

CROSS-SECTIONAL STUDY OF *SALMONELLA* PREVALENCE AND ANTIMICROBIAL RESISTANCE PROFILE IN SINGLE AND MIXED POULTRY–FISH FARMS IN UMUAHIA AGRICULTURAL ZONE, NIGERIA

¹NDIANA L.A., ¹ERUMAKA I.G., ²KALU E., ¹IKPENDU C.N., ¹OZIOKO C. & ¹OBI C.S.

¹Department of Veterinary Microbiology, ²Department of Veterinary Public Health and Preventive Medicine, Michael Okpara University of Agriculture, Umudike, Nigeria

*Correspondence: laukagwu@gmail.com

ABSTRACT

Salmonella is an important zoonotic pathogen that causes foodborne illness globally and is commonly transmitted through contaminated animal products and farm environments. Mixed livestock farming systems may increase the spread of antimicrobial-resistant bacteria due to close contact between different animal species and shared environments. This study determined the prevalence and antimicrobial resistance patterns of *Salmonella* isolates from single-species and mixed-species (poultry–fish) farms in the Umuahia Agricultural Zone. A cross-sectional survey was conducted in four Local Government Areas: Umuahia North, Umuahia South, Ikwuano, and Obingwa. A total of 146 samples were collected, including cloacal swabs (n = 92), fish anal swabs (n = 27), and pond water samples (n = 27). *Salmonella* isolation and identification followed standard microbiological methods, and antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion technique. Data were analysed using SPSS. Overall *Salmonella* prevalence was 13.0%, with higher rates in mixed-species farms (16.3%) compared to single-species farms (8.3%), though this difference was not statistically significant. Questionnaire results indicated moderate biosecurity weaknesses, including limited veterinary input and the use of animal waste in feed systems. Antimicrobial testing showed reduced effectiveness of commonly used antibiotics, with high resistance to ceftazidime and dominance of intermediate and resistant phenotypes to ciprofloxacin. Most antibiotics showed no significant differences between farming systems, except trimethoprim/sulfamethoxazole. The findings indicate ongoing circulation of *Salmonella* with emerging antimicrobial resistance across study areas. This highlights the need for improved farm biosecurity, responsible antimicrobial use through stewardship programs, and continuous surveillance to limit further spread.

Keywords: antimicrobial resistance, food safety, Nigeria, poultry-fish, *Salmonella*, zoonotic pathogens

INTRODUCTION

The emergence of antimicrobial-resistant (AMR) *Salmonella* constitutes a significant global public health threat, contributing to increased morbidity, mortality, and treatment failure in both human and animal populations, particularly in low- and middle-income countries where surveillance and control measures remain limited. The growing demand for animal protein has led to rapid expansion and intensification of livestock and aquaculture production systems, often

accompanied by increased antimicrobial use and suboptimal biosecurity practices. Within this context, mixed-species livestock farming—defined as the co-location of two or more animal species such as poultry and fish—has gained prominence as a strategy to enhance resource efficiency and productivity (Allsopp *et al.*, 2013). Unlike fully integrated systems, these farms maintain physically distinct but ecologically interconnected production units, allowing practices such as the reuse of poultry waste in aquaculture

while facilitating microbial exchange across species boundaries (Little & Edwards, 2003). Such interactions may create favourable conditions for the persistence and dissemination of *Salmonella* and antimicrobial-resistant strains within farm environments.

The increasing adoption of such systems among smallholder farmers reflects their economic and ecological advantages, including income diversification, improved nutrient recycling, and reduced input costs (Akanmu *et al.*, 2023). Poultry manure, rich in essential nutrients, supports primary productivity in pond ecosystems by promoting plankton growth and nutrient cycling (Gabriel *et al.*, 2007; Banerjee *et al.*, 2014). However, these same interactions create ecological interfaces that may facilitate the transmission of microorganisms, including zoonotic pathogens, across animal species and into surrounding environmental compartments (Jones *et al.*, 2013; Abdugheni *et al.*, 2023; Ramirez-Plascencia *et al.*, 2025).

