate III: 1.5% Agarose Gel Electrophoresis Showing Tet B gene PCR amplicon of 206bp from *Salmonella* species (Lane 1) with 1500bp ladder (Lane ML)

Science World Journal Vol. 21(No 2) 2026
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University
https://dx.doi.org/10.4314/swj.v21i2.32

The agarose gel electrophoresis image of the PCR amplicons, resolved on a 1.5% agarose gel in 1x TAE buffer, was run at 75 mV for 30 minutes. Lanes 1-4 represent the unamplified Tet C gene fragments from *Salmonella enterica*. There was no amplicon migration at 207bp. The negative control (Lane NC) showed no amplification. The DNA ladder (Lane ML) indicates the molecular weight standards, ranging from 100-1500bp. There was background noise in all lanes due to nonspecific binding/dimer artifacts, visible as faint bands below 100 bp. This is shown in Plate IV.

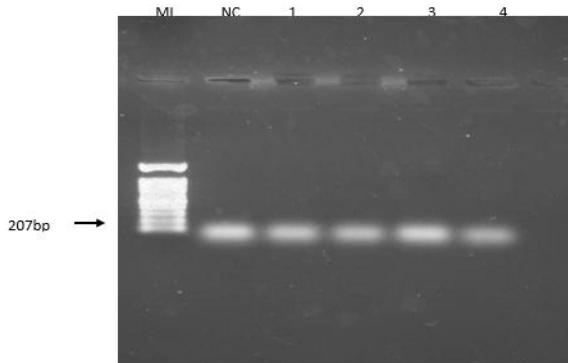

Plate IV: 1.5% Agarose Gel Electrophoresis Showing Absence of Tet C genes PCR amplicons of 207bp from *Salmonella* species with 1500bp ladder (Lane ML).

The agarose gel electrophoresis image of the PCR amplicons, resolved on a 1.5% agarose gel in 1x TAE buffer, was run at 75 mV for 30 minutes. Lanes 1-4 represent amplified TetD gene fragments from *Salmonella enterica*. The amplicons migrated at 187bp. The negative control (Lane NC) showed no amplification. The DNA ladder (Lane ML) indicates the molecular weight standards, ranging from 100-1500bp. There was background noise in all lanes due to nonspecific binding/dimer artifacts, visible as faint bands below 100 bp. This is shown in Plate V.

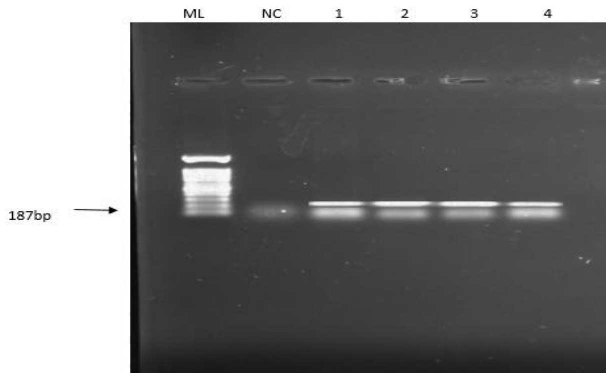

Plate V: 1.5% Agarose Gel Electrophoresis Showing Tet D genes PCR amplicons of 187bp from *Salmonella* species (Lanes 1-4) with 1500bp ladder (Lane ML)

The agarose gel electrophoresis image of the PCR amplicons, resolved on a 1.5% agarose gel in 1x TAE buffer, was run at 75 mV for 30 minutes. Lanes 1-4 represent the unamplified Tet O gene fragments from *Salmonella enterica*. There was no amplicon migration at 171bp. The negative control (Lane NC) showed no amplification. The DNA ladder (Lane ML) indicates the molecular weight standards, ranging from 100-1500bp. There was background noise in all lanes due to nonspecific binding/dimer artifacts, visible as faint bands below 100 bp. This is shown in Plate VI.

