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Original Research Article

Antibiotic Susceptibility of *Salmonella* Species Isolated from Stool Samples of Patients Attending Ahmadu Bello University, Medical Centre, Zaria

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ABSTRACT
Salmonella infection remains a major global public health concern. The effective treatment of salmonellosis and other enteric infections has been severely compromised by the emergence of antibiotic-resistant *Salmonella* strains, largely due to decades of indiscriminate and inappropriate antibiotic use. This study was designed to isolate *Salmonella* species from stool samples of patients attending the Ahmadu Bello University Medical Centre, Zaria, and to evaluate their antibiotic susceptibility patterns. A total of 100 stool samples were collected and screened for *Salmonella*. Initial enrichment was carried out using Selenite F broth, followed by inoculation onto *Salmonella-Shigella* agar, and subsequent subculture onto nutrient agar slants for storage and pure culture preparation. The isolates were subjected to a series of biochemical tests, including motility, catalase, citrate utilization, sugar fermentation, and methyl red–Voges-Proskauer (MR-VP) tests. Out of the 100 samples analyzed, 17 (17.0%) tested positive for *Salmonella*. Antibiotic susceptibility testing revealed the highest sensitivity to ceftazidime (64.7%) and ceftriaxone (47.1%), while resistance was most pronounced against ciprofloxacin (76.5%), amoxicillin (70.6%), chloramphenicol (52.9%), and gentamicin (47.1%). Although no statistically significant associations were observed between infection rate and socio-demographic or risk factors, a higher prevalence was noted among male patients, children aged 4–12 years, individuals consuming unwashed raw vegetables, households with pets, persons exposed to livestock and their waste, those who washed hands infrequently, individuals not using hand sanitizers, and those relying on well water as their primary drinking source. Strengthening public awareness on safe food handling practices, improved hygiene, and environmental sanitation is essential in reducing the burden of *Salmonella* infection. In addition, routine antibiotic susceptibility surveillance should be prioritized to monitor resistance trends and guide effective treatment strategies.

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INTRODUCTION

Antimicrobial resistance (AMR) in *Salmonella enterica*—including typhoidal (S. Typhi/Paratyphi) and non-typhoidal serovars—remains a major public health concern across low- and middle-income countries, where enteric infections cause substantial outpatient visits and hospitalizations (Van Puyvelde *et al*., 2023; Wang *et al*., 2025). Recent global genomic syntheses show widening geographic hotspots of resistance—fluoroquinolone non-susceptibility in particular—with Nigeria listed among countries with high predicted AMR burdens in *Salmonella* (Wang *et al*., 2025). Within Nigeria, genomic surveillance and hospital-based studies confirm circulation of multidrug-resistant (MDR) lineages of typhoidal and non-typhoidal *Salmonella*, underscoring therapeutic challenges and the need for locally generated susceptibility data to guide empiric therapy (Ikhimiukor *et al*., 2022).

Nigeria launched a second National Action Plan on AMR to strengthen stewardship, surveillance, and laboratory capacity in 2024 (Nigeria CDC, 2024; WHO AFRO, 2024). Yet, gaps persist at facility level, especially for stool culture–based surveillance that reflects community-acquired gastroenteritis rather than invasive disease alone (Laboratory Epidemiology of Salmonella Infections in Nigeria, 2025). Emerging hospital studies from southern Nigeria report MDR *Salmonella* from patient stool, including resistance determinants such as *sul1/sul2* and reduced fluoroquinolone susceptibility—patterns that can compromise first-line typhoid and gastroenteritis management (Osose *et al*., 2025). Given ongoing antimicrobial availability without prescription and variable stewardship implementation, timely, site-specific antibiograms remain critical to optimize care and curb resistance spread (Van Puyvelde *et al*., 2023; Imade *et al*., 2024).

Ahmadu Bello University Medical Centre, Zaria, serves a large catchment in Northwestern Nigeria where enteric infections are common; however, recent stool-based susceptibility profiles for *Salmonella* from this setting are limited in the literature. This study, therefore, determines the antibiotic susceptibility of *Salmonella* species isolated from stool samples of patients attending Ahmadu Bello University Medical Centre, Zaria, using current CLSI standards, to generate actionable evidence for local empiric treatment guidelines and antimicrobial stewardship (CLSI, 2024).