From a One Health perspective—recognizing the interconnectedness of human, animal, and environmental health—mixed-species farming systems represent critical nodes for the emergence and dissemination of AMR. The use of untreated or minimally treated animal waste introduces enteric pathogens such as *Salmonella* spp. into aquatic systems, where they may persist, proliferate, and spread through water, sediment, and biota (Edwards, 2015). This creates multiple exposure pathways, including direct animal contact, environmental contamination, and consumption of contaminated food products (Ukwueze, 2019). The risks are particularly pronounced in settings with limited biosecurity infrastructure, inadequate antimicrobial stewardship, and weak regulatory oversight, as is common in many parts of sub-Saharan Africa, including Nigeria (Okocha *et al.*, 2018). Gram-negative bacteria, especially members of the family Enterobacteriaceae, play a central role in the ecology of AMR due to their capacity for horizontal gene transfer and environmental persistence. Genera such as *Salmonella*, *Escherichia*, *Klebsiella*, *Proteus*, and *Citrobacter* have been widely reported in aquaculture environments, including pond water, sediments, and fish tissues (Akinbowale *et al.*, 2006; Ruzauskas *et al.*, 2018). Among these, *Salmonella* remains a leading cause of foodborne illness globally, contributing substantially to morbidity, mortality, and economic burden (Crump *et al.*, 2015; Ayuti *et al.*, 2024; Billah & Rahman, 2024). The convergence of animal waste reuse, aquatic ecosystems, and human exposure underscores the role of mixed-species farms as potential reservoirs and amplification points for resistant pathogens.

Despite the growing prevalence of poultry–fish farming systems in Nigeria, there is a paucity of data on the occurrence and antimicrobial resistance profiles of *Salmonella* spp. within these environments. This knowledge

gap limits the ability to assess risks and design context-specific interventions. Investigating AMR patterns in such systems is essential for understanding transmission dynamics across animal–environment interfaces and for informing integrated surveillance strategies aligned with One Health principles.

The present study therefore examines the antimicrobial susceptibility profiles of *Salmonella* spp. isolated from mixed-species livestock farms in Umuahia agricultural zone, Abia State, Nigeria. By analyzing isolates obtained from poultry droppings, pond water, and fish, this study aims to elucidate potential pathways of bacterial exchange and resistance dissemination within these interconnected systems. The findings have important implications for public health, particularly in guiding antimicrobial stewardship, strengthening farm-level biosecurity, and informing policies that address AMR at the human–animal–environment interface.

MATERIALS AND METHODS

STUDY AREA

The study was conducted in Umuahia Agricultural Zone located in Abia State, south-eastern Nigeria. The area comprises four Local Government Areas: Umuahia North, Umuahia South, Ikwuano, and Obingwa.

The zone lies approximately between latitude 5°25'–5°40'N and longitude 7°20'–7°35'E and is characterized by a humid tropical climate with distinct wet and dry seasons. The region is an important agricultural hub where mixed poultry–fish farming systems are commonly practiced, allowing poultry waste to be utilized in fish ponds as a nutrient source for plankton production, a practice which may facilitate microbial exchange between poultry, fish, and aquatic environments.

STUDY DESIGN

A cross-sectional study design was employed to assess the occurrence and antimicrobial susceptibility patterns of *Salmonella* species in poultry–fish farming systems, as it allows for the efficient estimation of prevalence and resistance profiles at a specific point in time without the need for prolonged follow-up. Sampling was carried out between March and June 2024. A convenience sampling approach was used to select farms, based on their accessibility and the willingness of farmers to participate in the study.

SAMPLE COLLECTION

A total of 146 samples were collected, which consisted of 92 cloacal swabs from poultry, 27 anal swabs from fish, and 27 pond water samples. Out of the samples collected, 86 were obtained from mixed-species farms, whilst 60 were from fish only and poultry only farms.

Cloacal swabs were obtained from poultry birds using sterile swab sticks inserted gently into the cloaca and rotated before removal. Anal swabs were collected from fish using sterile swabs inserted into the anal opening. Pond water samples were collected aseptically into sterile sample bottles from different points within the fish ponds. The rationale for the selection and distribution of samples in this study is grounded in the need to comprehensively assess the presence and potential transmission pathways of *Salmonella* across different ecological niches within poultry–fish production systems.

