

Emergence of Multidrug-Resistant *Salmonella enterica* in Lagos: Insights from MALDI-TOF MS and β -Lactamase Profiling

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Abstract: Non-typhoidal *Salmonella* (NTS) is a major cause of foodborne illness and invasive infections, particularly in low-resource settings. This study aimed to identify and characterise *Salmonella enterica* isolates from human, animal, and environmental sources in Lagos, Nigeria, using Matrix-Assisted Laser Desorption Ionisation–Time of Flight Mass Spectrometry (MALDI-TOF MS), and to evaluate their antimicrobial resistance profiles and β -lactamase production. A total of 2,522 samples were collected and processed using culture-based methods, biochemical profiling (Microbact 24E), and MALDI-TOF MS. Antimicrobial susceptibility testing was performed via the Kirby–Bauer disc diffusion method. AmpC β -lactamase and extended-spectrum β -lactamase (ESBL) production were assessed using disc antagonism and double disc synergy tests. *Salmonella* was detected in 1.9% of samples, with the highest prevalence in environmental effluents (12%), followed by animal sources (4.2%) and human clinical samples (0.95%). Forty-eight isolates were recovered, predominantly *Salmonella* Newbrunswick, alongside Bredeney, Choleraesuis, Abortusequi, Montevideo, and untyped *Salmonella* spp. All isolates were resistant to ampicillin, cefoxitin, amikacin, gentamicin, tetracycline, and chloramphenicol. Imipenem showed the highest susceptibility (93.75%), while ciprofloxacin and nitrofurantoin had moderate activity. AmpC β -lactamase production was confirmed in 22.9% of isolates, mainly among Choleraesuis and Newbrunswick; no ESBL producers were detected. The emergence of multidrug-resistant *Salmonella* and AmpC producers across clinical and environmental settings highlights the need for enhanced surveillance, antimicrobial stewardship, and rapid diagnostic tools such as MALDI-TOF MS to guide effective treatment and containment strategies.

Key word: Antimicrobial resistance, AmpC β -lactamase, MALDI-TOF MS, Multidrug resistance (MDR), Non-typhoidal *Salmonella* (NTS).

INTRODUCTION

The genus *Salmonella*, a member of the family Enterobacteriaceae, remains one of the leading causes of foodborne illnesses worldwide (Akinyemi *et al.*, 2017; Lucca *et al.*, 2019). Non-typhoidal *Salmonella* (NTS) serovars are a major source of human infections, typically causing self-limiting gastroenteritis that resolves without antimicrobial therapy and carries a low fatality rate (Stanaway *et al.*, 2017; WHO, 2018). However, NTS can sometimes breach normally sterile body sites, leading to invasive non-typhoidal *Salmonella* (iNTS) disease, a condition associated with severe clinical outcomes. Factors such as malaria, HIV infection, and malnutrition have been identified as major predisposing conditions for iNTS infection (Crump *et al.*, 2015; Park *et al.*, 2016; Stanaway *et al.*, 2017). Clinical manifestations of iNTS are often non-

specific and may include fever, hepatosplenomegaly, and respiratory distress, which, if untreated, can be fatal (Uche *et al.*, 2017). Approximately 6% of children under five years, patients with diarrheal disease, and immunocompromised individuals develop bacteremia (Parry *et al.*, 2013). Globally, iNTS infections are estimated at 2.1–6.5 million cases annually, with nearly 25% of related deaths occurring in low- and middle-income countries. In sub-Saharan Africa, mortality is particularly high among children under three years, primarily due to severe anaemia, malnutrition, HIV, and malaria (Ao *et al.*, 2015). Accurate detection and identification of *Salmonella* are essential for epidemiological investigations. Although, culture-based methods remain the gold standard, but are labour-intensive, time-consuming, and require well-trained personnel. Moreover, these methods often