MATERIALS AND METHODS

Study Area

This study was carried out at the Ahmadu Bello University Medical Centre (ABUMC), Zaria, Kaduna State, Nigeria. Kaduna State is located in Northwestern Nigeria between latitudes 10.52°N and 11.75°N, and longitudes 7.44°E and 8.50°E. Zaria, a major city in Kaduna State, lies approximately at latitude 11.08°N and longitude 7.71°E.

Study Population

The study population comprised patients of all ages attending ABUMC who presented with clinical symptoms of gastroenteritis, including diarrhea, fever, dysentery (with mucus or blood), and abdominal pain.

Sample Size Determination

A total of 100 stool samples were collected and analyzed. The sample size was determined based on convenience sampling.

Ethical Considerations

Ethical approval for the study was obtained from the Ethical Committee of Ahmadu Bello University (NHREC/10/12/2015; D-U-N-S NUMBER: 954524802). Informed consent was also obtained from all participating patients prior to sample collection.

Sample Collection

Fresh stool specimens were collected from patients into sterile wide-mouth universal sample bottles. The samples were immediately transported in ice packs to the Department of Microbiology Laboratory, Ahmadu Bello University, for analysis.

Media Preparation

Selenite F broth, Salmonella-Shigella (SS) agar, nutrient agar, and Mueller-Hinton agar were prepared according to the manufacturer's specifications.

Sample Analysis

Enrichment in Selenite F Broth

A loopful of each stool specimen was inoculated into 10 mL of Selenite F broth and incubated at 37 °C for 24 hours (Inabo *et al*., 2016).

Culture on Salmonella-Shigella Agar

A loopful of the enriched broth culture was streaked onto Salmonella-Shigella agar plates and incubated at 37 °C for 24 hours. Presumptive *Salmonella* colonies appeared colorless with black centers due to hydrogen sulfide production (Inabo *et al*., 2016).

Subculture on Nutrient Agar Slants

Suspected colonies were subcultured onto nutrient agar slants for storage and pure culture isolation, followed by incubation at 37 °C for 24 hours (Inabo *et al*., 2016).

Gram Staining

After incubation, colonies were smeared on clean glass slides, air-dried, and heat-fixed. The smears were

sequentially stained with crystal violet (60 seconds), Gram's iodine (60 seconds), acetone (rinsed immediately), and safranin (counterstain). Slides were examined under an oil immersion lens (×100 objective). Gram-negative short rods were considered presumptive *Salmonella* isolates (Cheesbrough, 2010).

Biochemical Identification

Biochemical characterization of the isolates was performed using motility, catalase, citrate utilization, sugar fermentation, methyl red (MR), and Voges-Proskauer (VP) tests, following standard procedures (Cheesbrough, 2010).

Antibiotic Susceptibility Testing

The antibiotic susceptibility patterns of the isolates were determined using the Kirby-Bauer disc diffusion method. The antibiotics tested included: chloramphenicol (30 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), amoxicillin (30 µg), gentamicin (10 µg), and ceftriaxone (30 µg) (Cheesbrough, 2010).

Data Analysis

Patient data and laboratory findings were analyzed using Chi-square (χ^2) and Odds Ratio (OR) at a 95% confidence interval, with statistical significance set at $p \leq 0.05$. Data were processed using IBM SPSS version 20, and results were presented in tables and charts as appropriate.

RESULTS AND DISCUSSION

The characteristics of *Salmonella* species isolated from stool samples of patients at ABUMC, Zaria are presented in Table 1. Out of 100 stool samples analyzed, 17 tested positive for *Salmonella*, representing an overall incidence rate of 17% (Figure 1).

The socio-demographic distribution of *Salmonella* infections among patients is summarized in Table 2. The highest prevalence was observed among children aged 4–12 years (25.8%), followed by adolescents aged 13–21 years (14.6%), while the lowest infection rate (10.7%) was recorded among patients aged 22–30 years. However, the differences across age groups were not statistically significant ($p = 0.266$).

In terms of sex distribution, male patients showed a higher occurrence of infection (20.5%) compared to females (14.8%), though the difference was not statistically significant ($p = 0.455$). Interestingly, females were found to have a relatively higher risk of infection with an odds ratio (OR) of 1.491 (Table 2).