Firstly, cloacal swabs from poultry ($n = 92$) were collected because the cloaca is the primary site of *Salmonella* shedding in infected birds. Poultry are well-established reservoirs of *Salmonella*, and cloacal sampling provides a direct and reliable indicator of intestinal colonization and carriage status within flocks.

Secondly, anal swabs from fish ($n = 27$) were included to investigate the possible presence or carriage of *Salmonella* in aquaculture species. Although fish are not classical primary hosts of *Salmonella*, they can act as mechanical carriers or become contaminated through shared water sources, particularly in integrated farming systems where cross-contamination may occur.

Thirdly, pond water samples ($n = 27$) were collected because water serves as a critical environmental reservoir and transmission medium for enteric bacteria. In integrated farming systems, pond water can facilitate the persistence and dissemination of *Salmonella* between animal species and the surrounding environment, making it essential for understanding indirect transmission pathways.

The unequal distribution of samples (92 poultry vs. 54 fish/water-related samples) reflects both the expected epidemiological burden and biological relevance of poultry as the primary host of *Salmonella*, while still ensuring inclusion of environmental (water) and secondary host (fish) samples to capture potential cross-species transmission dynamics.

Finally, the higher number of samples from mixed-species farms (86 vs. 60 from single-species farms) was intentional to better evaluate the influence of integrated farming practices on bacterial distribution, antimicrobial exposure, and potential interspecies transmission, which are central to the study objectives. All samples were clearly labeled and transported in insulated containers to the Veterinary Microbiology Laboratory of Michael Okpara University of Agriculture for immediate bacteriological analysis.

QUESTIONNAIRE SURVEY

A well-structured questionnaire was administered to farmers operating single-species and mixed poultry–fish farms. The questionnaire captured information on farm management

practices, stocking density, biosecurity measures, antibiotic usage, waste management practices and farmer awareness of zoonotic diseases.

ISOLATION AND IDENTIFICATION OF *SALMONELLA*

Isolation of *Salmonella* species was performed using standard bacteriological procedures. Each sample was inoculated onto MacConkey agar plates using the streak plate method and incubated aerobically at 37 °C for 24 hours. Colonies appearing as non-lactose fermenting (pale or tan) colonies were selected and sub-cultured onto *Salmonella–Shigella* agar and incubated at 37°C for another 24 hours. Colonies producing black centres indicating hydrogen sulfide (H₂S) production were considered presumptive *Salmonella* isolates. Presumptive isolates were further subjected to biochemical confirmation tests using Simmons citrate agar and urease agar. Citrate-positive and urease-negative reactions were used as supporting criteria for the identification of *Salmonella* species following standard microbiological procedures.

ANTIMICROBIAL SUSCEPTIBILITY TESTING

Antimicrobial susceptibility testing was performed using the following antibiotics: cefuroxime (CEF), streptomycin (S), trimethoprim/sulfamethoxazole (TRX), ciprofloxacin (CPX), gentamicin (CN), ceftazidime (CTZ), pefloxacin (PEF), amoxicillin–clavulanate (AU), ofloxacin (OFX), and cephalixin (CEP) by the Kirby–Bauer disk diffusion method in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2024, 2025), specifically following CLSI document M02 (14th ed., 2024) for test methodology and CLSI M100 (35th ed., 2025) for interpretation of inhibition zone diameters and quality control criteria. For antimicrobials lacking CLSI interpretive criteria, EUCAST (2024) guidelines were consulted. However, EUCAST provides only a screening cut-off for pefloxacin (24 mm) and does not define clinical breakpoints for streptomycin, cephalixin or ofloxacin in Enterobacterales. Therefore, Ofloxacin susceptibility was interpreted using zone diameter breakpoints proposed by Sjölund-Karlsson *et al.* (2014), where susceptibility was defined as ≥ 21 mm, intermediate as 16–20 mm, and resistance as ≤ 15 mm. for cephalixin, interpretive criteria were adapted from published disc diffusion regression studies due to the absence of standardized breakpoints for cephalixin in CLSI/EUCAST systems (Crosse & Burt, 1984). Streptomycin was included for comparative susceptibility profiling; however, interpretation was not performed due to the absence of standardized disk diffusion breakpoints in CLSI, EUCAST, and published literature

specific to *Salmonella* spp. Results were therefore reported as inhibition zone diameters only.

