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Antibiotic Resistant Patterns of *Salmonella* Serovars Isolated from Clinical Stool Samples in Nasarawa State, Nigeria

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Abstract

Salmonella infection is a major public health concern, especially in resource-limited countries like Nasarawa State, Nigeria, where drug-resistant strains are emerging. This study investigated the prevalence, antibiotic resistance patterns, and risk factors associated with Salmonella serotypes isolated from clinical stool samples. A total of 450 stool samples were cultured using standard microbiological techniques, and serotyping was performed. Eighteen Salmonella isolates were identified: *S. typhimurium* (33%), *S. enteritidis* (22%), *S. typhi* (11%), *S. heidelberg* (11%), and four others. Antibiotic susceptibility testing showed 100% resistance to Augmentin and erythromycin across all serovars. However, most isolates were susceptible to ciprofloxacin, gentamicin, and nitrofurantoin. Intermediate resistance to cotrimoxazole was observed in *S. typhimurium* and *S. heidelberg*. These findings highlight the need for continuous surveillance and informed antibiotic prescription to combat antimicrobial resistance in the State. The antibiogram data from this study offer crucial insights for improving empirical treatment strategies and guiding public health interventions.

Keywords: *Salmonella* serovars, antibiotic resistance, epidemiology, Nigeria

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Introduction

Salmonella species are Gram-negative, rod-shaped, facultative anaerobic bacteria belonging to the family Enterobacteriaceae. They are significant pathogens responsible for a broad spectrum of infections in humans and animals, ranging from self-limiting gastroenteritis to invasive systemic diseases such as typhoid fever and bacteremia [1]. Globally, *Salmonella* infections cause substantial morbidity and mortality, particularly in developing regions where inadequate sanitation, poor hygiene, and antimicrobial misuse contribute to widespread transmission and resistance development [2]. The burden of *Salmonella* infections is particularly high in tropical and subtropical regions, including sub-Saharan Africa. Each year, non-typhoidal *Salmonella* (NTS) infections result in millions of cases of diarrhoea, while typhoidal *Salmonella* (*S. enterica* ser. *Typhi and S. enterica* ser. *Paratyphi*) cause invasive infections leading to an estimated 135,000 deaths annually [3]. The World Health Organization (WHO) recognizes *Salmonella* infections as a major public health concern, necessitating ongoing surveillance, improved diagnostic capabilities, and effective antimicrobial stewardship to mitigate their impact [4].

The genus *Salmonella* comprises two primary species: *S. enterica* and *S. bongori*. While *S. bongoriis* mainly associated with cold-blooded animals and is less commonly implicated in human infections, *S. enterica* contains multiple serovars that are responsible for human diseases. These serovars can be broadly classified into typhoidal and non-typhoidal groups. Typhoidal *Salmonella* (*S. enterica* ser. Typhi and *S. enterica* ser. Paratyphi A, B, and C) are human-restricted pathogens that cause systemic infections characterized by prolonged fever, abdominal discomfort, and complications such as intestinal perforation [5]. Typhoid fever remains endemic in many developing countries due to contaminated water sources and inadequate sanitation infrastructure [6]. Non-typhoidal *Salmonella* (NTS), including *S. enterica* ser. *Typhimurium* and *S. enterica ser. Enteritidis* are zoonotic pathogens that cause gastroenteritis and, in some cases, invasive infections, particularly in immunocompromised individuals [7]. In sub-Saharan Africa, NTS bacteremia is a leading cause of mortality among children under five years and HIV-infected individuals [8]. The emergence of multidrug-resistant (MDR) *Salmonella* serovars further complicates treatment and disease management. The increasing prevalence of antimicrobial-resistant strains necessitates

comprehensive studies to determine serovar distribution, antibiotic resistance profiles, and epidemiological trends [9].

In Nigeria, *Salmonella* infections remain a significant health problem, with outbreaks frequently reported in different regions. Poor hygiene, consumption of contaminated food and water, and indiscriminate use of antibiotics contribute to the persistence of *Salmonella* infections in the population [10]. Several studies have documented the prevalence of *Salmonella* serovars in Nigeria, with *S. enterica* ser. *Typhimurium* and *S. enterica ser. Enteritidis* is the most commonly isolated strain [11]. Previous research has highlighted regional variations in *Salmonella* infections, with urban areas showing higher prevalence rates due to increased population density and environmental contamination [12]. However, rural areas are also at risk due to limited access to clean water and healthcare services. In Nasarawa State, little is known about the specific serovars circulating in the population and their antibiotic resistance patterns, highlighting the need for targeted epidemiological studies [13].