Educational status also appeared to influence infection rates. Patients with only primary education recorded the highest prevalence (28.6%), followed by those with secondary education (19.4%), while the lowest infection rate was among patients with tertiary education (9.3%). Nonetheless, these differences were not statistically significant ($p = 0.139$).

Table 1: Cultural, Microscopic and Biochemical Characteristics of *Salmonella* Species Isolates from Stool Samples of Patients at ABUMC, Zaria

Culture on SSA	Microscopy	Citrate utilization	MR	VP	M	C	TSI	Inference
Colourless colonies with black centers	Gram negative bacilli	+	+	-	+	+	K/A: H_2S+G	*Salmonella* species

Keys: MR = Methyl red, VP = Voges-Proskauer, K=Alkaline, A = Acid, M = Motility, C = Catalase, TSI = Triple Sugar Iron, H_2S = Hydrogen sulfide, G = Gas production, SSA= Salmonlella-Shigella agar

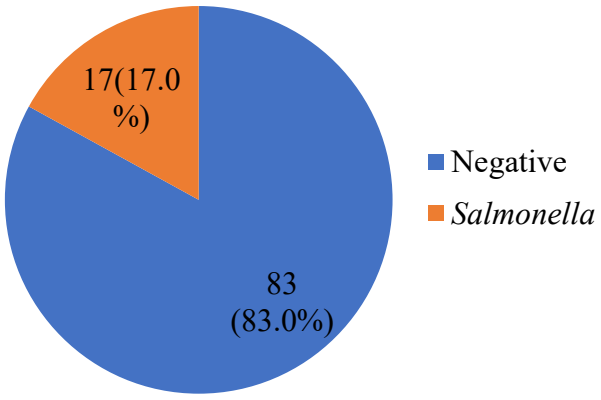

Figure 1: Overall Incidence of *Salmonella* Species from Stool Samples of Patients at ABUMC, Zaria

Table 2: Socio-Demographic Distribution of *Salmonella* Species Infections among the Patients

Socio-demographic factor	No. examined	No positive (%)	χ^2	df	*P-value*	OR
Age (years)						
4-12	31	8(25.8)	2.651	2	0.266	N.A
13-21	41	6(14.6)				
22-30	28	3(10.7)				
Gender						
Female	61	9(14.8)	0.559	1	0.455	1.491
Male	39	8(20.5)				0.671
Level of education						
Primary	21	6(28.6)	3.951	2	0.139	N.A
Secondary	36	7(19.4)				
Tertiary	43	4(9.3)				

N.A = Not applicable, dfv=v degree of freedom, OR = odds ratio

The highest occurrence of *Salmonella* infection (42.9%) was observed among patients who experienced diarrhea three times within 24 hours, compared to 17.1% among those without diarrhea. However, this difference was not statistically significant ($p = 0.234$) (Table 3).

Analysis of stool characteristics revealed that patients with mucus in their stool had the highest prevalence of infection (23.8%), followed by those with undigested food particles (21.1%). Blood-stained stools recorded 9.1%, while the lowest occurrence was seen in patients with normal (formed) stools (5.9%). These variations were not statistically significant ($p = 0.254$) (Table 3).

Patients presenting with fever (18.2%), vomiting (19.4%), and abdominal pain (19.2%) also showed higher rates of *Salmonella* infection compared to those without these symptoms. Nonetheless, none of these clinical features were statistically associated with infection ($p > 0.05$; OR < 1) (Table 3).

Potential risk factors for *Salmonella* infection are presented in Tables 4a and 4b. Higher prevalence was recorded among patients with a history of recent travel (19.4%), those who consumed raw vegetables (20.3%), individuals who did not wash vegetables before consumption (27.3%), and patients exposed to livestock or their waste (21.7%), though these associations were not statistically significant ($p > 0.05$). Hand hygiene appeared to influence infection rates: patients who washed their hands several times daily had the lowest prevalence (10.4%), compared to those who washed once daily (23.1%), a few times per week (27.3%), or rarely (20.0%). Similarly, the source of drinking water showed variations, with the highest infection recorded among patients using well water (20.0%), followed by tap water (18.2%) and rainwater (16.7%), while the lowest prevalence was among borehole users (7.7%). None of these distributions were statistically significant ($p > 0.05$).