Pure bacterial cultures were inoculated onto Mueller–Hinton agar plates using sterile swabs to obtain a uniform bacterial lawn. Antibiotic discs were aseptically placed on the inoculated agar surface and plates were incubated at 37 °C for 24 hours. After incubation, the diameter of the zone of inhibition around each disc was measured in millimetres using a meter rule and interpreted as susceptible, intermediate, or resistant according to CLSI/EUCAST standards where possible.

STATISTICAL ANALYSIS

Data obtained from laboratory analyses were analyzed using IBM SPSS Statistics (Version 27.0; IBM Corp, 2020).

Descriptive statistics including frequencies and percentages were used to summarize the data and a Chi-square test of independence was performed to determine the association between farming system and *Salmonella* prevalence.

ETHICAL CONSIDERATIONS

Ethical clearance for the study was granted by the Research Ethics Committee of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike (Ref: MOUAU/CVM/REC/202614).

RESULTS

PREVALENCE OF SALMONELLA IN SINGLE AND MIXED-SPECIES FARMS

The overall prevalence of *Salmonella* spp. was 13.0% (19/146), with 95% CI: 8.1–18.0%. When categorized by farming system, mixed-species farms recorded a higher proportion of positive samples (16.3%; 14/86) compared to single-species farms (8.3%; 5/60). A Chi-square analysis revealed that the difference in *Salmonella* prevalence between farming systems was not statistically significant (p = 0.16). This indicates that although a higher prevalence was observed in mixed-species farms, the association between farming system and *Salmonella* occurrence was not significant (Table I).

Table I: Prevalence of *Salmonella* in Single and Mixed-species farms

Farming System	Number of Samples Collected (n)	of <i>Salmonella</i> -Positive Samples (%)
Single-species farms	60	5 (8.3%)
Mixed-species farms	86	14 (16.3%)
Total	146	19 (13.0%)

Chi-square test showed no significant association between farming system and *Salmonella* prevalence ($\chi^2 = 1.97$, $df = 1$, $p = 0.16$).

QUESTIONNAIRE SURVEY DEMOGRAPHIC CHARACTERISTICS OF RESPONDENTS

A total of nine farm operators were willing to participate in the questionnaire survey conducted among farmers managing single-species and mixed-species farms in Umuahia Agricultural Zone.

Most respondents (78%) were between 20–40 years of age, while 22% were between 41–60 years. Male farmers constituted the majority (67%) of respondents. Regarding education level, most farmers (78%) were university graduates, whereas 22.2% had secondary school education (W.A.E.C).

In terms of farming experience, 44% had been practicing for two years, while another 44% reported three or more years of farming experience (Table II).

TABLE II: DEMOGRAPHIC CHARACTERISTICS OF FARM RESPONDENTS (N = 9)

Variable	Frequency	Percentage (%)
Age (years)		
20–40	7	78
41–60	2	22
Sex		
Male	6	67
Female	3	33
Educational Level		
W.A.E.C	2	22
University graduate	7	78
Years of Practice		
1 year	1	11
2 years	4	44
≥ 3 years	4	44

FARM MANAGEMENT CHARACTERISTICS

Poultry stocking density varied widely among farms, ranging from 80 birds to 1,936 birds, while fish stocking density ranged from 100 to 2,000 fish.

Most farmers (66.7%) reported feeding animals with commercial feed, whereas 33.3% used droppings from other animals as part of the feeding system (Table III).

TABLE III: FARM CHARACTERISTICS OF RESPONDENTS

Variable	Frequency	Percentage (%)
Type of Animals Raised		
Poultry and fish	6	67
Poultry	2	22
Fish	1	11
Poultry population		
No birds	1	11
80	1	11
100	1	11
200	2	22
300	2	22
500	1	11
1936	1	11
Fish Density		
100	1	11
105	1	11
150	1	11
200	2	22
500	2	22
2000	1	11
No response	1	11
Feeding Method		
Commercial feed	6	67
incorporated animal waste into feed	3	33

BIOSECURITY AND HEALTH MANAGEMENT PRACTICES

Most farmers (77%) reported vaccinating their animals, whereas 22% did not practice vaccination. Disease outbreaks were experienced in 44% of farms, while 56% reported no outbreaks. Among those reporting disease outbreaks, diarrhoea was the most observed clinical sign.