yield ambiguous results due to overlapping phenotypic traits among Enterobacteriaceae members such as *Citrobacter freundii* and *Proteus* spp. (Bell *et al.*, 2016; Xiong *et al.*, 2017; Pillar *et al.*, 2020). Serotyping remains a valuable tool, but demands expert interpretation of complex agglutination patterns and involves high costs and extended turnaround time (Diep *et al.*, 2019). Molecular and proteomic techniques have been developed to improve accuracy and turnaround time in *Salmonella* identification. These include pulsed-field gel electrophoresis (PFGE) (Santana *et al.*, 2020), multilocus sequence typing (MLST) (Ranjbar *et al.*, 2017), and whole-genome sequencing (WGS) (Pornsukarom *et al.*, 2018). Matrix-Assisted Laser Desorption Ionisation Time-of-Flight Mass Spectrometry (MALDI-TOF MS) has emerged as a rapid and high-throughput alternative, capable of identifying bacterial species and subspecies by analysing characteristic protein ionisation profiles (Kang *et al.*, 2017; Kim *et al.*, 2020; Mangmee *et al.*, 2020).

Effective management of *Salmonella* infections depends on timely diagnosis and the use of appropriate antimicrobial agents. Cephalosporins and fluoroquinolones are considered first-line therapies for salmonellosis. In Nigeria, treatment commonly involves chloramphenicol, third-generation cephalosporins (3GC), and fluoroquinolones. Unfortunately, the emergence of multidrug-resistant *Salmonella* strains has compromised treatment efficacy, prolonged hospital stays, and increased morbidity and mortality (WHO, 2021).

The AmpC β -lactamases, which belong to Ambler class C, are particularly concerning as they confer resistance to cephalosporins, oxyimino-cephamycins, monobactams, and penicillins (Sawa, 2020). These enzymes can be chromosomally encoded or plasmid-mediated and are widely distributed among members of the Enterobacteriaceae family. Rapid and cost-effective identification of the etiological agent, coupled with reliable antimicrobial susceptibility testing (AST),

remains crucial for the effective management of bacterial infections (Bari *et al.*, 2020). In Nigeria, the recent rise in diarrheal cases and pyrexia of unknown origin underscores the need for improved surveillance of *Salmonella* infections. The wide host range, genetic diversity, and antimicrobial resistance exhibited by non-typhoidal *Salmonella* make it a pathogen of significant epidemiological importance. This study employed MALDI-TOF mass spectrometry for the identification and characterisation of *Salmonella* subtypes from diverse sources. Additionally, it investigated the prevalence of antimicrobial-resistant *Salmonella* isolates and screened for inducible AmpC β -lactamase production to provide insights into resistance mechanisms circulating within the population.

MATERIALS AND METHODS

Study Design and Patient Population: This cross-sectional study was conducted between December 2018 and April 2019 among patients seeking medical care at three public health facilities in Lagos State, Nigeria: Lagos State University Teaching Hospital (LASUTH), Alimosho General Hospital, and Massey Street Children's Hospital. A total of 700 patients were recruited. Demographic and clinical data were collected using a structured questionnaire.

Inclusion Criteria: Participants were eligible for inclusion if they met at least two of the following criteria: fever persisting for 3–7 days ($\geq 37.9^\circ\text{C}$), diarrhoea within the preceding two weeks, or absence of an alternative confirmed diagnosis. There was no restriction on age or sex. Both hospitalised and outpatient cases were included.

Animal Population: Animal sampling was carried out concurrently at four major municipal abattoirs located along Governor Road and Oko-Afo in Badagry, Lagos. Between December 2017 and April 2019, a total of 150 cattle dung samples and 270 poultry dropping samples were collected.

Lagos State lies in southwestern Nigeria (latitude 6.5227° N, longitude 3.6218° E) at an elevation of approximately 41 m above sea level, with an estimated metropolitan population exceeding 21 million (Idris and Fagbenro, 2019).

Environmental Samples: A total of 100 hospital effluent samples were aseptically collected from surgical wards, pathology departments, and septic tanks of the selected hospitals. Sampling was conducted between 9:00 a.m. and 1:00 p.m. from December 2017 to April 2019.