Significant predictors of *Salmonella* infection included a history of diarrhea (OR = 1.932), lack of vegetable washing prior to consumption (OR = 2.284), presence of household pets ($p = 0.016$), and non-use of hand sanitizers ($p = 0.002$).

Table 3: Symptoms of *Salmonella* Species Infections among Patients at ABUMC, Zaria

Symptoms	No Examined	No positive (%)	χ^2	df	p-value	OR
Frequency of diarrhoea in 24 h						
None	82	14(17.1)	5.570	4	0.234	N.A
1	3	0(0.0)				
2	5	0(0.0)				
3	7	3(42.9)				
4	0	0(0.0)				
5	3	0(0.0)				
Constituent in Stool:						
Blood	22	2(9.1)	4.066	3	0.254	N.A
Mucus	42	10(23.8)				
Undigested food	19	4(21.1)				
Normal	17	1(5.9)				
Fever						
No	78	13(16.7)	0.028	1	0.867	1.111
Yes	22	4(18.2)				0.900

Symptoms	No Examined	No positive (%)	χ^2	df	p-value	OR
Vomiting						
No	64	10(15.6)	0.238	1	0.626	1.303
Yes	36	7(19.4)				0.767
Abdominal pain						
No	74	12(16.2)	0.124	1	0.725	1.230
Yes	26	5(19.2)				0.813

N.A = Not applicable, df = degree of freedom, OR = odds ratio

Table 4a: Risk Factors of *Salmonella* Species Infections among Patients at ABUMC, Zaria

Risk factor	n	No positive (%)	χ^2	df	*p-value*	OR
History of diarrhoea						
No	81	15(18.5)	0.697	1	0.404	0.518
Yes	19	2(10.5)				1.932
Recent travel history						
No	33	4(12.1)	0.831	1	0.362	1.745
Yes	67	13(19.4)				0.573
Consumption of raw vegetables						
No	41	5(12.2)	1.137	1	0.286	1.838
Yes	59	12(20.3)				0.544
Washing of vegetables						
No	22	6(27.3)	2.109	1	0.146	0.438
Yes	78	11(14.1)				2.284
Presence of pet at home						
No	44	3(6.8)	5.773	1	0.016	4.556
Yes	56	14(25.0)				0.220

n = Number examined, df = degree of freedom, OR = odds ratio

Table 4b: Risk Factors of *Salmonella* Species Infections among the Patients (Cont'd)

Risk factor	n	Number positive (%)	χ^2	df	p-value	OR
Exposure to livestock and their wastes						
No	77	12(15.6)	0.475	1	0.490	1.505
Yes	33	5(21.7)				0.665
Frequency of hand washing						
Many time a day	48	5(10.4)	3.073	3	0.380	N.A
Once a day	26	6(23.1)				
Few times a week	11	3(27.3)				
Rarely	15	3(20.0)				
Use of hand sanitizer						
No	69	17(24.6)	9.202	1	0.002	N.A
Yes	31	0(0.0)				
Source of drinking water						
Borehole	13	1(7.7)	0.960	3	0.811	N.A
Rain water	6	1(16.7)				
Tap	66	12(18.2)				
Well	15	3(20.0)				

n = Number examined, N.A = Not applicable, df = degree of freedom, OR = odds ratio

The antibiotic susceptibility patterns of *Salmonella* isolates from patient stool samples are presented in Table 5. Of the 17 isolates tested, the highest sensitivity was observed to ceftazidime (64.7%) and ceftriaxone (47.1%). In contrast, high levels of resistance were recorded against ciprofloxacin (76.5%), amoxicillin (70.6%), chloramphenicol (52.9%), and gentamicin (47.1%).