Approximately 44% of farmers reported mortality events on their farms. Similarly, 44% reported consulting a veterinarian when disease problems occurred, while the same proportion indicated they did not seek veterinary assistance. Sample collection for laboratory diagnosis was reported by 44% of farmers. Among the reported diagnostic samples, fecal samples were the most collected (33%), followed by blood samples (11%). (Table IV).

TABLE IV: BIOSECURITY AND DISEASE MANAGEMENT PRACTICES

Variable	Frequency	Percentage (%)
Vaccination		
Yes	7	78
No	2	22
Disease Outbreak		
Yes	4	44
No	5	56
Observed Clinical Signs		
Diarrhea	3	33
No response	6	67
Mortality Recorded		
Yes	4	44
No	4	44
No response	1	11
Veterinary Consultation		
Yes	4	44
No	4	44
No response	1	11
Diagnostic Samples Collected		
Yes	4	44
No	4	44
No response	1	11
Type of Sample Collected		
Fecal	3	33
Blood	1	11
No response	5	56

ANTIMICROBIAL RESISTANCE PATTERNS OF SALMONELLA ISOLATES**ANTIMICROBIAL RESISTANCE PATTERNS OF ISOLATES FROM SINGLE-SPECIES FARMS**

The antimicrobial resistance patterns of *Salmonella* isolates from single-species farms across the four study areas; Umuahia North, Umuahia South, Ikwuano, and Obingwa revealed notable variations in susceptibility profiles.

In Umuahia North, all isolates exhibited complete intermediate susceptibility (100%) to ofloxacin (OFX), pefloxacin (PEF), and ciprofloxacin (CPX). Augmentin (AU) and ceftazidime (CTZ) each showed a 50% intermediate and 50% resistant pattern. Complete susceptibility (100%) was observed only with gentamicin (CN) in two locations while cefuroxime (CEF) showed complete resistance (100%).

TABLE V: ANTIMICROBIAL RESISTANCE PATTERNS OF *SALMONELLA* ISOLATES FROM SINGLE SPECIES FARMS

Drug	UMUAHIA NORTH			UMUAHIA SOUTH			IKWUANO			OBINGWA		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
OFX	0 (0)	2 (100)	0 (0)	0 (0)	2 (100)	0 (0)	–	–	–	0 (0)	1 (100)	0 (0)
AU	0 (0)	1 (50)	1 (50)	0 (0)	0 (0)	2 (100)	–	–	–	0 (0)	0 (0)	1 (100)
PEF	0 (0)	2 (100)	0 (0)	0 (0)	0 (0)	2 (100)	–	–	–	0 (0)	1 (100)	0 (0)
CTZ	0 (0)	1 (50)	1 (50)	0 (0)	2 (100)	0 (0)	–	–	–	0 (0)	0 (0)	1 (100)
CN	2 (100)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)	–	–	–	0 (0)	1 (100)	0 (0)
CPX	0 (0)	2 (100)	0 (0)	0 (0)	2 (100)	0 (0)	–	–	–	0 (0)	1 (100)	0 (0)
CEF	0 (0)	0 (0)	2 (100)	0 (0)	2 (100)	0 (0)	–	–	–	0 (0)	0 (0)	1 (100)
TRX	0 (0)	0 (0)	2 (100)	0 (0)	2 (100)	0 (0)	–	–	–	0 (0)	0 (0)	1 (100)
S*	–	–	–	–	–	–	–	–	–	–	–	–
CEP	0 (0)	0 (0)	2 (100)	0 (0)	2 (100)	0 (0)	–	–	–	0 (0)	0 (0)	1 (100)

Cefuroxime (CEF), Streptomycin (S), Trimethoprim/Sulfamethoxazole (TRX), Ciprofloxacin (CPX), Gentamicin (CN), Ceftazidime (CTZ), Pefloxacin (PEF), Amoxicillin–clavulanate (Augmentin) (AU), and Ofloxacin (OFX), Cephalexin (CEP), S=sensitive, I= intermediate, R= resistant*