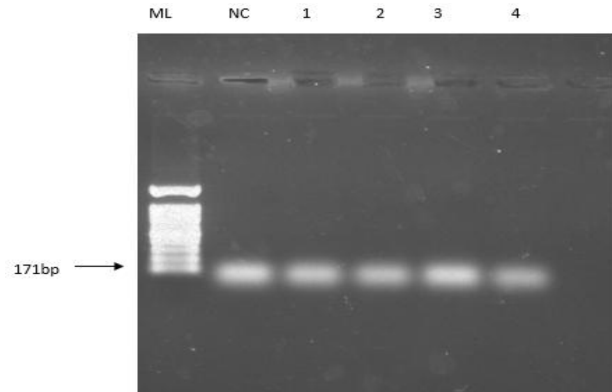

Plate VI: 1.5% Agarose Gel Electrophoresis Showing Absence of Tet O genes PCR amplicons of 171bp from *Salmonella* species with 1500 bp ladder (Lane ML)

The agarose gel electrophoresis image of the PCR amplicons, resolved on a 1.5% agarose gel in 1x TAE buffer, was run at 75 mV for 30 minutes. Lanes 1-4 represent the unamplified Tet S gene fragments from *Salmonella enterica*. There was no amplicon migration at 169bp. The negative control (Lane NC) showed no amplification. The DNA ladder (Lane ML) indicates the molecular weight standards, ranging from 100-1500bp. There was background noise in all lanes due to nonspecific binding/dimer artifacts, visible as faint bands below 100 bp. This is shown in Plate VII.

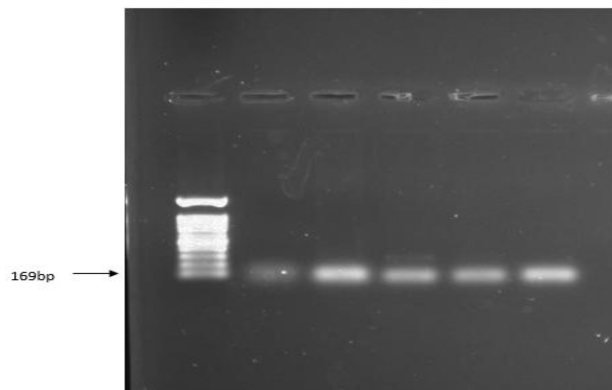

Plate VII: 1.5% Agarose Gel Electrophoresis Showing Absence of Tet S genes PCR amplicons of 169bp from *Salmonella* species with 1500bp ladder (Lane ML).

The percentage frequency of tetracycline-resistant genes in *Salmonella* species is shown in Figure 2, indicating that Tet A, Tet C, Tet O, and Tet S had a frequency of 0% (0/4), Tet B 25% (1/4), and Tet D 100% (4/4).

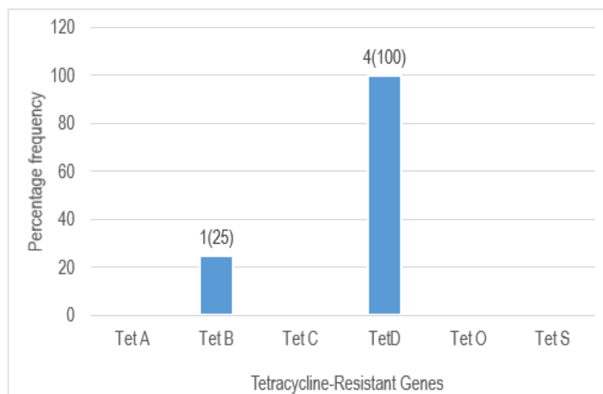

Figure 2: Percentage Frequency of Tetracycline-Resistant Genes in *Salmonella* species (N=4).

