

Molecular Fingerprinting and Antibiotic Resistance Profiling of Multidrug-Resistant *Salmonella enterica* Isolates Using RAPD Markers in a Typhoid-Endemic Region

Okafor, A. C.,¹ Uba, C. U.,^{12*} Eze, E. A.³

¹ Department of Biological Science, Godfrey Okoye University, Enugu State, Nigeria

² DNA Learning Center Nigeria, Ugwuomu-Nike, Enugu state, Nigeria.

³ Department of Microbiology, University of Nigeria, Nsukka, Enugu State, Nigeria

Abstract

Salmonella Typhi is endemic in developing countries at poor resource areas and poses a serious concern in the health sector. This study assessed the genetic diversity and antibiotic resistance patterns of *S. Typhi* isolates from stool samples of diagnosed patients. Isolates were confirmed biochemically and tested against ten commonly used antibiotics. Furthermore, five RAPD primers (797, 784, AP7, NSC I, P5) were evaluated for their discriminatory power based on banding pattern polymorphism and clustering analysis. The results revealed that all isolates exhibited multidrug resistance (MDR), with multiple antibiotics resistance (MAR) indices exceeding the 0.2 threshold, indicating high antibiotic selection pressure. One strain (A12) was resistant to all tested antibiotics and had the highest MAR index of 1.0. The RAPD analysis generated between 9 and 17 bands per isolate with RAPD profiles revealing genetic heterogeneity among isolates. Among the studied primer, 797 showed the highest polymorphic potential suggesting its utility for strain differentiation. This finding highlights significant genetic diversity among MDR *S. Typhi* in this endemic setting, underscoring the utility of RAPD markers for epidemiological investigations. Also, it provides insight on the need for surveillance strategies targeting MDR strains to support public health efforts in endemic regions.

Keywords: *Salmonella*, *Salmonella Typhi*, Antibiotic Susceptibility, Typhoid Fever, RAPD Profile.

Corresponding author's email: *charlesuba192@gmail.com

Introduction

Salmonella species is a significant food-borne pathogen that cause disease in both people and animals. It is responsible for 93 million instances of gastrointestinal tract infections or illnesses with more than 2,500 identified *Salmonella* serotypes collectively causing between 200 million to over a billion infections annually (Orooba et al., 2023). Typhoid fever is caused by *Salmonella enterica* serotype Typhi (*S. Typhi*), while paratyphoid fever is caused by *Salmonella enterica* serotype Paratyphi A, B, and C (*S. Paratyphi A*, *S. Paratyphi B*, and *S. Paratyphi C*), these two serotype are the two most well-known invasive *Salmonella* serovars. Due to their high morbidity, *Salmonella* serovars are the cause of infections in both the developing and developed worlds, and they have become a significant economic burden (Obaro et al., 2017). Typhoid fever, a fatal widespread infection is caused by *Salmonella enterica* serovar affecting both adults and children at a high incidence (Paul et al., 2017).

Contaminated food or water are the primary source of transmission of typhoid when consumed by human. The most reliable way to diagnose typhoid is to look for *S. Typhi* in blood or bone marrow (WHO 2018). Isolating *S. Typhi* from patients urine and stool samples displaying significant clinical symptoms, is crucial for diagnosing typhoid. Thus, a common challenge for healthcare providers in developing nations like Nigeria is making treatment decisions solely on compatible clinical symptoms or a combination of clinical symptoms and widal test results from a single acute-phase sample (Khoharo et al., 2010; Wasihun et al., 2015). Despite a number of limitations, the Widal test is most frequently used in Nigeria to determine the presence of typhoid fever (Abuobeida, 1996; Moro et al., 2000; Moro et al., 2004). The DNA typing is typically done using two primary techniques. The first technique uses restriction fragment length polymorphism (RFLP), which is based on the precise cutting of DNA by restriction enzymes to create DNA patterns known as fingerprints. The second technique is random amplified polymorphic DNA (RAPD), which amplifies specific genomic areas using short oligonucleotide primers of arbitrary octamer lengths (Thangaraj et al., 2011). Therefore, the discriminating power of RFLP is relatively

low because it targets a limited genomic region, compared with other techniques like RAPD (Cheah et al., 2008).

Typhoid fever poses a significant public health issue globally, mostly affecting developing countries due to factors like poverty, poor sanitation, and limited access to clean water (Mogasale et al., 2014). In the year 2000, about 5.4 million cases of paratyphoid fever, 21.6 million new cases of typhoid fever, and 210,000 deaths from the illness was reported (Crump et al., 2004). *Salmonella* infections and co-infections are assuming endemic proportions in parts of Nigeria and other resource-poor areas of Africa with Nigeria believed to have one of the highest rates of typhoid fever in the region. *Salmonella* infections have assumed endemic status in Nigeria creating the need for the evaluation of clonal relationship among the causative species as an epidemiology steps towards its containment. Due to its high rates of morbidity and mortality, many cases of pyrexia with no apparent cause, and endemic character, typhoid fever is a serious socio-medical concern in Nigeria (Ramyil et al., 2014). According to Enabulele and Awunor (2016) the disease is caused by a causative agent that is co-endemic with other non-bacterial febrile diseases and viruses, making a clinical diagnosis difficult.

There is limited information on the incidence of invasive *Salmonella* disease in sub-Saharan African with standardized, multicounty data required for understanding the nature and burden of disease in the region. The lack of adequate diagnosis laboratory resources and inadequate infrastructure to support epidemiological and clinical research, have made typhoid fever less known in Africa when compare to Asia. Thus, in Nigeria particularly areas like Enugu state, timely and accurate diagnosis of infections remains a major challenge. Diagnosis, multidrug resistance and tracking of disease dissemination are major parts of the problem in the containment of the infections. Techniques including serotyping, plasmid profile analysis, and PCR with both molecular employed as well as cultural and non-cultural procedures.

RAPD when compared to other molecular typing techniques, is faster and less technically demanding because it doesn't

require any previous knowledge of the target organism's DNA sequence, making it a versatile instrument with great power and applicability (Noha et al., 2011). Vital step in tracking the spread of infectious agents is the evaluation, through molecular procedures, of the clonal relationships existing among isolates obtained from different parts of an environment. RAPD has become a veritable genetic typing tool in this regard. The effectiveness of *Salmonella* epidemiological surveillance studies is connected with the typing procedures used to distinguish them. RAPD is a commonly applied technique for comparative genome analysis and genetic diversity assessment, especially in non-model species. Thus, this study's main focus is to (i) determine the effectiveness of RAPD molecular markers for DNA profiling of *Salmonella* isolates and (ii) evaluate the discriminatory potential of different RAPD primers in differentiating these isolates.