Table 5: Antibiotic Susceptibility Pattern of *Salmonella* Species Isolated from Stool Samples of Patients from ABUMC, Zaria

Antibiotic (µg) (n=17)	Number (%)		
	S	I	R
Gentamicin (10µg)	4(23.5)	5(29.4)	8(47.1)
Ceftazidime (30µg)	11(64.7)	1(5.9)	5(29.4)
Chloramphenicol (30µg)	4(23.5)	4(23.5)	9(52.9)
Amoxicillin (30µg)	3(17.6)	2(11.8)	12(70.6)
Ceftriaxone (30µg)	8(47.1)	3(17.6)	6(35.3)
Ciprofloxacin (5µg)	0(0.0)	4(23.5)	13(76.5)

Discussion

This study recorded an incidence of 17.0% for *Salmonella* species. The prevalence observed is lower than that reported in Lagos (26%) (Smith *et al*., 2014), Sokoto (58.9%) (Ameh *et al*., 2004), and Niger State (67.8%) (Adogo *et al*., 2015). Conversely, it is higher than the prevalence reported in Zaria (6.4%) (Anchau *et al*., 2016), Lagos (16.2%) (Akinyemi *et al*., 2004), and Kano (13.7%) (Abdullahi, 2010). These variations may be attributed to differences in study design, time periods, levels of hygiene, environmental conditions, and geographical factors.

The highest infection rate was observed among patients aged 4–12 years. This finding aligns with reports by Abdullahi *et al*. (2010) and Mengistu *et al*. (2014), who also noted a higher prevalence of infection in children. In contrast, Ohalete *et al*. (2011) documented higher infection rates among middle-aged individuals. The increased susceptibility of children may be linked to their underdeveloped immune systems, which make them more vulnerable, as only a few *Salmonella* cells are required to initiate infection. The low infective dose of the bacilli thus places children at greater risk (Gallies, 2007).

Male patients showed a higher infection rate than females, a finding consistent with Ohalete *et al*. (2011) and Ifeanyi *et al*. (2013). However, Mengistu *et al*. (2014) reported the opposite trend, with higher prevalence in females. Such gender differences in *Salmonella* infection may be explained by variations in hygiene practices, occupational exposure, awareness levels, and behavioral factors (Anchau *et al*., 2016).

The antibiotic susceptibility patterns revealed that the isolates showed the greatest susceptibility to ceftazidime (64.7%) and ceftriaxone (47.1%), while high resistance was observed against ciprofloxacin (76.5%), amoxicillin (70.6%), chloramphenicol (52.9%), and gentamicin (47.1%). These differences in susceptibility may be attributed to the inherent pharmacological properties of the antibiotics and the resistance mechanisms developed by the bacteria. Ceftazidime and ceftriaxone are broad-spectrum cephalosporins with strong activity against Gram-negative organisms and relative resistance to enzymatic degradation, which may explain their higher effectiveness. In contrast, resistance to amoxicillin, gentamicin, ciprofloxacin, and chloramphenicol is likely due to bacterial adaptations, including the production of inactivating enzymes and target-site mutations that reduce drug efficacy.

In terms of risk factors, patients who consumed raw or unwashed vegetables and those exposed to livestock and animal waste recorded higher infection rates. This can be explained by the high bacterial load typically found in raw vegetables and the natural colonization of *Salmonella* in animal intestines (Mengistu *et al*., 2014).

Hand hygiene was also associated with infection risk. Patients who washed their hands infrequently or did not use hand sanitizers exhibited higher prevalence of *Salmonella* infection compared to those who washed their hands multiple times daily or used sanitizers regularly. This supports the role of poor hygiene in facilitating transmission, as hands may harbor high bacterial loads (Van Immerseel *et al*., 2009).

Finally, the source of drinking water appeared to influence infection rates. Patients who relied on well water had the highest prevalence, followed by those consuming tap and rainwater, while the lowest prevalence was observed among borehole water users. This may be due to the ability of *Salmonella* to survive for extended periods in untreated well water, thereby increasing the risk of infection among its consumers (Lee *et al*., 2019).

CONCLUSION

Incidence of 17.0% was obtained for *Salmonella* species in patients attending ABUMC. Male and children belonging to age group of 4-12 years were observed to have the highest *Salmonella* infection with 20.5% and 25.8% respectively. The *Salmonella* isolates were susceptible to Ceftazidime (30µg) and Ceftriaxone (30µg) but resistance to Ciprofloxacin (5µg), Amoxicillin (30 µg), Chloramphenicol (30µg) and Gentamicin (10µg). In addition, antibiotic susceptibility test should be carried out regularly on *Salmonella* isolates to monitor development of resistance. Public health awareness on personal, food and environmental hygiene should be practiced to prevent or control *Salmonella* infections. Although there was no statistical difference between the infection rate and the sociodemographic and the risk factors determined.

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