Vol 6 No 3 September 2025 Pages 64-68 e-ISSN 2708-5694

Journal of Agriculture, Food and Environment (JAFE)

Journal Homepage: https://journal.safebd.org/index.php/jafe

https://doi.org/10.47440/JAFE.2025.6309

Research Article

Prevalence and antibiotic resistance profile of *Salmonella spp.* isolated from intestine of cattle sold in Abraka and environment

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Article History

Received: 09 July 2025

Accepted: 26 September 2025

Published online: 30 September 2025

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Keywords

Cattle, Salmonella spp, antibiotics, resistance, health, zoonosis

How to cite: Odogwu D A 2025: Prevalence and antibiotic resistance profile of *Salmonella spp.* isolated from intestine of cattle sold in Abraka and environment. J. Agric. Food Environ. 6(3): 64-68.

ABSTRACT

Salmonella spp. remain a leading cause of foodborne illnesses globally, with cattle serving as key reservoirs for zoonotic transmission, particularly in low-resource settings like Nigeria where poor hygiene and antimicrobial misuse exacerbate risks. This study investigated the prevalence and antibiotic resistance profiles of *Salmonella spp.* isolated from the intestines of cattle sold in Abraka, Delta State, Nigeria. Using a cross-sectional design, intestinal samples were collected post-slaughter from five market sites with varying hygiene levels and processed via standard microbiological methods, including enrichment on MacConkey agar, and biochemical tests (e.g., citrate utilization, TSI. Results revealed a high prevalence of *Salmonella spp.* at 60% among the 10 samples collected (n=6 isolates), dominating over other microbes like *Bacillus spp.* and *Acinetobacter* (40%). Biochemical profiles confirmed classic Salmonella traits: Gram-negative, citrate-positive, and glucose-fermenting with gas production. Antimicrobial susceptibility testing via Kirby-Bauer disk diffusion (CLSI guidelines) against 10 antibiotics showed widespread multidrug resistance (MDR), with Multiple Antibiotic Resistance Index (MARI) values of 0.6–0.8. Isolates exhibited high resistance to streptomycin, augmentin, and cephalexin, with reduced inhibition zones visualized in assays, signaling threats to veterinary and human treatments. These findings show that informal markets are hotspots for MDR *Salmonella* dissemination, driven by unsatisfactory sanitation and over-the-counter antibiotic use. Urgent interventions, including biosecurity enhancements, stewardship programs, and surveillance programs, are recommended to mitigate zoonotic risks and safeguard public health in Nigeria's cattle industry.

INTRODUCTION

Foodborne illnesses constitute a major global public health challenge, with *Salmonella* species recognized as one of the leading causative agents (Billah and Rahman, 2024). These Gram-negative, bacillary bacteria, belonging to the family Enterobacteriaceae, are notorious for infecting both animals and humans (Aljasir and Allam, 2025). Human infections typically occur through the ingestion of contaminated foods, especially animal-derived products such as beef, poultry, eggs, and dairy (Eng *et al.*, 2015). Cattle serve as a primary reservoir for *Salmonella* species, harboring the pathogen asymptomatically in their intestines (Moraes *et al.*, 2024). This silent carriage poses a serious risk, facilitating undetected zoonotic transmission to humans via mishandled or undercooked contaminated meat.

In Nigeria and other sub-Saharan African countries, cattle farming is a cornerstone of the agricultural economy and rural livelihoods, with beef consumption being widespread (Ekpunobi *et al*, 2024). However, cattle management—from transport and slaughter to market sales, often occurs under suboptimal hygienic conditions. In Abraka, Delta State, cattle are frequently transported from northern Nigeria for local sale and slaughter, yet limited surveillance and microbiological screening of these animals heighten the potential for zoonotic spread.

The burden of *Salmonella* infections is particularly acute in low-resource settings, compounded by weak public health infrastructure, poor sanitation, and limited access to clean water (Moraes *et al.*, 2024). This challenge is further intensified by the rising threat of antimicrobial resistance (AMR). The indiscriminate use of antibiotics in veterinary practice, such as over-the-counter dispensing without prescriptions, their application as growth promoters, and failure to observe withdrawal periods, has driven the emergence of resistant *Salmonella* strains (Pop and Papa, 2021; Pławińska-Czarnak *et al.*, 2022; Kumar *et al*, 2025). Such resistant isolates jeopardize both animal and human health, leading to treatment failures, increased morbidity, and higher mortality rates (Van Boeckel *et al.*, 2015).