MATERIALS AND METHOD

Ethics Statement

The research ethical clearance was obtained from the Research Ethics Committee of Enugu State University Teaching Hospital (ESUTH), Parklane, Enugu, Nigeria. Institutional approval was also granted by the university authorities, and the research was conducted in full compliance with all relevant ethical guidelines and regulatory requirements as outlined by the committee. The reference number or code is ESUTHP/C-MAC/RA/034/VOL.3/220.

Bacteriological analysis and Characterization

Sixty *Salmonella* isolates were randomly collected from patients in Enugu State, Nigeria, comprising samples obtained from a university teaching hospital (n = 50) and a private hospital (n = 10). Bacteriological examination was performed on isolates obtained from patients with a clinical diagnosis of typhoid fever. The preparation of *Salmonella* Shigella Agar (SSA) followed the manufacturer's instructions. Using a sterile wire loop, stool samples from individuals who had been clinically diagnosed with typhoid fever were streaked onto agar plates that had already solidified and were incubated at 37 °C for 18–24 h using the inoculated plates. Colonies that showed presumptive morphological

characteristics were purified on nutrient agar slants and stored at 4 °C for subsequent experimental procedures. Isolates obtained on nutrient agar were characterized based on colonial morphology, cultural characteristics, Gramstain reaction, and biochemical tests (Indole test, Simmon citrate test, Sugar fermentation test, Catalase test, Oxidase test, Voges-Proskauer test and Methyl red test).

Preparation of MacFarland, Standardization of Test Bacteria and Antibiotic Susceptibility Test

MacFarland was prepared according to Sagar (2022) and used to standardize, by comparison, each bacterial inoculums (Cheesbrough, 2006). Antimicrobial susceptibility tests were conducted on *Salmonella* isolates using the Kirby-Bauer disk diffusion technique on Nutrient agar (Merck, Germany) with antibiotic disks in accordance with the requirements published by the Clinical Laboratory Standards Institute (CLSI 2015). The studied antibiotic were Amoxicillin (30 µg), Chloramphenicol (10 µg), Nitrofurantoin (100 µg), Gentamycin (10 µg), Ciprofloxacin (10 µg), Ofloxacin (10µg), Streptomycin (30 µg), Perfloxacin (10 µg), Ceftriazone (30 µg), and Meropenem (10 µg).

DNA Extraction, RAPD profiling and PCR Electrophoresis

DNA Extraction was done using the Quick-DNA TM Miniprep Plus Kit (Qiagen, Canada). A microcentrifuge tube was filled with the sample, Biofluid and Cell Buffer (Red), and Proteinase K at volumes of 200 µl, 200 µl and 20 µl respectively. After fully mixing it for ten to fifteen seconds, the tube was incubated at 55 °C for ten min. Subsequently, an equal volume of Genomic Binding Buffer was added to the lysate and vortexed again for 10–15 seconds before transferring the mixture onto a collection tube and was centrifuged for 1 min at > 12,000 x g. Then 400 µl of DNA Pre-Wash Buffer was added to the spin column and centrifuged for 1 min at > 12000 x g, after which the collecting tube was emptied. Next, 700 µl of g-DNA Wash Buffer was added to the spin column and centrifuge at > 12000 xg for 1 min. Finally, the Spin Column was moved into a clean micro centrifuge tube, and the matrix immediately received the addition of ≤50 µl⁴ DNA Elution Buffer. It was incubated for 5 min at normal temperature. The DNA was extracted and stored at –20 °C until use. The oligonucleotides were Inqaba Biotec West Africa Ltd.'s commercially produced products. RAPD-PCR typing was

performed using five 10-mer primers (Table 1). The DNA binding patterns was visualized under UV illumination using a transilluminator (Bio-Rad Laboratories, USA).

Data Analysis

The data collected from the antibiotic susceptibility testing was cleaned using Microsoft excel (Microsoft Corporation, 2013), thereafter the data were evaluated using IBM SPSS version 23. Descriptive statistics, including mean inhibition zone diameter, frequency, and percentage susceptibility profiles, were calculated and presented in tables and figures. Ten (10) strains of *Salmonella* were selected to undergo RAPD analysis. Selection was based on simple random sampling from confirmed isolates obtained across different sample sources with each isolate assigned a unique code. Inclusion criteria

were confirmed *Salmonella* identity, high-quality genomic DNA, and complete antibiotic susceptibility data; isolates not meeting these criteria were excluded. The RAPD-PCR profile were analyzed using Bio-Rad software (Bio-Rad Laboratories, 2020). Percentage antibiotic resistance profiles of the different *Salmonella* strains were calculated and visualized using graphical analysis in R software (R Core Team, 2022). Furthermore, hierarchical clustering analysis using Euclidean distance was used to perform the cluster analysis with R software (R Core Team, 2022). An homology cut-off was done with the R software and used to cluster the strains; strains exceeding this cut-off were deemed to be closely related and placed in the same cluster.

Table 1: Oligonucleotides used as primers in RAPD PCR

Name	Sequence (5-3)	Bases	GC(%)	Tm(°C)	Mol Wt (Da)	Reference
AP7	GTGGATGCGA	10	60	32.2	3188.39	Ji-Dong et al. (2006); Habtamu et al. (2011); Surendra et al. (2019); Thmer et al. (2021)
NSC I	AGGACCAGGG	10	70	38.0	3174.37	Habtamu et al. (2011); Surendra et al. (2019)
784	GCGGAAATAG	10	50	30.0	3181.41	Quintaes et al. (2002); Jegadeeshkumar et al. (2010); Smith et al. (2011)
P5	AACGCGCAAC	10	60	32.0	3086.34	Quintaes et al. (2002); Jegadeeshkumar et al. (2010); Thmer et al. (2021)
797	AGCGTCACTC	10	60	32.0	3086.3	Quintaes et al. (2002); Jegadeeshkumar et al. (2010); Smith et al. (2011)

Results

Morphological and biochemical characteristics

Table 2 displays the morphological characteristics of the colonies from each plate, including colour, shape, texture, elevation, Gram reaction, and probable organism. All of the isolates were Gram-negative rods. Samples A12, B12, C12, D12, C18, and E18 have the same colour, which is blue black, while the other samples have the same colour (pink). Samples A12 and B12 exhibited a wet, mucoid appearance, whereas the remaining samples showed a wet, smooth texture. Sugar fermentation patterns varied among the isolates,

with 70% producing both acid and gas (A.G) during lactose fermentation, whereas 30% produced gas only (G). In glucose fermentation, 80% of isolates produced acid and gas, while 20% produced only gas. Similarly, sucrose fermentation showed that 80% of isolates produced acid and gas, whereas 20% produced gas only. The cluster analysis of the biochemical analysis of the sample isolates (Figure 1) showed that it forms three major group with group A comprising of C12 and B18 isolates, group B (A18 and H12) and group C (B12, A12, D12, F18, C18 and E18) isolates.