DISCUSSION

This study investigated the occurrence of *Salmonella species* among other enteric bacteria recovered from 300 stool samples collected in three major hospitals in Kano (Aminu Kano Teaching Hospital, Muhammad Abdullahi Wase Teaching Hospital, and Murtala Muhammad Specialist Hospital), providing local data that broadly align with patterns reported across sub-Saharan Africa. *Escherichia coli* was the most frequent isolate, 27.7% (83/300), consistent with reports from Nigeria, Cameroon, Kenya, and Ethiopia, where *E. coli* typically predominates among stool and other enteric isolates (Akinkunmi *et al.,* 2025; Tchientcheu *et al.,* 2021; Mbuthia *et al.,* 2018; Abebe *et al.,* 2019). Other genera recovered included *Salmonella, Citrobacter, Yersinia, Proteus,* and *Enterobacter,* mirroring the spectrum of pathogens commonly associated with diarrhoeal disease in African settings (Tchientcheu *et al.,* 2021; Nas *et al.,* 2017; Mbuthia *et al.,* 2018; Abebe *et al.,* 2019). Similar organism distributions have been described in Douala (Cameroon), central Kenya, and Port Harcourt, Nigeria, underscoring a broadly shared regional enteric pathogen profile (Tchientcheu *et al.,* 2021; Mbuthia *et al.,* 2018; Agi & Mbatha, 2025).

The proportion of *Salmonella* species (1.3%) in this study is comparable to some hospital-based Nigerian and East African studies, where *Salmonella* species generally occurs at lower frequencies than *E. coli* but remains a significant cause of enteric and invasive disease (Akinkunmi *et al.,* 2025; Nas *et al.,* 2017; Mbuthia *et al.,* 2018; Abebe *et al.,* 2019). The recovery of *Citrobacter freundii* and *Yersinia* species is likewise consistent with earlier African reports documenting these genera as less frequent yet established enteric pathogens (Tchientcheu *et al.,* 2021; Mbuthia *et al.,* 2018).

All four phenotypically identified *Salmonella* isolates were confirmed as *Salmonella enterica* by amplification and sequencing of the 16S rRNA gene, reinforcing the value of 16S rRNA sequencing as a robust tool for genus and species-level identification of enterobacteria (Umesha & Narayanaswamy, 2017; Fadlalla *et al.,* 2021; Jamal *et al.,* 2024; Sadiq & Othman, 2022; Christensen *et al.,* 1998). The isolates showed 96.05–97.64% sequence identity with reference *Salmonella enterica* subsp. *enterica* strains, slightly below the ≥99% similarity often reported for fully characterized strains, suggesting possible local strain variation and that these represent previously unregistered strains. Bacterial species typically harbor at least one copy of the 16S rRNA gene, which contains conserved and hypervariable regions, making it highly suitable for taxonomic characterization.

Phylogenetic analysis (cladogram) demonstrated that all isolates clustered within *S. enterica* subsp. *enterica,* though in distinct serovar-associated clades. Isolates AK33 and MA123 clustered closely with South African strains, including serovars Heidelberg and Paratyphi B (MH356683.1; MH352212.1; MH356708.1), while AK34 grouped with serovar Rissen strains from South Africa (MH548513.1; MH548519.1; MH548509.1). Isolate MA86 clustered with strains from India and China, including strain E40 (MG725952.1) and serovar Shubra (MK809201.1; MK809202.1). These findings are comparable to reports of serovar Heidelberg and Rissen from commercial layer farms in Korea (Im *et al.,* 2015) and serovar Shubra from ducklings in Egypt (Osman *et al.,* 2014). Similar phylogenetic relatedness across continents has been documented in studies from Iraq, Tehran, and other regions, demonstrating that human and animal *S. enterica* strains often share highly conserved 16S rRNA sequences with global strains, suggesting common ancestral lineages and international circulation (Tajbakhsh *et al.,* 2011; Sadiq & Othman, 2022; Jamal *et al.,* 2024; Cao *et al.,* 2023). The short horizontal branch lengths observed in the phylogenetic tree further indicate minimal nucleotide divergence among the isolates.