Antibiotic resistance among *Salmonella* isolates is an escalating global health challenge. The widespread use of antibiotics in human medicine, agriculture, and veterinary practices has led to the selection and dissemination of resistant strains, complicating treatment and increasing morbidity and mortality rates [14]. The WHO has classified fluoroquinolone-resistant and extended-spectrum beta-lactamase (ESBL)-producing *Salmonella* among the highest-priority pathogens requiring urgent intervention [15]. Resistance to commonly used antibiotics such as ampicillin, cotrimoxazole, and chloramphenicol has been widely documented in many parts of Africa, leading to increased reliance on third-generation cephalosporins and fluoroquinolones [16]. However, resistance to these last-resort antibiotics is also emerging, necessitating continuous monitoring and the development of alternative treatment strategies [17]. In Nigeria, studies have reported high rates of multidrug-resistant *Salmonella* strains, particularly among clinical isolates. Resistance to amoxicillin-clavulanic acid (Augmentin), erythromycin, and tetracyclines has been observed in several studies, raising concerns about treatment failure and the need for revised treatment guidelines [18]. The increasing resistance to fluoroquinolones and cephalosporins is particularly worrisome, as these antibiotics are often used for severe *Salmonella* infections, especially in immunocompromised patients [19]. Understanding the local resistance patterns is essential for guiding empirical therapy and improving patient outcomes.

Salmonella infections are primarily transmitted through the faecal-oral route via contaminated food, water, or direct contact with infected animals or humans. Several risk factors contribute to the spread of *Salmonella* in endemic regions, including inadequate hand washing, improper food handling, and consumption of unclean water contribute to the high burden of *Salmonella* infections in developing countries [20]. Undercooked poultry, eggs, and dairy products are common sources of *Salmonella* infections [21]. Street food vendors, where hygiene standards are often poor, also play a role in transmission.Livestock, especially poultry, cattle, and pigs, can serve as reservoirs for *Salmonella*, leading to zoonotic transmission [22]. Hospital outbreaks of *Salmonella* infections have been reported, often due to contaminated medical equipment or cross-infection between patients [23]. The inappropriate use of antibiotics in both human and veterinary medicine has led to the emergence of resistant *Salmonella* strains, making infections harder to treat [24].

Despite the significant public health burden of *Salmonella* infections in Nigeria, there is limited data on the distribution of serovars and their antibiotic resistance profiles in Nasarawa State. The lack of comprehensive surveillance and epidemiological data hampers effective disease control and treatment strategies. Given the increasing prevalence of antimicrobial-resistant *Salmonella* strains, there is a critical need to investigate the serovar diversity and resistance patterns in clinical isolates.

Materials and Methods

Study area

This study was conducted in Nasarawa State, Nigeria. The State is located in the North-Central geopolitical zone of the country. To ensure a representative sample, the study was carried out in three selected Local Government Areas (LGAs) across the three Senatorial Districts of the state. Samples were collected from General Hospital Doma, (Doma LGA), located in Nasarawa South Senatorial District, General Hospital Akwanga, (Akwanga LGA), located in Nasarawa North Senatorial District and General Hospital Garaku (Kokona LGA) located in Nasarawa West Senatorial District. These healthcare facilities were purposively selected based on patient inflow, diagnostic capacity, and geographical representation across Nasarawa State.

Ethical considerations

Ethical approval for the study was obtained from the Nasarawa State Hospital Management Board Ethics Committee, following guidelines for biomedical research involving human subjects [4]. Written informed consent was obtained from all participants or their legal guardians before sample collection. The study adhered to the ethical principles outlined in the Declaration of Helsinki, ensuring participant confidentiality, voluntary participation, and the right to withdraw at any stage without consequence [5].

Study design and population

A cross-sectional study design was employed to determine the prevalence, serovar diversity, and antibiotic resistance patterns of *Salmonella* isolates from clinical stool samples. The study population comprised patients presenting with gastrointestinal symptoms suggestive of salmonellosis, including diarrhoea, abdominal cramps, fever, nausea, and vomiting. Patients attending the selected health facilities during the study period and meeting the inclusion criteria were enrolled.

Inclusion criteria

- Patients of all age groups and genders presenting with symptoms of gastroenteritis.
- Patients who had not taken antibiotics within two weeks before sample collection.
- Patients or guardians who provided informed consent.

Exclusion criteria

- Patients without gastrointestinal symptoms.
- Patients who had received antibiotic treatment within two weeks before the study.
- Patients unwilling to provide informed consent.