Table 2: Phenotypic characterization of isolates based on colonial and morphological features

Sample Isolates	Colour	Texture/ Appearance	Elevation	Gram stain	Shape	Probable Organism
A12	Blue black	Wet/Mucoid	Raised	Gram –ve	Rod	<i>Salmonella typhi</i>
B12	Pink	Wet/Mucoid	Flat	Gram –ve	Rod	<i>Salmonella enteritidis</i>
C12	Blue black	Wet/Smooth	Slightly raised	Gram –ve	Rod	<i>Salmonella typhi</i>
D12	Blue black	Wet/Smooth	Slightly raised	Gram –ve	Rod	<i>Salmonella typhi</i>
H12	Pink	Wet/Smooth	Flat	Gram –ve	Rod	<i>Salmonella enteritidis</i>
A18	Pink	Wet/Smooth	Flat	Gram –ve	Rod	<i>Salmonella enteritidis</i>
B18	Blue black	Wet/Smooth	Slightly raised	Gram –ve	Rod	<i>Salmonella typhi</i>
C18	Blue black	Wet/Smooth	Slightly raised	Gram –ve	Rod	<i>Salmonella typhi</i>
E18	Blue black	Wet/Smooth	Slightly raised	Gram –ve	Rod	<i>Salmonella typhi</i>
F18	Pink	Wet/Smooth	Flat	Gram –ve	Rod	<i>Salmonella enteritidis</i>

Table 3 Biochemical characterization and fermentation profiles of bacterial isolates

Sample Isolate	Catalase	Oxidase	Citrate utilization	Indole	Methyl red	Voges-Proskauer	Sugar fermentation		
							Lactose	Glucose	Sucrose
A12	+	-	+	+	-	-	A.G	A.G	A.G
B12	+	-	+	+	-	-	G	A.G	A.G
C12	+	-	+	+	-	-	A.G	A.G	G
D12	+	-	+	+	-	-	A.G	A.G	A.G
H12	+	-	+	+	-	-	A.G	G	A.G
A18	+	-	+	+	-	-	G	G	A.G
B18	+	-	+	+	-	-	G	A.G	G
C18	+	-	+	+	-	-	A.G	A.G	A.G
E18	+	-	+	+	-	-	A.G	A.G	A.G
F18	+	-	+	+	-	-	A.G	A.G	A.G

Key: AG = Acid and gas; G = Gas; + = Positive; - = Negative

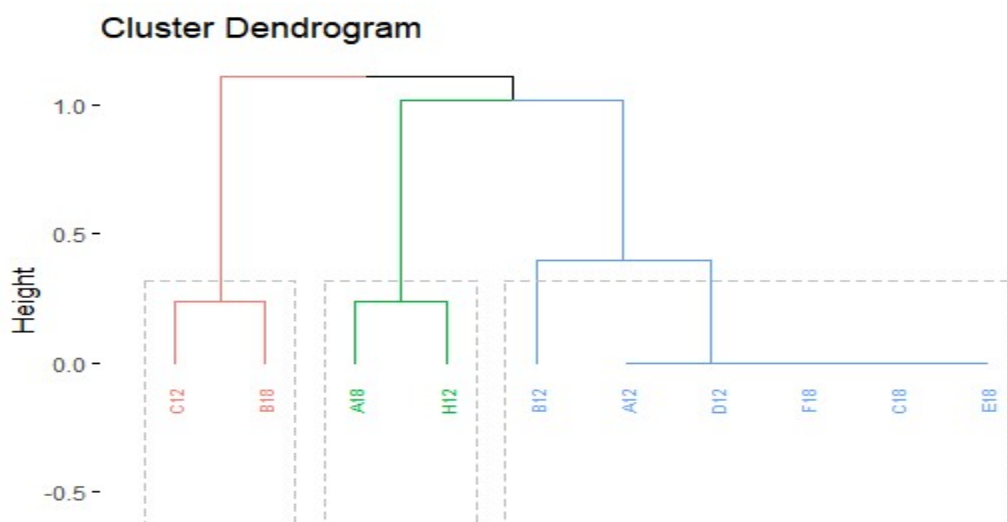


Figure 1: Cluster dendrogram showing the relationship among the isolates based on the biochemical analysis

Susceptibility and resistance of the bacterial isolates

Ten (10) antimicrobial discs were used to test the bacterial isolates' antibiotic susceptibility and resistance (Table 4). Some isolates showed zones of inhibition, while others didn't. Chloramphenicol, Meropenem, Ciprofloxacin, Ceftriaxone Pefloxacin and Nitrofurantoin, were all resistance to all bacterial isolates. Gentamycin was effective against sample isolates B12, C12, H12, E18, and F18, while Streptomycin worked against B12, C12, D12, A18, B18, and C18. Furthermore, only isolates

B12 were responsive to Ofloxacin. The cluster analysis (Figure 2) grouped the antibiotic drug into five groups (i.e A to E) with group C (Ciprofloxacin, Chloramphenicol, Pefloxacin, Meropenem, Ceftriaxone, and Nitrofurantoin) having most of the drug and showed 100% resistant these antibiotics. In contrast, Gentamycin (group D) and Streptomycin (group E) showed the highest rate or number of antibiotic susceptible when compared to other antibiotics.