Sample size: The sample size was estimated using the formula:

$$N = \frac{Z^2 p (1-p)}{d^2}$$

Where, N = sample size, Z = 1.96 (standard error) at a 95% confidence interval, p = The Prevalence of the disease as reported by Smith *et al.* (2012) = 74%

Case Definition - Human Cases: Blood (n = 1,042) and stool (n = 960) samples were obtained from patients presenting with one or more of the following symptoms: fever ($\geq 37.9^\circ\text{C}$) lasting three to seven days, diarrhoea, vomiting, abdominal pain, loss of appetite, or headache.

Animal Cases: A total of 150 cattle dung and 270 poultry stool samples were collected from animals exhibiting diarrhoea or bloody faeces.

Sample Collection and Bacterial Isolation from Blood: Venous blood samples were collected from study participants and analysed according to Akinyemi *et al.* (2021a).

Human Stool, Cattle Dung, and Poultry Faecal Sample: Approximately 2–4 g of each stool or dung sample was inoculated into 10 ml of buffered peptone water (BPW) for pre-enrichment and incubated at 37°C for 18–24 hours. One millilitre of the enriched suspension was then transferred into 9 ml of selenite F broth (HiMedia, Mumbai, India) for selective enrichment, followed by incubation at 37°C for 18–24 hours. The enriched culture was subcultured onto selective media, including xylose lysine deoxycholate (XLD) agar (HiMedia, India),

Bismuth sulfite agar, and *Salmonella–Shigella* agar (SSA) (Lab M, UK) and Rambach agar (Merck KGaA, Darmstadt, Germany). All plates were incubated at 37°C for 18–24 hours, and presumptive *Salmonella* colonies were selected for further characterisation described by Akinyemi *et al.* (2021).

Environmental Samples (Wastewater and Effluent): A total of 100 wastewater samples were analysed using a modified multiple-tube fermentation method (McCartney *et al.*, 1993). Briefly, 3 ml of each water sample was added to 27 ml of selenite F broth and incubated at 37°C for 18–24 hours. The enriched broth was subcultured onto xylose lysine deoxycholate (XLD) agar (HiMedia, India), Bismuth sulfite agar, and *Salmonella–Shigella* agar (SSA) (Lab M, UK) and Rambach agar (Merck KGaA, Darmstadt, Germany). All plates were incubated at 37°C for 18–24 hours, and presumptive *Salmonella* colonies were selected for further characterisation.

MICROBACT 24E Identification System: Presumptive *Salmonella* isolates were identified using the Microbact 24E Identification Kit (Oxoid, Basingstoke, UK), which contains dehydrated substrates for biochemical reactions. Results were analysed using the Microbact 2009 software package (v2.04, Windows), which automatically generates species identification profiles.

Protein Profiling by MALDI-TOF MS: All *Salmonella* isolates were further confirmed using Matrix-Assisted Laser Desorption Ionisation–Time of Flight Mass Spectrometry (MALDI–TOF MS) following the protocol of Bizzini and Greub (2010). Briefly, bacterial colonies from overnight cultures were suspended in 300 µl of bi-distilled water and mixed with 900 µl of 96% ethanol (Carl Roth GmbH, Karlsruhe, Germany) for protein precipitation. After centrifugation at 10,000 × g for 5 minutes, the pellet was resuspended in 50 µl of 70% formic acid (Sigma-Aldrich, Germany) and 50 µl of acetonitrile (Carl Roth GmbH). The mixture was centrifuged again, and 1.5 µl of

the supernatant was spotted on a polished steel target plate (MTP 384, Bruker Daltonik GmbH, Bremen, Germany) and air-dried. Each sample spot was overlaid with 2 µl of a saturated α -cyano-4-hydroxycinnamic acid (HCCA) matrix solution (Sigma-Aldrich) prepared in 50% acetonitrile and 2.5% trifluoroacetic acid and air-dried. Spectra were acquired using an Ultraflex II MALDI-TOF instrument (Bruker Daltonik GmbH) calibrated with the IVD Bacterial Test Standard. Data were analysed using Biotyper 3.1 software (Bruker), and identification scores were interpreted according to manufacturer guidelines: ≥ 2.3 for high-confidence species identification, 2.0–2.29 for probable species, 1.7–1.99 for probable genus, and ≤ 1.7 as unreliable (Lüthje *et al.*, 2017).