Sample collection and processing

A total of 450 stool samples were collected from enrolled patients. Each patient provided a freshly passed stool sample in sterile, leak-proof, wide-mouthed containers. The collected samples were transported immediately to the microbiology laboratory under appropriate conditions using Cary-Blair transport medium to preserve bacterial viability [6].

In the laboratory, the stool samples were processed according to standard microbiological procedures. Macroscopic examination was performed to assess stool consistency, presence of blood, mucus, or unusual colouration. Each sample was then subjected to selective and differential culturing to isolate *Salmonella* species.

Isolation and identification of *Salmonella*

The isolation of *Salmonella* was performed using conventional cultural techniques in accordance with the WHO Global Foodborne Infections Network Laboratory Protocol [7]. The stool samples were first pre-enriched in buffered peptone water and incubated at 37°C for 18–24 h. Following enrichment, the samples were inoculated onto selective media, including *Salmonella-Shigella* (SS) Agar,which was used for isolating *Salmonella* and *Shigella* species. *Salmonella* colonies typically appeared colourless with black centres due to hydrogen sulfide (H_2S) production, in Xylose Lysine Deoxycholate (XLD) Agar.*Salmonella* colonies appeared as red colonies with black centres, distinguishing them from other enteric bacteria and Hektoen Enteric (HE) Agar was used for further differentiation based on lactose fermentation and H_2S production. Presumptive *Salmonella* colonies were subcultured onto nutrient agar for purity and further biochemical identification.

Biochemical characterization

Biochemical identification of *Salmonella* isolates was performed using standard biochemical tests [8] such as: Gram staining, Triple Sugar Iron (TSI) Test, Citrate Utilization, Indole Test, Oxidase, Catalase Test, Urease and Methyl Red and Voges-Proskauer (MR-VP). Further confirmation of *Salmonella* species was done using the API-20E biochemical identification system (bioMérieux, France), a standardized test strip system for enteric bacteria [9].

Serotyping of *Salmonella*

Serological identification of *Salmonella* isolates was carried out using the slide agglutination test based on the Kauffmann-White scheme, following established protocols [10]. The isolates were tested with commercial polyvalent and monovalent *Salmonella* antisera (Denka Seiken, Japan) to identify specific O (somatic) and H (flagellar) antigens. The identified serovars were classified into typhoidal and non-typhoidal *Salmonella* groups.

Antibiotic susceptibility testing

The antibiotic resistance profile of *Salmonella* isolates was determined using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar, following Clinical and Laboratory Standards Institute (CLSI) guidelines [11]. The following antibiotics were tested, based on their clinical relevance in treating *Salmonella* infections: Each isolate was classified as susceptible (S), intermediate (I), or resistant (R) based on the zone of inhibition measured in millimetres. Multidrug resistance (MDR) was defined as resistance to three or more classes of antibiotics [12].

Data analysis

Data were analyzed using SPSS version 25.0 (IBM, USA). Descriptive statistics, including frequency distributions and percentages, were used to summarize categorical variables. Chi-square (χ^2) tests were employed to determine associations between infection prevalence and demographic variables. The Kruskal-Wallis test was used to assess variations in antibiotic resistance across different age groups. A p-value <0.05 was considered statistically significant [13].

Results and Discussion

The result of the study is presented in Tables 1 – 5 and Figs 1 – 2. As shown in Table 1, out of the 450 stool samples analyzed, 18 (4.0%) tested positive for *Salmonella*. The highest prevalence (5.3%) was recorded in Doma, followed by Garaku (4.0%) and Akwanga (2.7%). The variations in prevalence across these locations could be linked to differences in sanitation practices, hygiene levels, water quality, and healthcare access. Doma, with the highest prevalence, may have greater exposure to risk factors such as contaminated food and water sources. The 4.0% prevalence observed in this study is relatively low compared to previous reports in other parts of Nigeria. For instance, a study in Lagos recorded a higher prevalence of *Salmonella* infections, potentially due to overcrowding, poor sanitation, and higher population density [9]. However, the findings align with studies conducted in some rural regions of Nigeria, where improved sanitation practices and healthcare interventions have contributed to a decline in prevalence [10]. Despite this, the detection of *Salmonella* serovars in clinical stool samples underscores the ongoing public health concern and the need for continuous surveillance.