Table 4: Antibiotic resistance and susceptibility patterns of bacterial isolates

ANTIBIOTICS	A12	B12	C12	D12	H12	A18	B18	C18	E18	F18
Gentamycin	R	S	S	R	S	R	S	R	S	S
Ciprofloxacin	R	R	R	R	R	R	R	R	R	R
Ofloxacin	R	S	R	R	R	R	R	R	R	R
Amoxicillin	R	R	R	R	R	R	I	R	R	R
Streptomycin	R	S	S	S	R	S	S	S	R	R

Chloramphenicol	R	R	R	R	R	R	R	R	R	R
Pefloxacin	R	R	R	R	R	R	R	R	R	R
Meropenem	R	R	R	R	R	R	R	R	R	R
Ceftriaxone	R	R	R	R	R	R	R	R	R	R
Nitrofurantoin	R	R	R	R	R	R	R	R	R	R

S = Susceptible, I = Intermediate, R = Resistant

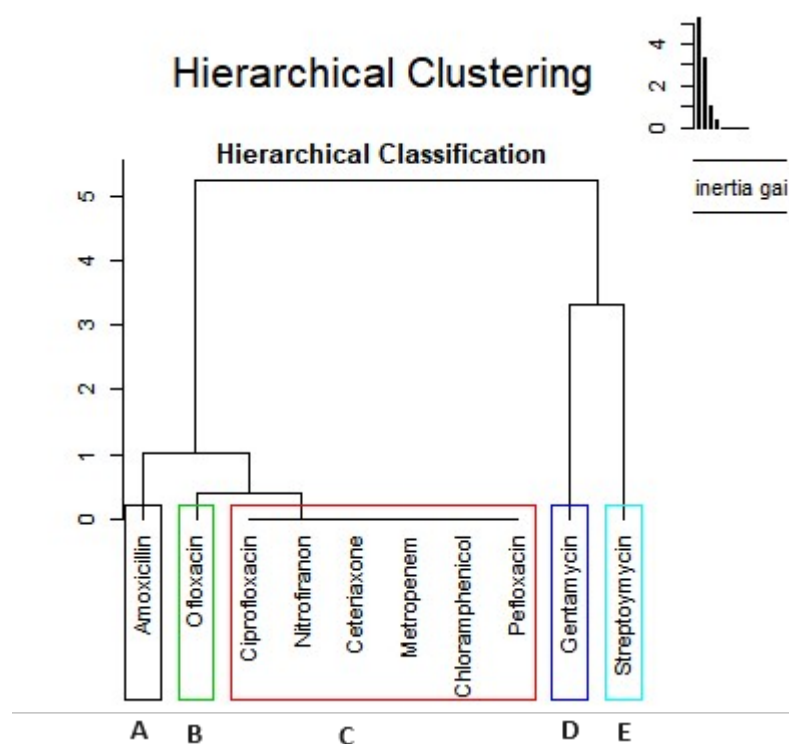


Figure 2: Hierarchical clustering of antibiotic resistance and susceptibility patterns of bacterial isolates showing distinct antibiotic groupings.

Effectiveness of the primers

The highest number of bands (17) was observed in strain B12, followed by strain C12 while the

least is strain E18 with 15 and 9 bands respectively (Table 5). The 797 primer produced the most polymorphic bands (34) among the ten salmonella strains, followed by the NSC I primer, and the least (18) with the P5 primer. The cluster analysis clearly delineated the primers into three groups (I, II and III) based on their discriminatory power (Figure 3). Group I (797 primer) showed the most discriminatory power while group III (P5 and 784 primer) and group II (AP7 and NSC I) had low discriminatory power. The image of the primer bands showing the isolates is shown in Figure 4.

Table 5: RAPD-PCR banding profiles of bacterial isolates generated using different primers

Primers	Sequence	Base pairs	GC %	Isolates										Total bands
				A12	B12	C12	D12	H12	A18	B18	C18	E18	F18	
NSC I	GCGGAAATAG	10	50	2	4	4	4	2	3	2	2	2	1	26
AP7	AACGCGCAAC	10	60	2	3	4	3	2	2	2	3	2	1	24
P5	AGCGTCACTC	10	60	2	3	1	0	1	3	2	1	3	2	18
784	GTGGATGCGA	10	60	2	2	2	1	3	2	3	2	2	3	22
797	AGGGCCCCGGG	10	90	4	5	4	3	3	2	2	1	4	6	34
Total				12	17	15	11	11	12	11	9	13	13	124

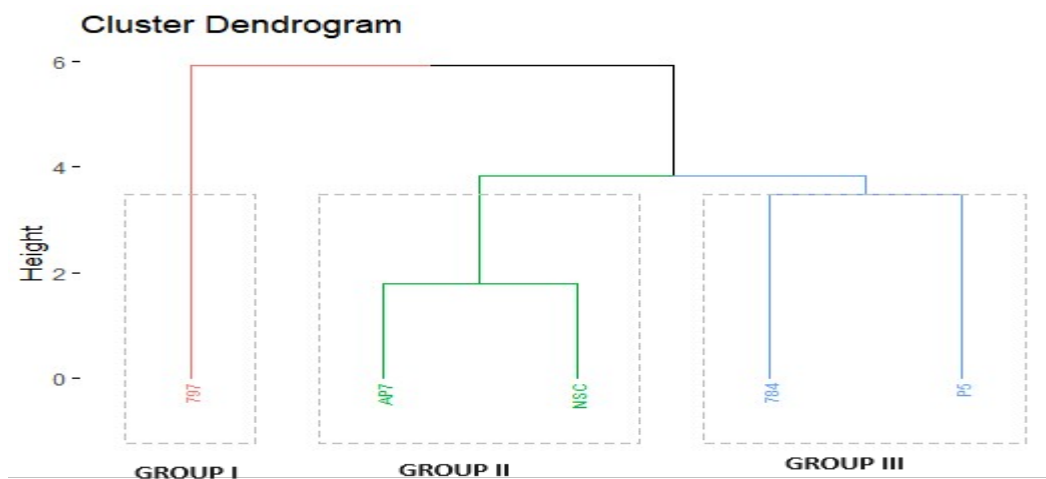


Figure 3: Cluster dendrogram showing the relationship among the studied primer discriminatory power