The successful deposition of the *Salmonella enterica* strains in this study has their 16S rRNA sequences in the GenBank (PP857911.1–PP857914.1). This ensures their inclusion in global comparative and surveillance datasets, as recommended in previous molecular characterisation studies (Umesha & Narayanaswamy, 2017; Fadlalla *et al.,* 2021; Jamal *et al.,* 2024; Sadiq & Othman, 2022).

Polymerase Chain Reaction detection of tetracycline resistance genes among the four *S. enterica* isolates revealed the universal presence of tetD (4/4), the presence of tetB in one isolate, and the absence of *tetA*, *tetC*, *tetO,* and *tetS*. The predominance of *tetD* contrasts with many global surveillance studies in which *tetA* and *tetB* are typically the most prevalent tetracycline resistance determinants in *Salmonella* from animal and human sources (Pavelquesi *et al.,* 2021; Toure *et al.,* 2025; Khoshbakht *et al.,* 2018; Pezzella *et al.,* 2004; Adesiji *et al.,* 2014; Gargano *et al.,* 2021; Guillaume *et al.,* 2000). However, detection of *tetB* and *tetD* aligns with findings from Kenya, where both genes were identified in *Salmonella Typhi* (Miruka *et al.,* 2011).

TetA, *TetC*, and TetD encode membrane-associated efflux proteins that export tetracycline from the bacterial cell, lowering intracellular drug concentrations and protecting ribosomal targets, but generally do not confer resistance to minocycline (Chopra & Roberts, 2001). *TetB*, also an efflux pump, confers resistance to both tetracycline and minocycline, while *TetO* and *TetS* encode ribosomal protection proteins that provide broader resistance to tetracyclines, including doxycycline and minocycline (Chopra & Roberts, 2001). The presence of both *tetB* and *tetD* in isolate AK34, and *tetD* alone in AK33, MA86, and MA123, suggests efflux-mediated resistance as the dominant mechanism in these strains. Similar observations linking tetracycline resistance to efflux genes have been reported elsewhere (Waghamare *et al.,* 2018; Uzairue, 2023).

The predominance of *tetD* in the Kano isolates suggests a distinct local resistance gene profile that may reflect region-specific antibiotic selection pressures, plasmid content, or mobile genetic elements. Tet genes are frequently located on conjugative plasmids and transposons, facilitating horizontal gene transfer and

https://dx.doi.org/10.4314/swj.v21i2.32

rapid dissemination within bacterial populations (Pavelquesi *et al.,* 2021; Khoshbakht *et al.,* 2018; Pezzella *et al.,* 2004; Chen *et al.,* 2016). High antibiotic usage in both clinical and agricultural settings may further select for such resistant strains (Molino *et al.,* 2022; Adzitey *et al.,* 2019). The detection of tetracycline-resistant *Salmonella enterica* strains is concerning, given the documented high rates of tetracycline resistance among enteric pathogens in Cameroon, Ethiopia, and Nigeria (Akinkunmi *et al.,* 2025; Tchientcheu *et al.,* 2021; Adesiji *et al.,* 2014; Abebe *et al.,* 2019).
Beyond tetracycline resistance, the identification of multiple resistance genes within a single isolate highlights the genetic adaptability of *S. enterica,* which may facilitate the emergence of multidrug-resistant (MDR) strains (Adzitey *et al.,* 2020; Waghamare *et al.,* 2018; Mathew *et al.,* 2017; Lu *et al.,* 2014). Such MDR strains complicate therapeutic management, increase the risk of treatment failure, and pose significant public health challenges (Adzitey *et al.,* 2020; Adzitey, 2018; Yousaf, 2024).