Numerous studies in Nigeria have reported substantial *Salmonella* prevalence in cattle populations. For instance, Aworh *et al.* (2023) documented a 4.8% prevalence in beef cattle from Abuja and Lagos abattoirs, linking low rates to urban slaughter practices but noting 81.5% multidrug resistance among isolates. Similarly, Tamba *et al.* (2021) identified multiple *Salmonella* serovars contaminating water sources in cattle-rearing areas of Jos. These observations underscore the role of cattle and their environments as key pathogen reservoirs. In parallel, assessing antibiotic susceptibility profiles is crucial for gauging the public health implications of *Salmonella* infections. Alhaji *et al.* (2023) revealed that over 70% of Nigerian cattle farmers disregard proper antimicrobial stewardship, often administering broad-spectrum drugs without veterinary oversight (Orum *et al.*, 2022). Despite these insights, data on *Salmonella* prevalence and resistance patterns remain scarce for cattle traded in Abraka and its environs, highlighting the need for targeted investigations to inform risk mitigation and surveillance strategies.

This study aimed to isolate and identify antibiotic resistance in strains of *Salmonella spp.* isolated from the intestines of cattle purchased in Abraka to provide epidemiological data for the risk analysis of antibiotic resistance in the Nigerian cattle industry.

MATERIALS AND METHOD

Study Area

This study was conducted in Abraka, a semi-urban town in Ethiope East Local Government Area, Delta State, Nigeria (latitude 5°47′N–5°50′N; longitude 6°05′E–6°09′E). Located in the tropical rainforest belt of southern Nigeria, Abraka hosts Delta State University and supports a mixed population engaged in academic, agricultural, and small-scale commercial activities. It serves as a regional hub for cattle slaughter and sales, with livestock often sourced from northern Nigeria and neighboring West African countries. Climatic conditions, including average temperatures of 24–33°C and annual rainfall of approximately 2,500 mm, favor the survival of enteric pathogens such as *Salmonella* spp. Suboptimal sanitation in cattle markets and slaughter areas, characterized by open animal defecation and inadequate waste management, creates reservoirs for zoonotic bacteria and heightens the risk of antimicrobial-resistant *Salmonella* transmission to humans via direct contact or the food chain. Abraka was selected due to its high cattle slaughter volume and central role in the regional meat supply chain.

Study Design

A cross-sectional descriptive design was employed to assess the prevalence and antibiotic resistance profiles of *Salmonella* spp. in cattle. This approach allows simultaneous evaluation of exposure (presence of *Salmonella* spp.) and outcome (antibiotic resistance) at a single time point among cattle in Abraka markets (Setia, 2016). It is particularly suitable for epidemiological studies of foodborne pathogens and antimicrobial resistance in resource-limited settings. Intestinal samples were collected from cattle randomly selected at five slaughter points in Abraka main market, representing diverse geographical and hygiene conditions.

Sampling Technique

Simple random sampling was used to select cattle from five market points with varying hygiene levels: (1) a seller's table near a tarred road; (2) a table adjacent to a waste dumpsite; (3) a table beside a gutter; (4) a relatively hygienic site; and (5) a site near the abattoir. This method ensured representation of the target population and supported prevalence estimation within the cross-sectional framework.

Sample Collection

Intestinal samples were aseptically collected post-slaughter, placed in sterile labeled containers, and stored in an insulated cold box with ice packs at 4°C to preserve bacterial viability. Samples were transported to the Microbiology Laboratory, Delta State University, Abraka, within 4–6 hours for processing.

Laboratory Analyses

All analyses were performed to isolate, identify, and characterize the antibiotic resistance of *Salmonella* spp. from cattle intestinal contents. Materials included sterile swab sticks, syringes with needles, distilled water, beakers, weighing scale, conical flasks, universal sterile containers, cover slips, micropipettes, aluminum foil, spatulas, measuring cylinders, autoclave, Petri dishes, MacConkey agar, nutrient agar, Mueller-Hinton agar, test tubes, Bunsen burner, cotton wool, spirit, sensitivity discs, inoculating loops, filter paper, oxidase reagent, Kovac's reagent, hydrogen peroxide, and meter rule. An incubator maintained at 37°C was used for all cultures.