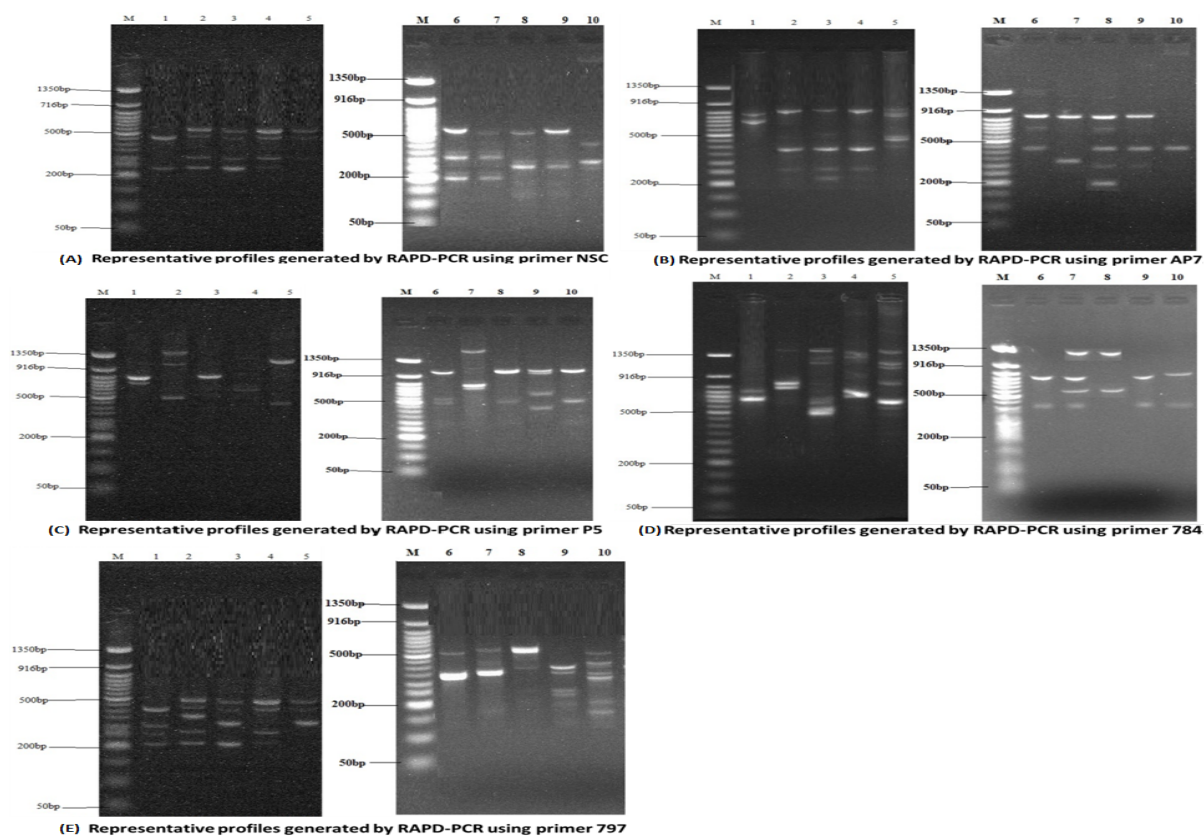


Figure 4: RAPD-PCR gel electrophoresis profiles of bacterial isolates using five primers studied.

Multiple antibiotic resistance index of different isolates

All strains analyzed through RAPD-PCR demonstrated resistance to multiple antibiotic classes, indicating multidrug-resistant phenotypes as shown in Table 6. Strain A12 was resistant to all ten (10) drugs and had the highest multiple antibiotic resistance index of 1.0. The

antibiotics with 100% resistant against the strains of salmonella used for RAPD-PCR amplification were Ciprofloxacin, Chloramphenicol, Pefloxacin, Metropenem, Ceftriaxone, and Nitrofurantoin. However, 60% of the strains were susceptible to Gentamycin and Streptomycin antibiotics (Figure 5).

Table 6: Antibiotics resistance pattern and multiple antibiotics resistance (MAR) index of different strains of Salmonella isolates

S/N	Strain	Resistance pattern	MAR index
1	A12	GN, CIP, OF, AX, ST, C, PF, MP, CT, N	1
2	B12	CIP, AX, C, PF, MP, CT, N	0.7
3	C12	CIP, OF, AX, C, PF, MP, CT, N	0.8
4	D12	GN, CIP, OF, AX, C, PF, MP, CT, N	0.9
5	H12	CIP, OF, AX, ST, C, PF, MP, CT, N	0.9
6	A18	GN, CIP, OF, AX, C, PF, MP, CT, N	0.9
7	B18	CIP, OF, C, PF, MP, CT, N	0.7
8	C18	GN, CIP, OF, AX, C, PF, MP, CT, N	0.9
9	E18	CIP, OF, AX, ST, C, PF, MP, CT, N	0.9
10	F18	CIP, OF, AX, ST, C, PF, MP, CT, N	0.9

GN= Gentamycin, CIP= Ciprofloxacin, OF= Ofloxacin, AX= Amoxicillin, ST= Streptomycin, C= Chloramphenicol, PF= Pefloxacin, MP= Metropenem, CT= Ceftriaxone, N= Nitrofurantoin.

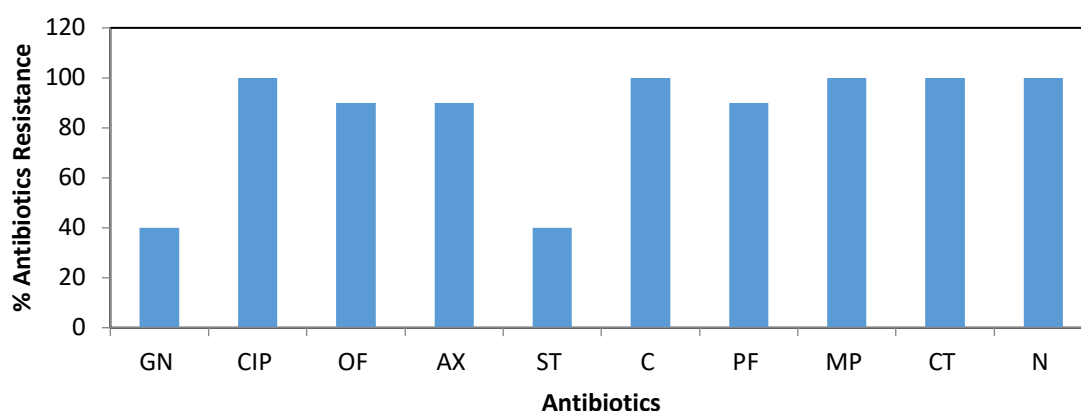


Figure 5: Distribution of antibiotic resistance patterns and multidrug-resistant phenotypes among Salmonella strains. (GN= Gentamycin, CIP= Ciprofloxacin, OF= Ofloxacin, AX= Amoxicillin, ST= Streptomycin, C= Chloramphenicol, PF= Pefloxacin, MP= Metropenem, CT= Ceftriaxone, N= Nitrofurantoin)