Disc Diffusion Method (DDM) for Antibiotic Susceptibility Testing (AST):

Antimicrobial susceptibility testing of *Salmonella* isolates was performed using the Kirby–Bauer disc diffusion method (DDM) following the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2019). Briefly, bacterial suspensions were prepared in sterile saline and adjusted to match the 0.5 McFarland standard (approximately 1×10^8 cfu/ml). A sterile cotton swab was used to inoculate the suspension uniformly onto the surface of Mueller–Hinton agar (MHA) plates. After allowing the surface to dry for 3–5 minutes, antibiotic discs (Oxoid, Basingstoke, UK) representing different classes of antimicrobials were aseptically placed on the agar surface using sterile forceps, ensuring adequate spacing to prevent overlapping of zones of inhibition. The plates were incubated aerobically at 37°C for 18–24 hours. Following incubation, the diameter of each zone of inhibition was measured in millimetres using a digital calliper, and the values were interpreted as susceptible, intermediate, or resistant based on CLSI breakpoints (CLSI, 2019).

Extended-Spectrum Beta-lactamase (ESBL) Detection (Double Disc Synergy Test): The production of extended-spectrum β -lactamases (ESBLs) among *Salmonella*

isolates was detected using the double disc synergy test (DDST), following the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2019). Briefly, bacterial isolates were inoculated onto Mueller–Hinton agar (MHA) plates to form a uniform lawn using a standardised 0.5 McFarland turbidity sterile cotton swab. Antibiotic discs containing third-generation cephalosporins cefotaxime (30 µg) and ceftazidime (30 µg) were placed 20 mm (centre to centre) from a disc containing amoxicillin–clavulanic acid (20/10 µg) (Oxoid, Basingstoke, UK). The plates were incubated aerobically at 37°C for 18–24 hours. After incubation, the plates were examined for enhancement of the zones of inhibition. Between any of the cephalosporin discs and the amoxicillin–clavulanic acid disc. The presence of a distinct keyhole or “champagne cork” shaped zone of inhibition toward the clavulanate disc was interpreted as a positive result for ESBL production. *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as ESBL-negative and ESBL-positive control strains, respectively.

Ampicillin Class C β -lactamase (AmpC) Production (Disk Antagonism Test):

The AmpC β -lactamase production among *Salmonella* isolates was detected using the disk antagonism test in accordance with CLSI guidelines (CLSI, 2019). Briefly, bacterial isolates were inoculated onto Mueller–Hinton agar (MHA) plates to obtain a uniform lawn culture standardised to 0.5 McFarland turbidity. A cefoxitin (30 µg) disc was placed at the centre of the agar plate, while cefotaxime (30 µg) and ceftazidime (30 µg) discs were positioned 20 mm (centre to centre) from the cefoxitin disc. The plates were incubated aerobically at 37°C for 18–24 hours. After incubation, AmpC β -lactamase production was indicated by a blunting or flattening of the zones of inhibition of cephalosporin discs adjacent to the cefoxitin disc, suggesting enzyme-mediated inactivation. *Escherichia coli* ATCC 25922 and *Enterobacter cloacae*

ATCC 13047 were used as negative and positive control strains, respectively.

Ethical Approval: Ethics approval from the ethics committee of the following institution was obtained before patients' enrolment: Human Research and Ethics Committee of the Lagos State University Teaching Hospital with reference number LREC/06/10/1012 and the Lagos State Health Service Commission with reference number LSHSC/2222/VOL.VC/352.

Consent of Participants: Informed consent was obtained from all individual participants included in the study. Written informed consent was obtained from the parents of underage children. Permission was obtained from farm managers before samples from animals were collected. Environmental samples used in this study do not require ethical approval.