Isolation and Culturing

Isolation and identification of organisms was carried out as described by Shivaprasad (2000) and Menghistu *et al.* (2011) with little modification. A sterile swab was used to collect intestinal contents, which were streaked onto MacConkey agar and incubated aerobically at 37°C for 24 h. Presumptive *Salmonella* colonies; colorless, smooth, convex with serrated margins were sub cultured onto nutrient agar for pure isolates.

The following biochemical tests were carried out: Indole test, triple sugar iron test, citrate test, methyl-red test,Voges-Proskauer test, lysine decarboxylase test, urease test, sugar (trehalose, sucrose, inositol, mannitol) fermentation test and motility test.

Antimicrobial Susceptibility Testing

Susceptibility was determined by the Kirby-Bauer disk diffusion method on Mueller-Hinton agar per Clinical and Laboratory Standards Institute guidelines (CLSI, 2020). Bacterial suspensions were adjusted to 0.5 McFarland turbidity, swabbed onto plates, and antibiotic discs applied: ofloxacin (10 µg), amoxicillin-clavulanate (30 µg), pefloxacin (10 µg), ceftazidime (30 µg), gentamicin (10 µg), ciprofloxacin (10 µg), cephalexin (10 µg), ceftriaxone (30 µg), streptomycin (30 µg), and cefuroxime (30 µg). Plates were incubated at 37°C for 18–24 h, inhibition zones measured in mm with a meter rule, and interpreted as susceptible, intermediate, or resistant using CLSI breakpoints. Multidrug resistance was defined as non-susceptibility to ≥1 agent in ≥3 antibiotic classes (Magiorakos *et al*., 2012).

RESULTS

Prevalence of *Salmonella spp.*

A total of ten (10) samples were collected from cattle intestines. The distribution of microbial organisms isolated from these samples is summarized in Table 1, highlighting the dominance of *Salmonella* spp. with a prevalence rate of 60% (6 out of 10 samples), compared to 40% (4 out of 10) for other organisms. This result indicates a significant burden of *Salmonella* contamination in the cattle sampled, which is a public health concern due to the zoonotic potential of this pathogen and its link to foodborne illnesses in humans.

Table 1: Percentage prevalence of Isolates

Organism	% Prevalence
Salmonella spp	60
Bacillus cereus, Acinetobacter	40

Biochemical Characterization of Isolates

The isolates were made to undergo biochemical tests, which was used to confirm the identity of the ten isolated organisms from the cattle intestines. Notably, six isolates displayed biochemical profiles consistent with *Salmonella* spp., particularly the negative Gram stain, positive citrate utilization, and gas production with glucose fermentation. The other four organisms identified included *Bacillus* spp. and *Acinetobacter*, underscoring the microbial diversity present, as shown in Table 2.

Table 2: Biochemical test results

Sample	catalase	Gram staining	oxidase	indole	Citrate	H2S	Acid	gas	glucose	Lactose	organism
CTI MA (A1)	+	-	-	-	+	-	+	+	+	-	*Salmonella*
CTI MA (A2)	+	+	-	-	+	-	+	+	+	-	*Bacillus*
CTI MA (B1)	+	-	-	-	+	-	+	+	+	-	*Salmonella*
CTI MA (B2)	+	-	-	-	+	-	+	+	+	-	*Salmonella*
CTI MA (C1)	+	-	-	-	+	-	-	-	+	-	*Acinetobacter*
CTI MA (C2)	+	+	-	-	+	-	+	+	+	-	*Bacillus cereus*
CTI MA (D1)	+	+	-	-	+	-	+	+	+	-	*Bacillus cereus*
CTI MA (D2)	+	-	-	-	+	-	+	+	+	-	*Salmonella*
CTI MA (E1)	+	-	-	-	+	-	+	+	+	-	*Salmonella*
CTI MA (E2)	+	-	-	-	+	-	+	+	+	-	*Salmonella*

CTI = cattle intestine, MA = MacConkey Agar

Antibiotic Resistance Profile of the *Salmonella* Isolates

Table 3 presents the antibiotic resistance profiles of *Salmonella* isolates against a panel of antibiotics, including Streptomycin (S), Ceftriaxone (CEF), Ofloxacin (OFX), Augmentin (AU), Pefloxacin (PEF), Cefotaxime (CTZ), Gentamicin (CN), Ciprofloxacin (CPX), Cephalexin (CEP), and Trimethoprim (TRX). The results show varying resistance patterns, with several isolates resistant to multiple antibiotics, as indicated by the high Multiple Antibiotic Resistance Index (MARI) values ranging from 0.6 to 0.8. This suggests the presence of multidrug-resistant *Salmonella* strains, as shown in Figure 1, posing a significant threat to both veterinary and human health.