Discussion

Antibiotic resistance patterns and MAR indices

The high prevalence of *Salmonella* isolates underscores the endemic nature of salmonellosis in Enugu state, Nigeria. Among the studied antibiotics, Ciprofloxacin, Chloramphenicol, Pefloxacin, Meropenem, Ceftriaxone, and Nitrofurantoin showed 100% resistant to the isolates. The resistance rate among *Salmonella* isolates notably exceeded susceptibility rates, likely reflecting underlying genetic adaptations such as mutations in regulatory genes that control resistance mechanisms. This is consistent with Jegadeeshkumar et al. (2010), who reported that all isolates were resistant to Ciprofloxacin and Nitrofurantoin. The present findings contradict those of Martha et al. (2006), who reported 100% susceptibility to Ciprofloxacin, and Maharajan et al. (2021), who reported similar susceptibility to Chloramphenicol, and Amoxicillin implying that none of the isolates were multidrug-resistant. The rise of drug-resistant salmonella species poses new hurdles for typhoid fever prevention and treatment. In this investigation, the antibiotic bacterial isolates with the highest sensitivity were Gentamycin, Streptomycin, and Ofloxacin. However, typhoid fever continues to pose significant impact on global public health challenge in low and middle income countries. According to Mogasale et al. (2014), the ailment is mostly caused by poor hygiene and a lack of access to clean water. Typhoid fever spreads primarily due to poverty.

All the strain used for RAPD-PCR amplification and analyses were observed to have multiple antibiotics resistance capacities (Khoharo et al., 2010), with strain A12 showing resistance to all the ten (10) antibiotics used for this study, and having the highest multiple antibiotics resistance index of 1.0. Thus, all the strains used in this study exceeded the 0.2 MAR index threshold for microbes. This study's findings are in agreement with those of Poonia et al. (2014), who found that 36.2% of bacterial isolates had MAR values greater than 0.2. Gladys et al. (2025) reported that widespread self-

medication practices especially the purchase of antibiotics without prescriptions remain endemic in Nigeria, thereby worsening the burden of antimicrobial resistance (AMR). The major factor that contributes to antibiotic misuse and rising resistance is the inappropriate dispensing and sale of antibiotics without physician authorization, a practice common in many low income countries, like Nigeria (Akande-Sholabi et al., 2023; Oga-Omenka et al., 2023) and in poor resource areas like Enugu State. Many report have linked this phenomenon to factors such as patient pressure, inadequate enforcement of existing regulations, economic incentives, and the desire to retain customer loyalty (Belachew et al., 2021).

RAPD-PCR strain diversity

The RAPD test is considered advantageous since it can generate polymorphism and is simpler, less expensive, and faster when compared with other molecular typing method (Albufera et al., 2009; Bhowmick et al., 2012). This work established the RAPD fingerprinting approach, which uses short DNA primers to identify *Salmonella* genetic variability. All multidrug resistant isolates could be distinguished using RAPD, demonstrating the method's potential as an epidemiological and characterization study tool. Similar findings were made in previous research by Nath et al. (2010), Jegadeeshkumar et al. (2010), and Shabarinath et al. (2007). Yashwant et al. (2019) noted that using multiple sets of primers can improve RAPD's value for epidemiological study of *Salmonella* enterica serovar Typhi strains. The banding patterns are determined by the type of primer used, whereas PCR conditions influence the strength and quantity of RAPD products. Furthermore, the existence of identical sets of RAPD bands shared by two or more isolates for the same primer reveals that these isolates belong to the same strain. However, it is sufficient to disregard isolates that generate just one or two weak intensity bands, independent of primer. The present research used five primers to differentiate *Salmonella* sample isolates and discovered that all studied primers were capable of revealing

polymorphic amplification patterns across all isolates.

In this study, we describe how RAPD approach distinguished *Salmonella* strains. Thus, previous knowledge of the target organism's DNA sequence is not required for RAPD to function and this makes it very powerful and versatile tool. Only a few isolates and amplified fragments with band patterns ranging from 100 bp to over 1000 bp showed commonalities. These findings clearly demonstrate substantial genetic heterogeneity among the isolates. Figure 4 (A to E) shows the electrophoretic pattern of RAPD-PCR products. Strain identification was achieved through analysis of band resolution patterns produced by individual primers and RAPD fingerprinting generated distinct and interpretable banding profiles characteristic of each strain. Among the samples, Figure 4 (E) shows the highest number of bands using primer 797. This result is consistent with the findings of Jegadeeshkumar et al. (2010), who investigated the prevalence, and antibiotic resistance of *Salmonella* species in India and discovered that primer (5' AGCGTCACTC 3') gave good discriminatory power among various isolates. According to earlier reports of Chansiripornchai et al. (2000) and De-Cesare et al. (2001), RAPD has good discriminatory power for differentiating *Salmonella* strains. Additionally, some researchers have found that RAPD analysis is more effective at differentiating *Salmonella* strains than PFGE (Hudson et al., 2001; Muresu et al., 2001).

Implications for epidemiology and public health

The present study demonstrated that typhoid fever continues to pose a substantial public health challenge in low- and middle-income countries. The observed prevalence in Enugu State, Nigeria, underscores the persistent epidemiological burden of the disease and highlights the need for strengthened surveillance, improved sanitation, and targeted public health interventions to reduce transmission and associated morbidity. According to Mogasale et al. (2014), the ailment is mostly caused by poor hygiene and a lack of access to clean water. Typhoid fever spreads primarily

due to poverty. In Iran, a dependable discriminating method for separating *Salmonella* for epidemiological reasons was the combination of RAPD type and antibiotic susceptibility testing (Madadgar et al., 2008). The phylogenetic characterization of multidrug-resistant *Salmonella* strains through RAPD-PCR provides important epidemiological insights into their genetic relatedness, clonal clustering, and coexistence of both closely related and genetically diverse strains within the study area.