RESULTS

A total of 2,522 samples were collected from human, animal, and environmental sources to assess the prevalence of *Salmonella*. The overall detection rate was 1.90%, with environmental samples showing the highest prevalence (12.00%), followed by animal samples (4.70%) in cattle dung and 3.7% in poultry droppings, and human samples (1.20%) in blood and 0.73% in stool (Table

1). Forty-eight *Salmonella enterica* isolates were recovered and further characterised. Among clinical isolates, *Salmonella* Newbrunswick and *Salmonella* spp. were predominant. Animal sources yielded a broader diversity, including Newbrunswick, Bredeney, Choleraesuis, Abortusequi, and *Salmonella* spp, while environmental isolates were mostly Newbrunswick and *Salmonella* spp. (Figure 1). Antibiotic susceptibility testing revealed high levels of resistance across multiple drug classes. All isolates were resistant to ampicillin, cefoxitin, amikacin, gentamicin, tetracycline, and chloramphenicol. Imipenem showed the highest efficacy, with 93.75% of isolates susceptible. High susceptibility was observed with ciprofloxacin (72.90%) and nitrofurantoin (50.00%), while other fluoroquinolones and β -lactam antibiotics exhibited substantial resistance (Table 2). The AmpC- β -lactamase production was detected in 22.90% of isolates, primarily among *Salmonella* Choleraesuis (100.00%), Newbrunswick (28.60%), and Bredeney (25.00%). No extended-spectrum β -lactamase (ESBL) production was observed in Table 3. The AmpC production was confirmed by disc antagonism testing, as illustrated in Plate 1.

Table 1: Prevalence of *Salmonella* in Human, Animal, and Environmental Samples

Source	Sample Type	Number of Samples	<i>Salmonella</i> detected	prevalence
Human	Blood	1042	12	1.20%
	Stool	960	7	0.73%
Animal	Cattle dung	150	7	4.70%
	Poultry dropping	270	10	3.70%
Environmental	Effluent	100	12	12.00%
	Total	2522	48	1.90%