The coexistence of a typical African enteric pathogen spectrum with molecularly confirmed, internationally related *Salmonella enterica* strains harbouring tetracycline resistance genes underscores the need for integrated surveillance in northern Nigeria. Routine culture-based diagnostics should be complemented by molecular typing approaches (e.g., 16S rRNA sequencing and additional markers such as invA, gyrB, and virulence genes) and systematic monitoring of antimicrobial resistance determinants, as advocated in studies from Iran, Iraq, and other regions (Fadlalla *et al.,* 2021; Jamal *et al.,* 2024; Tajbakhsh *et al.,* 2011; Sadiq & Othman, 2022; Cao *et al.,* 2023).
Nevertheless, the small number of *Salmonella* isolates (n = 4), the absence of comprehensive serotyping, and the focus on selected tetracycline resistance genes limit the generalisability of the findings. Future studies should include larger multi-year collections, detailed serovar identification, phenotypic susceptibility profiling, and expanded resistance gene panels (e.g., sul, bla, qnr, aminoglycoside, and chloramphenicol genes) to define the antimicrobial resistance landscape in Kano (Malik *et al.,* 2025; Pavelquesi *et al.,* 2021; Toure *et al.,* 2025; Khoshbakht *et al.,* 2018; Adesiji *et al.,* 2014; Cao *et al.,* 2023).

Conclusion
This study reveals that *E. coli* 83/300 (27.7%) predominates among enteric isolates in some tertiary hospitals in Kano, while a small subset is *Salmonella enterica* 4/300 (1.3%). It also provided the molecular characterisation of stool-derived *Salmonella enterica*, revealing an unusual predominance of *tetD* among clinical isolates. The isolates showed phylogenetic relatedness to strains from Africa and Asia, highlighting the global spread of resistant strains. With all isolates carrying at least one tetracycline-resistance gene (tetB or tetD), these findings support strengthened antibiotic stewardship, the restriction of empirical tetracycline use, and sustained molecular surveillance to mitigate the emergence and spread of multidrug-resistant *Salmonella* in northern Nigeria