Table 1: Prevalence of *Salmonella* infection in clinical stool samples across health facilities

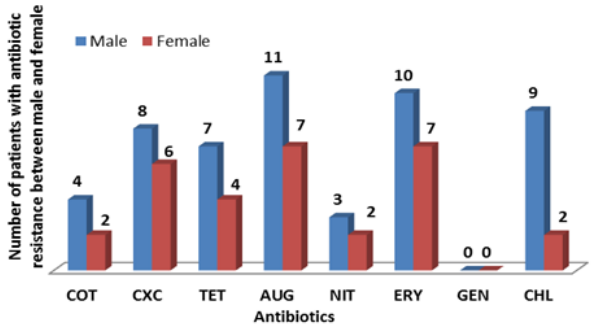
Health Facility	Sample Size	*Salmonella* Positive Cases n(%)	Prevalence (%)
Doma	150	8(44.4)	5.3
Akwanga	150	4(22.2)	2.7
Garaku	150	6(33.3)	4.0

$\chi^2_{(5)}$ (location and prevalence) = 1.89, P= 0.863(P>0.05)

Table 2: Prevalence of *Salmonella* serotypes in clinical stool samples

Salmonella species/serovars	Frequency of % Occurrence	Prevalence %
S. enterica ser. Agona	1 (5.56%)	0.24
S. enterica ser. Paratyphi B	1 (5.56%)	0.24
S. enterica ser. Heidelberg	2 (11.1%)	0.48
S. enterica ser. Typhi	2 (11.1%)	0.48
S. enterica ser. *Typhimurium*	6 (33.33%)	1.43
S. enterica ser. Enteritidis	4 (22.22%)	0.95
S. enteric ser. Huaian	1 (5.56%)	0.24
S. bongori	1 (5.56%)	0.24
Total	**18 (100%)**	**4.29**

$\chi^2_{(7)}$ (serovars and occurrence of infection) = 57.93, P= 0.000(P<0.05)



W (male and female) = 84.0, P=0.05 (P≤0.05)

Figure 1: Sex distribution of antibiotic resistance among *Salmonella* patients

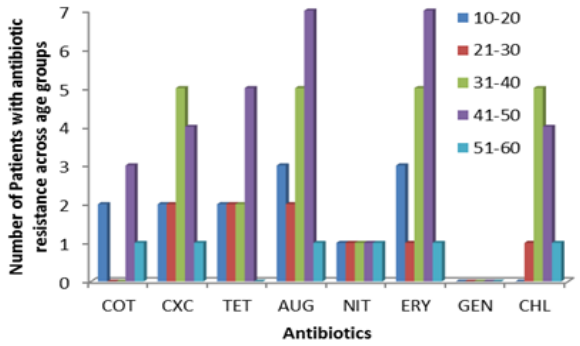
This study identified eight distinct *Salmonella* serovars, with *S. enterica* ser. *Typhimurium* (33%) and *S. enterica ser. Enteritidis* (22%) is the most prevalent (Table 2). Other detected serovars included *S. enterica* ser. Typhi (11%), *S. enterica* ser. Heidelberg (11%), *S. enterica* ser. Agona, *S. enterica* ser. Paratyphi B, *S. enterica* ser. *Huaian*, and *S. bongori*(6% each). The dominance of *S. enterica* ser. *Typhimurium* and *S. enterica ser. Enteritidis*aligns with global and regional studies, as these serovars are the most frequently implicated in non-typhoidal *Salmonella* (NTS) infections [11]. The detection of *S. enterica* ser. Typhi and *S. enterica* ser. Paratyphi B, which are responsible for typhoidal salmonellosis, indicates the presence of both typhoidal and non-typhoidal *Salmonella* infections in Nasarawa State. The presence of *S. bongori* is notable, as this species is more commonly associated with cold-blooded animals rather than human infections. The isolation of this serovar suggests potential zoonotic transmission or environmental contamination. Serovar diversity was higher among male patients, with all eight serovars detected in males, compared to only two (*S. enterica* ser. *Typhimurium* and *S. enterica ser. Enteritidis*) in females. This difference could be due to behavioural factors, occupational exposure, or dietary habits that increase male susceptibility to *Salmonella* infections. Similar gender variations have been reported in other studies, where males exhibited higher infection rates than females [12].