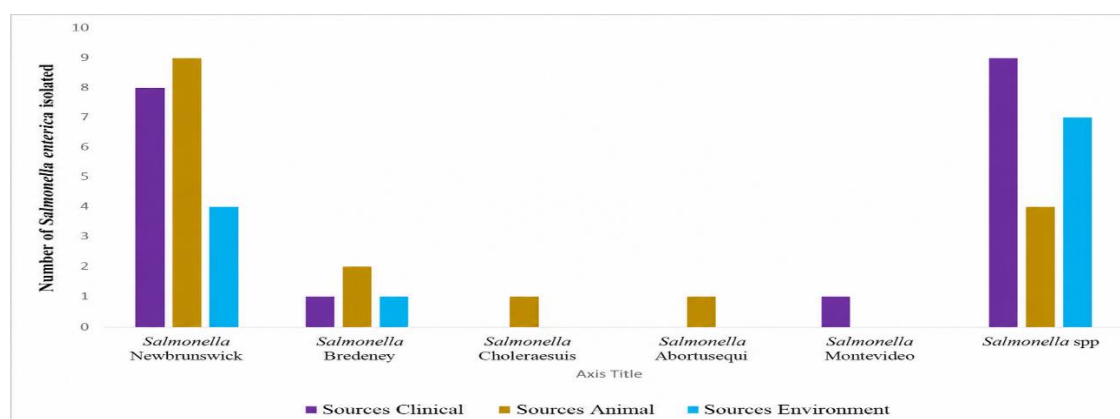


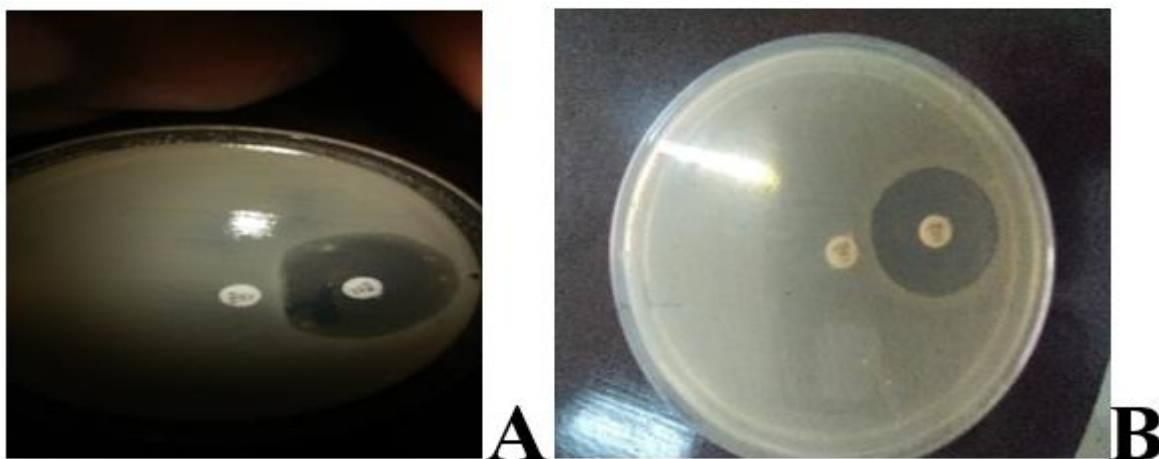
Figure 1: Identified Serovars of *Salmonella enterica* subspp *enterica*

Table 2: Antibiotic Susceptibility Profile of *Salmonella* Isolates

Antibiotic class	Antibiotics	Disc diffusion method (N-48)	
		Susceptible %	Resistant %
Penicillin	Ampicillin	0 (0.00)	48 (100.00)
	Amoxicillin+ Clavulanate	13 (27.00)	35 (72.90)
	Ceftriaxone	15 (31.30)	33 (68.70)
	Ceftriaxone-sulbactam	10 (21.00)	38 (79.00)
	Cefuroxime-Acetil	12 (25.00)	36 (75.00)
	Ceftazidime,	5 (10.40)	43 (89.60)
	Cefoxitin	0 (0.00)	48(100.00)
	Imipenem	45 (93.75)	3(6.25.00)
Aminoglycosides*	Amikacin *	0 (0.00)	48 (100.00)
	Gentamicin*	0 (0.00)	48 (100.00)
Quinolone /Fluoroquinolone	Ciprofloxacin	35 (72.90)	13 (27.10)
	Ofloxacin	3 (6.25)	45(93.75)
	Levofloxacin	10 (21.00)	38(79.00)
	Nalidixic acid	23 (48.00)	25(52.00)
Macrolide	Azithromycin	10 (21.00)	38(79.00)
Tetracycline	Tetracycline	0 (0.00)	48(100.00)
Phenicol	Chloramphenicol	0 (0.00)	48(100.00)
Nitrofurantoin	Nitrofurantoin	24 (50.00)	24(50.00)
Sulphanamide	Trimethoprim-Sulfamethoxazole	15 (31.30)	33(68.70)

Table 3: AmpC- β -lactamase Production among *Salmonella enterica* subsp *enterica* Isolated from Different Sources in Lagos

Number of Serovars screened	No of isolates positive for AmpC- β -lactamase production (%)	No of isolates positive for ES β L Production (%)
	Disc antagonistic test	Double disc synergy test
N-48		
<i>Salmonella</i> Newbrunswick (n-21)	6 (28.60)	0 (0.00)
<i>Salmonella</i> Bredeney (n-4)	1 (25.00)	0 (0.00)
<i>Salmonella</i> Choleraesuis (n-1)	1 (100.00)	0 (0.00)
<i>Salmonella</i> Abortusequi (n-1)	0 (0.00)	0 (0.00)
<i>Salmonella</i> Montevideo (n-1)	0 (0.00)	0 (0.00)
<i>Salmonella</i> spp (n-20)	3 (15.00)	0 (0.00)
Total (48)	11 (22.90)	0 (0.00)