Table 3: Multiple Antibiotic Resistance Index of *Salmonella*

Sample	S	CEF	OFX	AU	PEF	CTZ	CN	CPX	CEP	TRX	MARI
$CTIA_1$	R	R	R	R	R	R	S	S	R	R	0.8
$CTIB_1$	R	R	S	R	S	R	R	S	R	R	0.7
$CTIB_2$	R	R	S	R	S	R	S	S	R	R	0.6
$CTID_2$	R	R	S	R	S	R	S	S	R	R	0.6
$CTIE_1$	R	S	S	R	S	R	R	R	R	R	0.7
$CTIE_2$	R	R	S	S	S	R	R	S	R	R	0.6

MARI: Multiple antibiotic resistance index

CTI = cattle intestine, MA = MacConkey Agar, R = resistant, **S** *– Streptomycin,* **CEF** *– Cefuroxime,* **OFX** *– Ofloxacin,* **AU** *– Augmentin (Amoxicillin + Clavulanic Acid),* **PEF** *– Pefloxacin,* **CTZ** *– Ceftazidime,* **CN** *– Gentamicin,* **CPX** *– Ciprofloxacin,* **CEP** *– Cephalexin,* **TRX** *– Cotrimoxazole (Trimethoprim + Sulfamethoxazole)*

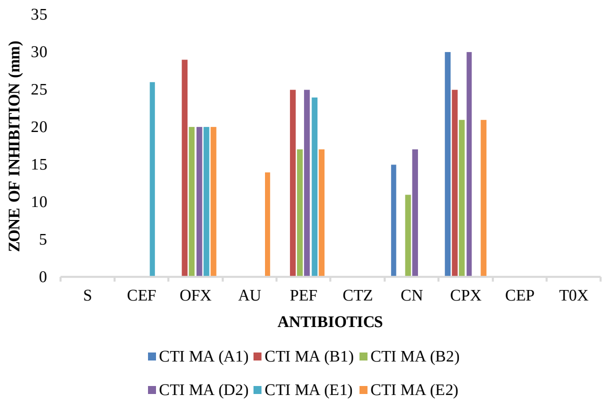

Figure 1: Zone of inhibition for Antibiotics Assay

DISCUSSION

The results from this study on the prevalence and antibiotic resistance profile of *Salmonella* spp. isolated from the intestines of cattle sold in Abraka and its environs reveal a concerning level of contamination and antimicrobial resistance, underscoring the zoonotic risks associated with cattle as a reservoir for foodborne pathogens. The high dominance of *Salmonella* spp. (60%) among isolates from cattle intestines, as summarized in Table 1, highlights a substantial public health burden in this region of Nigeria. This prevalence rate far exceeds those reported in several recent studies on similar cattle populations or beef products in Nigeria and neighboring African contexts. For instance, Aworh *et al.* (2023) documented a much lower prevalence of 4.8% for non-typhoidal *Salmonella enterica* in beef cattle sampled from abattoirs in Abuja and Lagos, Nigeria, attributing the lower rates to potentially better slaughter hygiene practices in urban settings compared to rural markets like Abraka. Similarly, Ekli *et al.* (2025) found a 26% prevalence in ready-to-eat beef (suya) samples from Minna Metropolis, Niger State, Nigeria, which, while elevated, still falls short of the 60% observed here, possibly due to post-slaughter processing that may reduce but not eliminate contamination. In a broader African comparison, Tadesse *et al.* (2024) reported an 8% prevalence in cattle carcasses at an Ethiopian abattoir, emphasizing environmental and handling factors as key contributors, factors likely amplified in the informal cattle trade around Abraka. The stark contrast suggests localized drivers in Delta State, such as inadequate sanitation in holding pens, overcrowding, or contaminated feed/water sources, warranting targeted interventions like improved biosecurity at local markets to mitigate zoonotic transmission to humans via undercooked meat or environmental exposure.