Table 3: Age/sex distribution of *Salmonella* infected patients

Age (year)	Frequency (%)	No of serotypes	Description	Sex distribution
11-20	3(16.7)	1(12.5)	*Typhimurium*	100%F
21-30	2(11.1)	2(25.0)	Heidelberg typhi	100%M
31-40	5(27.8)	3(37.5)	*Typhimurium* paratyphi B *Enteritidis*	40%M, 60%F
41-50	7(38.9)	7(87.5)	Typhi Heidelberg *Enteritidis* Huaian Agona *Typhimurium* Bongori	86%M, 14%F
51-60	1(5.6)	1(12.5)	*Enteritidis*	100%M
Total	18	14		

$\chi^2_{(4)}$ (age and occurrence of infection) = 35.74, P= 0.000 (P<0.05

As illustrated in Fig. 2 and Table 3, the highest infection rate was recorded among individuals aged 41–50 years, who also exhibited the greatest serovar diversity. This contrasts with studies that have reported a higher prevalence among children under ten years, likely due to weaker immune defences and increased exposure to contaminated food and water [13]. The lower prevalence among younger children in this study may be attributed to improved parental supervision and better hygiene practices. The high prevalence in the 41–50 age group may be associated with occupational exposure, dietary habits, or predisposing health conditions that increase vulnerability to infections. Additionally, increased consumption of high-risk foods such as street food, undercooked meat, and unpasteurized dairy products could contribute to higher *Salmonella* infection rates in middle-aged adults [14].



Kruskal Wallice H (Age groups) = 9.72, DF = 4, P = 0.045 (P<0.05)

Figure 2: Age distribution (years) of antibiotic resistance among *Salmonella* infected patients

Table 4: Antibiotic susceptibility profile of salmonella serovars isolated from clinical stool samples

SEROVAR	AUG	CIP	CEF	ERY	COT	GEN	NIT
S. Typhimurium (6)	R	S	S	R	I	S	S
S. Enteritidis (4)	R	S	S	R	S	S	S
S. Typhi (2)	R	I	S	R	S	I	S
S. Heidelberg (2)	R	S	S	R	I	S	S
S. Agona (1)	R	S	S	R	S	S	S
S. Paratyphi B (1)	R	S	S	R	I	S	S
S. Huaian (1)	R	S	S	R	S	S	S
S. bongori (1)	R	S	S	R	S	S	S

Table 5: Antibiotics and their concentration tested on *Salmonella* serotypes

Antibiotics	Code	Concentration (µg)
Ampicillin	AMP	10
Cotrimoxazole	COT	25
Ciprofloxacin	CIP	5
Cloxacillin	CXC	5
Tetracyclin	TET	30
Augmentin	AUG	20
Nitrofurantoin	NIT	300
Erythromycin	ERY	15
Gentamicin	GEN	10
Ceftriaxone	CEF	30

As detailed in Table 4, the antibiogram revealed significant resistance to Augmentin and erythromycin, with Augmentin showing no inhibitory effect on any of the isolates. This high resistance is concerning, as Augmentin is commonly used to treat bacterial infections, including *Salmonella*. The observed resistance may be due to widespread antibiotic misuse, self-medication, and inadequate regulatory control over antibiotic prescriptions in Nigeria [15]. The resistance to erythromycin is expected, as *Salmonella* species are known to exhibit intrinsic resistance to macrolides [16]. This finding is consistent with previous studies reporting high erythromycin resistance among *Salmonella* isolates from both clinical and environmental sources [17]. The ineffectiveness of these antibiotics highlights the urgent need to revise treatment guidelines for *Salmonella* infections in the study area. Conversely, cotrimoxazole, nitrofurantoin, and gentamicin demonstrated the highest efficacy against most isolates. Cotrimoxazole remains a viable treatment option despite emerging resistance in some regions [18]. Nitrofurantoin's effectiveness is noteworthy, as it is not a first-line treatment for *Salmonella* infections, which may explain the lower resistance rates. Gentamicin, an aminoglycoside, also exhibited strong efficacy, making it a potential choice for severe cases requiring intravenous treatment. Notably, ciprofloxacin was not tested in this study, but previous research has documented increasing fluoroquinolone resistance among *Salmonella* serovars in Nigeria [19]. Further studies should include fluoroquinolone susceptibility testing to assess emerging resistance trends in the study area. The findings of this study emphasize the need for enhanced public health interventions to control *Salmonella* infections and combat antibiotic resistance. The presence of multiple *Salmonella* serovars, including those associated with invasive infections highlights the importance of robust surveillance programs and improved diagnostic capabilities.

Conclusion

The total prevalence of *Salmonella* cases in this study was 4.0%. Doma recorded the highest prevalence of 5.3%. The dominant serovars were S. ser. *Typhimurium* and S. ser. *Enteritidis*, accounting for approximately 33 and 22% respectively. Serovar identity was more in malesthan in females. Infection was also pronounced between ages 41-50 years, which also recorded the highest diversity of *Salmonella* serovars. Cotrimoxazole, Nitrofurantoin and gentamicin are the recommended antibiotics in the treatment of strain-dependent *Salmonella* infections in the study area.

Conflict of interest: The authors wish to declare that there is no conflict of interest.

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