Conclusion

In conclusion, the high frequency of *Salmonella* isolates in the research region indicates that salmonellosis is endemic. Salmonellosis may be successfully diagnosed utilizing the pre-enrichment and enrichment of stool samples in the cultural approach. On the other hand, serotyping proved to be highly effective in distinguishing the *Salmonella* (in serotypes), and the fast PCR (RAPD) demonstration of high specificity and sensitivity in comparison to other techniques was remarkable and added to the body of knowledge regarding the diagnosis of salmonellosis. Some of the first-line medications were highly susceptible to *Salmonellae* in vitro, indicating that these medications are effective in treating salmonellosis. The pattern of antibiotic resistance and multiple antibiotic resistance to Ceftriaxone, Chloramphenicol, Metropenem, Ciprofloxacin, and Nitrofurantoin may be caused by gene mutations in regulators, drug misuse, self-administration of drugs, or incorrect administration of these over-the-counter medications. Therefore, it is also advised to set up surveillance systems to track antimicrobial resistance trends across the state and the entire nation. We conclude that RAPD PCR is an accurate and effective tool for the profiling of the strains of *Salmonella* species especially for epidemiological purposes.

Acknowledgement

Our special thanks goes to Enugu State University Teaching Hospital (ESUTH), Parklane, Enugu, Champion Diagnostic Centre Enugu, and Department of Biological Science, Godfrey Okoye

University, Enugu, Enugu State, Nigeria for their support.

Conflict of Interest

The authors declare no conflict of interest.

Consent

Written informed consent was obtained and consent of Enugu State University Teaching Hospital (ESUTH), Parklane Enugu, was obtained before starting data collection

References

Abuobeida, A. (1996) Typhoid and paratyphoid fever. *Afr. Health*; 18(5): 14-15

Akande-Sholabi, W., Oyesiji, E., and Adebisi, Y. A. (2023). Antimicrobial stewardship: Community pharmacists' antibiotic dispensing practices, knowledge, and perception regarding antibiotics and antibiotic resistance. *J. Pharm. Health Serv. Res.* 14(4), 383–391.

Albufera, U., Bhugaloo-Vial, P., Issack, M. and Jaufeerally-Fakim, Y. (2009). Molecular characterization of *Salmonella* isolates by REP-PCR and RAPD analysis. *Infect. Genet. Evol.* 9(3):322–327.

Aryal, S. (2022). Fermentation test – Principle, procedure, uses and interpretation. *Microbe Notes*.

Belachew, S. A., Hall, L., Erku, D. A., and Selvey, L. A. (2021). No prescription? No problem: Drivers of non-prescribed sale of antibiotics among community drug retail outlets in low and middle income countries: A systematic review of qualitative studies. *BMC Public Health*, 21(1), 1056.

Bhowmick, P. P., Srikumar, S., Devegowda, D., Shekar, M., Ruwandeepika, H. A. D. and Karunasagar, I. (2012). Serotyping and molecular characterization for study of genetic diversity among seafood associated nontyphoidal *Salmonella* serovars. *Indian Journal of Medical Research*; **135**(3):371–381.

Bio-Rad Laboratories. (2020). Image Lab Software (Version 6.1) [Computer software]. Bio-Rad Laboratories. <https://www.bio-rad.com/>

Chansiripornchai, N., Ramasoota, P., Bangtrakulnonth, A., Sasipreeyajan, J., and Svenson, S.B. (2000). Application of randomly amplified polymorphic DNA (RAPD) analysis for typing Avian *Salmonella enterica* subsp. *enterica*. *FEMS Immunol. Med. Microbiol*; 29, 221–225.

Cheah, Y-K., Salleh, N.A., Lee, L.H., Radu, S., Sukardi, S., and Sim, J.H. (2008). Comparison of PCR fingerprinting techniques for the discrimination of *Salmonella enterica* subsp. *enterica* serovar Weltevreden isolated from indigenous vegetables in Malaysia. *World J Microbiol. Biotechnol*; 24(3):327-35.

Cheesbrough, M. (2006). District Laboratory Practice in Tropical Countries. Part 2, 2nd Edition, Cambridge University Press Publication, South Africa; 1-434.

CLSI. (2015). Performance standards for antimicrobial susceptibility testing twenty fifth International Supplement. CLSI document M100-S25 Wayne, PA: *Clinical and Laboratory Standards Institute*.

Crump, J.A., Luby, S.P., and Mintz, E.D (2004). The global burden of typhoid fever. *Bull World Health Organ* 82: 346–53.

De-Cesare, A., Manfreda, G., Dambaugh, T. R., Guerzoni, M. E., and Franchini, A. (2001). Automated ribotyping and random amplified polymorphic DNA analysis for molecular typing of *Salmonella* Enteritidis and *Salmonella* Typhimurium strains isolated in Italy. *J. Appl. Microbiol*; 91:780-785.

Enabulele, O., and Awunor, S.N. (2016). Typhoid fever in a tertiary hospital in Nigeria: Another look at the Widal agglutination test as a preferred option in diagnosis. *Nig. Med. J.*; .57 (3):145-149

Faja, O. M., Mhyson, A. S., Atiyah, W. R., Mohammed, B. J., and Adnan, A. (2023). Molecular genotyping of *Salmonella* spp. isolated from cheese samples of local stores in Al-Diwaniyah city, *Open Vet. J.*; 13(10), 1277–1282.