The biochemical characterization of isolates (Table 2) further confirms the identity of *Salmonella* spp. through classic profiles, including Gram-negative staining, positive citrate utilization, and gas production from glucose fermentation, aligning with standard diagnostic criteria for the genus. This is consistent with protocols used in contemporary studies, such as those by Aworh *et al.* (2023), who employed similar biochemical and serological tests to identify rare serovars in Nigerian cattle samples. Notably, the co-isolation of other genera like *Bacillus* spp. and *Acinetobacter* (40% combined prevalence) reflects the microbial diversity in cattle intestines, a pattern echoed in Ekli *et al.* (2025), where diverse contaminants were noted alongside *Salmonella* in beef products, potentially exacerbating spoilage and cross-contamination risks during processing. Such diversity underscores the need for comprehensive microbiological surveillance beyond *Salmonella* alone, as these opportunistic pathogens could compound infection risks in immunocompromised consumers.

The antibiotic resistance profiles of the *Salmonella* isolates (Table 3) paint a particularly alarming picture, with multiple resistance patterns leading to high Multiple Antibiotic Resistance Index (MARI) values of 0.6–0.8, indicative of multidrug-resistant (MDR) strains capable of withstanding 60–80% of tested antibiotics. This is visualized in Figure 1, showing reduced zones of inhibition for key drugs like streptomycin (S), ceftriaxone (CEF), ofloxacin (OFX), and others, signaling a threat to both veterinary treatment efficacy and human therapy options. These findings resonate with recent evidence of escalating AMR in African livestock systems. For example, Dagah *et al.* (2024) reported MARI values up to 0.6 in *Salmonella* from roasted beef (suya) in Abuja, Nigeria, with 100% resistance to ampicillin, gentamicin, and other beta-lactams, mirroring the broad resistance here to augmentin (AU) and cefotaxime (CTZ). Likewise, Aworh *et al.* (2023) observed 81.5% MDR among Nigerian cattle-derived *Salmonella*, with over 85% resistance to gentamicin and 78% to tetracycline, often linked to mobile genetic elements disseminating resistance genes like *aac(6')-Ib-cr* and *tetA*. In Ethiopia, Tadesse *et al.* (2024) found comparable MDR rates (81.5%) in cattle isolates, including 100% resistance to ampicillin and 59% to trimethoprim-sulfamethoxazole, highlighting a regional trend driven by indiscriminate antibiotic use in agriculture. The elevated MARI in this Abraka study (>0.5 threshold for high-risk sources) suggests chronic selective pressure from over-the-counter veterinary antibiotics, a common issue in Nigeria's informal livestock sector (Ekli *et al.*, 2025). This juxtaposition emphasizes the urgency of antimicrobial stewardship programs, such as restricting non-essential prophylaxis in cattle rearing, to curb the emergence of "superbugs" that could render standard treatments like ciprofloxacin (CPX) ineffective.

Overall, these results position the Abraka cattle trade as a hotspot for *Salmonella* dissemination, with prevalence and resistance levels surpassing many recent benchmarks, likely due to socioeconomic and infrastructural gaps in rural Nigeria. While biochemical confirmation provides robust isolate validation, the high MDR burden aligns with global calls for One Health approaches integrating animal, human, and environmental surveillance (Aworh *et al.*, 2023; Tadesse *et al.*, 2024). Future research should incorporate genomic sequencing to trace resistance gene mobility, as demonstrated in these comparative studies, and advocate for policy reforms like hazard analysis critical control points (HACCP) in local abattoirs to safeguard food safety.

CONCLUSION

This study reveals a high prevalence of Salmonella spp. (60%) in the intestines of cattle sold in Abraka and its environs, coupled with alarming multidrug resistance profiles (MARI 0.6–0.8) against key antibiotics like ciprofloxacin and gentamicin, underscoring the urgent zoonotic and food safety threats in Nigeria's informal livestock trade. These findings, highlight the need for immediate interventions such as enhanced biosecurity in markets, antimicrobial stewardship to curb resistance, and routine surveillance under a framework to safeguard public health and mitigate the spread of resistant pathogens from animal to human populations.

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