REFERENCES
Abebe, M., Tadesse, S., Meseret, G., & Derbie, A. (2019). Type of bacterial isolates and antimicrobial resistance profile from different clinical samples at a Referral Hospital, Northwest Ethiopia: five-year data analysis. *BMC Research Notes, 12.* https://doi.org/10.1186/s13104-019-4604-6
Adamu, M., Musa, I., Abiodun, A., Uba, A., & Adamu, U. (2024). Antimicrobial susceptibility pattern and molecular characterization of ESBLs *Salmonella enterica* isolated from patients attending hospitals in Northeast Nigeria. *Microbes and Infectious Diseases.* https://doi.org/10.21608/mid.2024.256575.1722
Adesiji, Y., Deekshit, V., & Karunasagar, I. (2014). Antimicrobial-resistant genes associated with *Salmonella* spp. isolated from human, poultry, and seafood sources. *Food Science & Nutrition, 2*, 436–442. https://doi.org/10.1002/fsn3.119
Agi, V. N., & Mbata, C. A. (2025). Molecular Characterization of Enteric Bacteria in Diarrhoeic Stool Samples of Children Less than Five Years in Port Harcourt, Nigeria. *Microbiology Research Journal International, 35*(2), 92–105. https://doi.org/10.9734/mrji/2025/v35i31554
Ajayi, A., Smith, S., Kalpy, J., Bode-Sojobi, I., René, Y., & Adeleye, A. (2019). Molecular diversity and antibiotic resistance gene profile of *Salmonella enterica* serovars isolated from humans and food animals in Lagos, Nigeria. *Acta Microbiologica et Immunologica Hungarica,* 1–19. https://doi.org/10.1556/030.66.2019.034
Akinkunmi, I. J., Eborah, C. C., Apeh, E. D., Ugoeze, F. C., Aigbokhaode, A. Q., Obu, S. I., Eduvie, T. A., Emekekwe, C. I., Edet, S. E., Edom, A. C., Amefune, J. U., Nnanna, P. T., Dibogbo, I., N., A., B., & Akinkunmi, K. (2025). Antimicrobial Sensitivity Profile of Bacteria Isolated from Stool Samples in Federal Medical Center, Nigeria. *Journal of Advances in Microbiology, 25*(6), 65–76. https://doi.org/10.9734/jamb/2025/v25i6950
Aminov, R., Chee-Sanford, J. C., Garrigues, N., Mehboob, A., & Mackie, R. I. (2004). Detection of tetracycline resistance genes by PCR methods. *Methods in Molecular Biology, 268*, 3–13.
Aroye, M. O. (2004). *Research Methodology with Statistics for Health and Social Sciences.* Nathadex Publishers, Ilorin.
Borah, P., Dutta, R., Das, L., Hazarika, G., Choudhury, M., Deka, N., Malakar, D., Hussain, M., & Barkalita, L. (2022). Prevalence, antimicrobial resistance, and virulence genes of *Salmonella* serovars isolated from humans and animals. *Veterinary Research Communications, 46*, 799–810. https://doi.org/10.1007/s11259-022-09900-z
Cao, G., Zhao, S., Kuang, D., Hsu, C., Yin, L., Luo, Y., Chen, Z., Xu, X., Strain, E., McDermott, P., Allard, M., Brown, E., Meng, J., & Zheng, J. (2023). Geography shapes the genomics and antimicrobial resistance of *Salmonella enterica* Serovar Enteritidis isolated from humans. *Scientific Reports, 13.* https://doi.org/10.1038/s41598-022-24150-4
Christensen, H., Nordentoft, S., & Olsen, J. (1998). Phylogenetic relationships of *Salmonella* based on rRNA sequences. *International Journal of Systematic Bacteriology, 48*(2), 605–610. https://doi.org/10.1099/00207713-48-2-605
Chopra, I., & Roberts, M. (2001). Tetracycline antibiotics: Mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiology and Molecular Biology Reviews, 65*, 232–260.
Dubey, R. C. (2009). *Multicolor Textbook of Biotechnology* (4th ed.). S. Chand Co., India.
Fadlalla, I., Hamid, M., Arahim, A., & Osman, M. (2021). The efficacy of partial 16S rRNA gene sequencing for precise determination of phylogenetic relatedness among *Salmonellae*. *Scientific African.* https://doi.org/10.1016/j.sciaf.2021.e01004

Gargano, V., Sciortino, S., Gambino, D., Costa, A., Agozzino, V., Reale, S., Alduina, R., & Vicari, D. (2021). Antibiotic susceptibility profile and tetracycline resistance genes detection in *Salmonella* spp. strains isolated from animals and food. *Antibiotics, 10*, 809.

Guillaume, G., Verbrugge, D., Chasseur-Libotte, M., Moens, W., & Collard, J. (2000). PCR typing of tetracycline resistance determinants (Tet A–E) in *Salmonella enterica* serotype Hadar and in the microbial community of activated sludges from hospital and urban wastewater treatment facilities in Belgium. *FEMS Microbiology Ecology, 32*(1), 77–85.

Im, M. C., Jeong, S. J., Kwon, Y., Jeong, O., Kang, M., & Lee, Y. J. (2015). Prevalence and characteristics of *Salmonella* spp. isolated from commercial layer farms in Korea. *Poultry Science,* 1691–1698.

Jamal, U., Soomro, R., Huda, N., Zeeshan, F., Urooj, S., Khan, M., Abbas, T., & Mirani, Z. (2024). Evaluation and sequencing of 16S rRNA gene for molecular identification of prevalent *Salmonella* strains isolated from food and water. *Microbiological & Immunological Communications.* https://doi.org/10.55627/mic.003.002.0809

Khoshbakht, R., Derakhshandeh, A., Jelviz, L., & Azhdari, F. (2018). Tetracycline resistance genes in *Salmonella enterica* serovars with animal and human origin. *International Journal of Enteric Pathogens.* https://doi.org/10.15171/ijep.2018.17

Kullin, B., Meggersee, R., D'Alton, J., Galvao, B., Rajabally, N., Whitelaw, A., & Abratt, V. R. (2015). Prevalence of gastrointestinal pathogenic bacteria in patients with diarrhea attending Groote Schuur Hospital, Cape Town, South Africa. *South African Medical Journal, 105*(2), 121–125.