**Plate 1: *Salmonella* spp Showing D shape AmpC Production with Disc Antagonism Test (A). Negative Result (B)**

DISCUSSION

Treatment failure and prolonged hospitalisation remain a continued public health issue within the high-burden countries, especially among patients with *Salmonella* bloodstream infections and severe gastroenteritis (Butler-Laporte *et al.*, 2018; Akinyemi *et al.*, 2021). The detection of *Salmonella* in 1.90% of the total samples analysed highlights its persistent presence across human, animal, and environmental reservoirs. Although, the overall prevalence appears low, the disproportionately high detection rate in environmental samples (12.00%) suggests that effluent and waste systems may serve as critical reservoirs and transmission pathways. This aligns with findings from Talukder *et al.* (2023), who reported pooled prevalence rates of up to 14.47% in South Asia, with environmental sources contributing significantly to the burden. The observed 12.00% prevalence from wastewater in this study is at variance with the 99.00% prevalence reported in Japan (Yanagimoto *et al.*, 2020), 53.00% in Spain (Santiago *et al.*, 2018), and 27.70% in Nigeria (Akinyem *et al.*, 2018).

In Nigeria, the prevalence of *Salmonella* spp. from animal sources varies significantly by region, as Bawa *et al.* (2020) reported a 1.50% prevalence in Abuja, Nigeria. This study found a higher rate of 4.00%, and an even greater prevalence of 39.00% was documented in Maiduguri by Jajere *et al.* (2015). The current study recorded a 1.20% prevalence of invasive non-typhoidal *Salmonella* (iNTS) and 0.70% for *Salmonella*-associated gastroenteritis. These findings are notably lower than previous reports of 20.00% in 2018 and 3.90% in 2021 for *Salmonella*-associated bacteraemia in Nigeria (Akinyemi *et al.*, 2018; Akinyemi *et al.*, 2021). Comparatively, the prevalence observed in this study is also lower than the 26.10% reported in Kenya and 32.00% in Bangladesh. The high burden of iNTS in African adults has been strongly linked to HIV infection, with coinfection rates exceeding 95.00%. The predominance of *Salmonella* Newbrunswick across all sample

types, especially in clinical and animal isolates, highlights its potential zoonotic relevance and adaptability. However, serovars like Enteritidis and Typhimurium are more commonly reported globally (Nazir *et al.*, 2025), the emergence of Newbrunswick and Bredeney in this study suggests regional variation in serovar distribution, possibly influenced by local ecological and agricultural practices.

Antibiotic resistance profiles revealed alarming levels of multidrug resistance (MDR), with 100.00% resistance to commonly used antibiotics such as ampicillin, cefoxitin, amikacin, gentamicin, tetracycline, and chloramphenicol. These findings mirror global trends, where MDR *Salmonella* strains are increasingly reported due to indiscriminate antibiotic use in both human medicine and livestock production (Lamichhane *et al.*, 2024; Nazir *et al.*, 2025). The high susceptibility to imipenem (93.75%) offers a therapeutic alternative, but its cost and limited availability in low-resource settings may hinder widespread use. The detection of AmpC- β -lactamase production in 22.90% of isolates, particularly among *Choleraesuis* and Newbrunswick, further complicates treatment options. The absence of ES β L producers is noteworthy, although it may reflect limitations in detection sensitivity or regional strain dynamics. The presence of AmpC enzymes contributes to resistance against cephalosporins and underscores the need for molecular surveillance to track resistance mechanisms.

CONCLUSION

This study highlights the continued public health threat posed by *Salmonella*, particularly in environmental reservoirs and animal sources. The dominance of *Salmonella* Newbrunswick and widespread multidrug resistance, including AmpC- β -lactamase production, emphasises the urgent need for integrated surveillance and control strategies. Prudent antibiotic use, improved sanitation, and targeted vaccination programs are essential to curb the spread of resistant strains. The study relied on

phenotypic methods for resistance detection; molecular techniques could provide deeper insights into resistance genes and transmission dynamics. Sampling was cross-

sectional, limiting the ability to assess seasonal or longitudinal trends in *Salmonella* prevalence and resistance.

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