Similarly, in Umuahia South, isolates demonstrated 100% intermediate responses to OFX, CTZ, CPX, and CEF. However, complete resistance (100%) was recorded for AU and PEF. Gentamicin (CN) remained fully effective with 100% susceptibility. In Obingwa, all isolates showed complete intermediate susceptibility (100%) to OFX, PEF, CN, and CPX. In contrast, complete resistance (100%) was observed against AU, CTZ, and CEF. Notably, no isolates were fully susceptible to any of the tested antibiotics in this area. Overall, gentamicin (CN) demonstrated the highest level of effectiveness compared to the other antibiotics, particularly in Umuahia North and Umuahia South, while cefuroxime (CEF) and augmentin (AU) showed high levels of resistance across most locations. The predominance of intermediate responses to fluoroquinolones (OFX, PEF, CPX) suggests reduced susceptibility and potential emerging resistance among the *Salmonella* isolates (Table V).

ANTIMICROBIAL RESISTANCE PATTERNS OF ISOLATES FROM MIXED-SPECIES FARMS

The antimicrobial resistance patterns of *Salmonella* isolates from multi-species farms showed generally high levels of resistance with limited susceptibility across the study areas. In Umuahia North and Umuahia South, gentamicin (CN) demonstrated the highest activity, with 60% of isolates

susceptible in both locations, while ofloxacin (OFX) and ciprofloxacin (CPX) were characterized by predominantly intermediate responses (60–80%) and low susceptibility (0–20%). Augmentin (AU) and pefloxacin (PEF) exhibited moderate to high resistance, particularly in Umuahia South where resistance reached 60% and 80%, respectively.

A striking finding across both locations was the consistently high resistance (80%) to cephalosporins—ceftazidime (CTZ), cefuroxime (CEF), and cefepime (CEP)—with no susceptible isolates recorded. In Ikwuano and Obingwa, susceptibility was generally absent or minimal, with fluoroquinolones (OFX and CPX) showing complete intermediate responses (100%), and cephalosporins exhibiting complete resistance (100%). Gentamicin (CN) and trimethoprim (TRX) showed comparatively better performance in these areas, each with 50% susceptibility, although TRX also displayed 50% resistance.

Overall, the results indicate widespread multidrug resistance among *Salmonella* isolates from multi-species farms, with gentamicin remaining more effective than the other antibiotics, while cephalosporins and some β -lactam antibiotics demonstrated markedly reduced efficacy (Table VI).

TABLE VI: ANTIMICROBIAL RESISTANCE PATTERNS OF *SALMONELLA* ISOLATES FROM MIXED-SPECIES FARMS

DRUG	UMUAHIA NORTH			UMUAHIA SOUTH			IKWUANO			OBINGWA		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
OFX	1 (20)	3 (60)	1 (20)	1 (20)	3 (60)	1 (20)	0 (0)	2 (100)	0 (0)	0 (0)	2 (100)	0 (0)
AU	1 (20)	2 (40)	2 (40)	0 (0)	2 (40)	3 (60)	0 (0)	1 (50)	1 (50)	0 (0)	1 (50)	1 (50)
PEF	0 (0)	2 (40)	3 (60)	0 (0)	1 (20)	4 (80)	0 (0)	1 (50)	1 (50)	0 (0)	1 (50)	1 (50)
CTZ	0 (0)	1 (20)	4 (80)	0 (0)	1 (20)	4 (80)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)	2 (100)
CN	3 (60)	1 (20)	1 (20)	3 (60)	1 (20)	1 (20)	1 (50)	1 (50)	0 (0)	1 (50)	1 (50)	0 (0)
CPX	1 (20)	3 (60)	1 (20)	0 (0)	4 (80)	1 (20)	0 (0)	2 (100)	0 (0)	0 (0)	2 (100)	0 (0)
CEF	0 (0)	1 (20)	4 (80)	0 (0)	1 (20)	4 (80)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)	2 (100)
TRX	0 (0)	1 (20)	4 (80)	3 (60)	1 (20)	1 (20)	1 (50)	0 (0)	1 (50)	1 (50)	0 (0)	1 (50)
S*	–	–	–	–	–	–	–	–	–	–	–	–
CEP	0 (0)	1 (20)	4 (80)	0 (0)	1 (20)	4 (80)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)	2 (100)