Long, L., You, L., Wang, D., Wang, M., Wang, J., Bai, G., Li, J., Wei, X., & Li, S. (2022). Highly prevalent MDR, frequently carrying virulence genes and antimicrobial resistance genes in *Salmonella enterica* serovar 4,[5],12:i:- isolates from Guizhou Province, China. *PLoS ONE, 17.* https://doi.org/10.1371/journal.pone.0266443

Malik, M., Khan, S., Irshad, H., Tayh, G., Naz, W., Khan, M., Belkahia, H., Said, B., Khan, R., Abdel-Maksou, M., Alamri, A., Alrokayan, S., Alabbad, A., El-Tayeb, M., & Badshah, F. (2025). Antibiotic susceptibility pattern and resistance genes in *Salmonella* strains isolated from cattle. *BMC Veterinary Research, 21.* https://doi.org/10.1186/s12917-025-05081-4

Mbuthia, O., Mathenge, S., Oyaro, M., & Ng'ayo, M. (2018). Etiology and pathogenicity of bacterial isolates: A cross-sectional study among diarrheal children below five years in central regions of Kenya. *Pan African Medical Journal, 31.* https://doi.org/10.11604/pamj.2018.31.88.15644

Miruka, O. D., Kakai, R., & Nyandago, W. E. (2011). Tetracycline efflux pump in *Salmonella enterica* isolated from patients with diarrhea at two rural health centers in Western Kenya. *Iranian Journal of Clinical Infectious Disease, 6*(1), 24–30.

Monsi, T., Edamkue, E., & Ollor, O. (2025). Antimicrobial Resistant Genes in Non-Typhoidal *Salmonella* Species in Food and Stool Samples in Port Harcourt, Rivers State, Nigeria. *South Asian Journal of Research in Microbiology.* https://doi.org/10.9734/sajrm/2025/v19i3424

Nas, F., Ali, M., & Yahaya, A. (2017). Identification of some enteric bacteria from diarrhoeic stool samples of patients attending Murtala Muhammad Specialist Hospital, Kano, Nigeria. *International Journal of Advanced Academic Research, 3*(9), 28–36.

National Population Commission (NPC). (2006). *Nigeria National Census: Population Distribution by Sex, State, LGAs and Senatorial District: 2006 Census Priority Tables* (Vol. 3).

Nguyen, T., Le, H., Hai, H., Tuan, T., Nguyen, H., Xuan, D., Thanh, T., & Thi, H. (2023). Whole-genome analysis of antimicrobial-resistant *Salmonella enterica* isolated from duck carcasses in Hanoi, Vietnam. *Current Issues in Molecular Biology, 45*, 2213–2229. https://doi.org/10.3390/cimb45030143

Oko, J. O., Adeshina, G. O., & Onaolapo, J. A. (2020). Enterotoxigenicity of multidrug-resistant *Staphylococcus aureus* isolated from dry catfish sold in some open markets in Zaria, Nigeria. *Asian Journal of Biochemistry, Genetics and Molecular Biology*, 22–27.

Olusola, L. F., Baba, J., & Muhammad, I. L. (2025). Prevalence of *Salmonella enterica* and *Vibrio cholerae* in diarrhoeic stool samples of patients attending the General Hospital, Minna, Niger State, Nigeria. *Journal of Health Systems Research, 10*(3).

Osman, K. M., Marouf, S. H., Zolnikov, T. R., & AlAtfeehy, N. (2014). Isolation and characterization of *Salmonella enterica* in day-old ducklings in Egypt. *Pathogens and Global Health, 108*(1), 37–48.