- Gladys, O.U., Gabriella, C.N., Lawal, F.O., Bukola, F.O., Amaechi, V.C., and Augustine, O.N. (2025) Antibiotics stewardship: prevalence, nature, and factors associated with dispensing of antibiotics without prescription among community pharmacists in Nigeria. *J Pharm Policy Pract.*; 18(1):2498927.
- Habtmu, T.M., Rathore, R., Dhama, K., and Agarwal, R.K. (2011). Epidemiological characterization of *Salmonella* Gallinarum isolates of poultry origin in India, employing two PCR based typing methods of RAPD-PCR and PCR-RFLP. *Asian J. Anim. Vet.*; 6(11):1037–1051.
- Hudson, C. R., M., Garcia, R. K., Gast, and Maurer, J. J. (2001). Determination of close genetic relatedness of the major *Salmonella* Enteritidis phage types by pulse field gel electrophoresis and DNA sequence analysis of several *Salmonella* virulence genes. *Avian Dis*; 45:875–886.
- Jegadeeshkumar, D., Saritha, V., Moorthy, K. & Suresh, K. (2010). Prevalence, antibiotic resistance and RAPD analysis of food isolates of *Salmonella* species. *Int. J of Biological Tech.*; 1(3): 50–55.
- Ji-Dong, Jin., Dong-Seok, Lee., Eun-Kyung, Shin., Sun-Joong, Kim., and Tae-Wook, Hahn. (2006). Molecular typing by RAPD and detection of virulence genes of *Salmonella* enteric subspecies enterica serovar Gallinarum biovar Gallinarum. *Journal of Vet. Med. Sci.* 2006; 68 (12): 1321–1326.
- Kavaratty, R. M., Ajar, K. T., and Yashwant, K. (2019). Analysis of *Salmonella* enterica serovar Typhi by outer membrane protein (OMP) profiling, random amplification of polymorphic DNA (RAPD), and pulsed-field gel electrophoresis (PFGE). *Trop. Life Sci. Res.*; 30(1), 57–71.
- Koharo, H.K., Ansari, S., and Qureshi, F. (2010). Evaluating single acute phase Widal test for the diagnosis of typhoid fever. *Med Channel*; 16(1) 42–4.
- Madadgar, O., Tadjbakhsh, H., Salehi, T.Z., Mahzounieh, M., and Feizabadi, M. (2008). Evaluation of random amplified polymorphic DNA analysis and antibiotic susceptibility application in discrimination of *Salmonella* Typhimurium isolates in Iran. *New Microbiol.*; 31:211–216.
- Maharjan, A., Dhungel, B., Bastola, A., Shrestha, U.T., Adhikari, N., Banjara, M.R., Lekhak, B., Ghimire, P. and Rijal K.R. (2021). Antimicrobial Susceptibility Pattern of *Salmonella* spp isolated from Enteric Fever Patients in Nepal. *Infect Dis Rep*; 13(2): 388–400.
- Microsoft Corporation. (2013). Microsoft Excel [Computer software]. Microsoft. <https://www.microsoft.com/>
- Mogasale, V., Maskery, B., and Ochiai, R. L. (2014). Burden of typhoid fever in low-income and middle-income countries: A systematic literature-based update with risk-factor adjustment. *Lancet Glob. Health*, 2(10), e570–e580.
- Moro, D. D., Okiki, P. A., Grillo, J. A., and Raheem, T. Y. (2004). Concurrent infections of some pathogenic bacteria and parasites in patients diagnosed with gastroenteritis in Yaba, Lagos, Nigeria. *J. Res. Rev. Sci*, 3, 215–221.
- Moro, D. D., Salu, O. B., Oluduro, O. A., Famurewa, O., and Akinside, K. A. (2000). The prevalence of bacterial pathogens and parasitic intestinal worms among vendors in Ajegunle, Lagos. *J. Biol. Phys. Sci.* 1, 129–134.
- Muresu, E., Piana, A., Azara, A., Maida, I., Nastasi, A., Sajid, S. D., and Rubino, S. (2001). Clonal relations among *Salmonella* enteritidis phage type 3 outbreak isolates traced by DNA fingerprinting. *New Microbiologica*, 24, 371–377.
- Nath, G., Maurya, P., and Gulati, A. K. (2010). ERIC-PCR and RAPD-based fingerprinting of *Salmonella* Typhi strains isolated over a period of two decades. *Infect. Genet. Evol.*; 10(4), 530–536.
- Obaro, S. K., Iroh Tam, P. Y., and Mintz, E. D. (2017). The unrecognized burden of typhoid fever. *Expert Rev. Vaccines*, 16(3), 249–260.

- Oga-Omenka, C., Okafor, U., and Sulis, G. (2023). Engaging pharmacists and medicine vendors in antimicrobial stewardship in LMICs. *Lancet Infect. Dis.* 23(7), 786–787.
- Oliveira, M., Cardoso, A. R. R., Santos, L. R., Pilotto, F., Hamilton, L. S., de Moraes, C. T. P. S., Rocha, V. P. N., and Nascimento, S. L. da S. (2006). Antibiotic resistance in *Salmonella enteritidis* isolated from broiler carcasses. *Braz. J. Microbiol.* 37(3), 368–371.
- Paul, U. K., and Bandyopadhyay, A. (2017). Typhoid fever: A review. *Int. J. Adv. Med.* 4(2), 300–306
- Poonia, S., Singh, T. S., and Tsering, D. C. (2014). Antibiotic susceptibility profile of bacteria isolated from natural sources of water from rural areas of East Sikkim. *Indian J. Community Med.*, 39(3), 156–160.
- Quintaes, B. R., Leal, N. C., Reis, E. M. F., Fonseca, E. L., and Hofer, E. (2002). Conventional and molecular typing of *Salmonella* Typhi strains from Brazil. *Rev. Inst. Med. Trop. Sao Paulo* 44(6), 315–319.
- R Core Team. (2022). R: A language and environment for statistical computing [Computer software]. R Project for Statistical Computing
- Ramyil, S. M. Z., and Ogundeko, T. O. (2014). Use of stool culture as determinant parameters of enteric fever in adults attending Bingham University Teaching Hospital, Jos, Nigeria. *Clin. Med. Res.* 3(2), 31–35.
- Rezk, N. A., Mansour, H., Ghoneim, N. H., and Rifaat, M. M. (2011). Typing of *Salmonella* Typhi strains isolated from Egypt by RAPD-PCR. *3 Biotech*, 2, 17–25.
- Shabarinath, S., Sanath Kumar, H., Khushiramani, R., Karunasagar, I., and Karunasagar, I. (2007). Detection and characterization of *Salmonella* associated with tropical seafood. *Int. J. Food Microbiol.* 114, 227–233.
- Surendra, S., Abhishek, G., Bincy, J., Hitesh, K., and Nirmal, K. (2019). Random amplified polymorphic DNA-based molecular heterogeneity analysis of *Salmonella enterica* isolates from foods of animal origin. *J. Vet. World*, 12(1), 146–154.
- Thangaraj, M., Vishruth, P., Ramesh, T., and Lipton, A. (2011). RAPD fingerprinting and demonstration of genetic variation in three pathogens isolated from mangrove environment. *Asian J. Biotechnol.*, 3(3), 269–274.
- Thmer, O. H., and Inam, J. L. (2021). RAPD fingerprinting and genetic diversity of *Salmonella* spp. isolated from broiler and layer flocks in Karbala, Iraq. *Arch. Razi Inst.* 76(5), 1183–1190.
- Wasihun, A. G., Wlekidan, L. N., Gebremariam, S. A., Welderufael, A. L., Muthupandian, S., and Haile, T. D. (2015). Diagnosis and treatment of typhoid fever and associated prevailing drug resistance in northern Ethiopia. *Int. J. Infect. Dis.* 35, 96–102.
- World Health Organization. (2018). Typhoid vaccines: WHO position paper, March 2018 – Recommendations. *Vaccine*, 36, 214–216.