Pavelquesi, S., De Oliveira Ferreira, A., Rodrigues, A., De Souza Silva, C., Orsi, D., & Da Silva, I. (2021). Presence of tetracycline and sulfonamide resistance genes in *Salmonella* spp.: Literature review. *Antibiotics, 10.* https://doi.org/10.3390/antibiotics10111314

Petrin, S., Orsini, M., Massaro, A., Olsen, J., Barco, L., & Losasso, C. (2023). Phenotypic and genotypic antimicrobial resistance correlation and plasmid characterization in *Salmonella* spp. isolates from Italy reveal high heterogeneity among serovars. *Frontiers in Public Health, 11.* https://doi.org/10.3389/fpubh.2023.1221351

Pezzella, C., Ricci, A., Digiannatale, E., Luzzi, I., & Carattoli, A. (2004). Tetracycline and streptomycin resistance genes, transposons, and plasmids in *Salmonella enterica* isolates from animals in Italy. *Antimicrobial Agents and Chemotherapy, 48*, 903–908. https://doi.org/10.1128/AAC.48.3.903-908.2004

Sadiq, M., & Othman, R. (2022). Phylogenetic tree constructed from *Salmonella enterica* subspecies *enterica* isolates from animals and humans in the Basrah and Baghdad governorates, Iraq. *Iraqi Journal of Veterinary Sciences.* https://doi.org/10.33899/ijvs.2022.132478.2096

Sulaiman, A. N., Arzai, A. H., & Taura, D. W. (2024). Susceptibility pattern of tetracycline-resistant *Salmonella* species to other antibiotics and some plant extracts. *UMYU Journal of Microbiology Research, 9*(2), 115–128.

Tajbakhsh, M., Nayer, B., Motavaze, K., Kharaziha, P., Chiani, M., Zali, M., & Klena, J. (2011). Phylogenetic relationship of *Salmonella enterica* strains in Tehran, Iran, using 16S rRNA and *gyrB* gene sequences. *Journal of Infection in Developing Countries, 5*(6), 465–472. https://doi.org/10.3855/jidc.1504

Tchientcheu, R., Penda, C., Etame, R., Dalle, C., Mouokeu, R., & Ngane, R. (2021). Epidemiology and sensibility profile of some enteric bacteria isolated from stool samples in some Douala-Cameroon hospitals. *International Journal of Gastroenterology.* https://doi.org/10.11648/j.ijg.20210501.12

Toure, M., Aké, M., Amoikon, T., Bonny, A., & Djé, K. (2025). Characterization of virulence genes and antimicrobial resistance in *Salmonella enterica* from chicken gizzards in

Côte d'Ivoire. *Bulletin of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Food Science and Technology.* https://doi.org/10.15835/buasvmcn-fst:2025.0014

Umesha, S., & Narayanaswamy, B. (2017). Elucidation of rRNA secondary structure and phylogenetic analysis of *Salmonella enterica* based on 16S RNA. *International Journal of Current Microbiology and Applied Sciences, 6*, 4056–4063. https://doi.org/10.20546/ijcmas.2017.607.420

Uzairue, L., Shittu, O., Ojo, O., Obuotor, T., Olanipekun, G., Ajose, T., Arogbonlo, R., Medugu, N., Ebruke, B., & Obaro, S. (2023). Antimicrobial resistance and virulence genes of invasive *Salmonella enterica* from children with bacteremia in north-central Nigeria. *SAGE Open Medicine, 11.* https://doi.org/10.1177/20503121231175322

Vázquez, X., García-Fierro, R., Fernández, J., Bances, M., Herrero-Fresno, A., Olsen, J., Rodicio, R., Ladero, V., García, V., & Rodicio, M. (2023). Incidence and genomic background of antibiotic resistance in food-borne and clinical isolates of *Salmonella enterica* serovar Derby from Spain. *Antibiotics, 12.* https://doi.org/10.3390/antibiotics12071204