MEAN ZONE OF INHIBITION OF ANTIMICROBIAL AGENTS

The mean zones of inhibition of antimicrobial agents against *Salmonella* isolates showed no statistically significant difference between single and mixed-species farms for most antibiotics ($p > 0.05$). Ofloxacin, amoxicillin–clavulanate, pefloxacin, cefuroxime, gentamicin, ciprofloxacin, cefuroxime, streptomycin, and cephalexin all showed comparable activity between the two farming systems. However, trimethoprim/sulfamethoxazole demonstrated a significant difference ($p = 0.001$), with a higher mean zone of inhibition in isolates from mixed-species farms (20.86 ± 5.99 mm) compared to single farming systems (10.73 ± 10.64 mm).

DISCUSSION

This study investigated the prevalence and antimicrobial resistance patterns of *Salmonella* isolates obtained from single-species and mixed poultry–fish farms in the Umuahia Agricultural Zone, southeast tern Nigeria. The results provide insight into the potential role of integrated farming systems in the transmission and persistence of antimicrobial-resistant *Salmonella* in livestock production environments. The overall prevalence of *Salmonella* observed in this study was 13.0%, indicating that the pathogen is present in poultry

TABLE VII: MEAN ZONE OF INHIBITION OF ANTIMICROBIAL AGENTS AGAINST *SALMONELLA* ISOLATES

Drug	Single Farming (Mean ± SD)	Multiple Farming (Mean ± SD)	p-value
OFX	20.07 ± 9.74	16.17 ± 9.63	0.185
AU	16.13 ± 7.37	13.36 ± 8.65	0.273
PEF	21.27 ± 10.16	16.57 ± 10.75	0.147
CTZ	10.33 ± 8.30	10.36 ± 7.55	0.992
CN	17.27 ± 8.15	16.26 ± 9.49	0.717
CPX	24.33 ± 5.51	22.05 ± 8.43	0.334
CEF	14.20 ± 10.83	14.10 ± 10.33	0.974
TRX	10.73 ± 10.64	20.86 ± 5.99	0.001*
S	12.93 ± 9.20	12.55 ± 7.76	0.876
CEP	12.47 ± 8.80	13.24 ± 8.44	0.765

Statistical significance was set at $p \leq 0.05$.

and fish farms within the study area. This prevalence is lower than the 24% reported by Ekundayo & Ezeoke (2011), but higher than the 5.6% reported by Nwachukwu & Nwiyi (2011).

Similar variations in *Salmonella* prevalence have been reported in different poultry production systems, where prevalence rates may range widely depending on management practices, environmental conditions, and diagnostic methods used (Djeffal *et al.*, 2018; Adane *et al.*, 2025). Differences in prevalence between studies may be attributed to variations in sampling methods, farm management practices, environmental hygiene, biosecurity measures, and geographic location. Additionally, differences in the types of samples collected and laboratory detection techniques may contribute to variations in reported prevalence rates.

When stratified by farming system, the prevalence of *Salmonella* was higher in integrated (multi-species) farms (16.3%) compared to single-species farms (8.3%). This finding suggests that integrated farming systems may increase the risk of cross-contamination between animal species and their surrounding environment. In integrated poultry–fish production systems, poultry droppings are often used as feed or fertilizer in fish ponds, creating opportunities for the transfer of enteric pathogens from poultry to aquatic environments. Such interactions may facilitate the persistence and circulation of *Salmonella* within the farm ecosystem. Previous studies have indicated that the use of untreated animal manure in aquaculture can introduce enteric pathogens into aquatic environments and contribute to microbial contamination (Petersen *et al.*, 2002; Jie-yi *et al.*, 1998; Elsaidy *et al.*, 2015). Similar observations have also been reported where mixed farming systems were associated with increased microbial contamination due to shared environmental resources and reduced biosecurity barriers (Akanmu *et al.*, 2003; Jeon *et al.*, 2025).

The antimicrobial susceptibility pattern of *Salmonella* isolates in this study revealed a generally reduced and heterogeneous response to most of the tested antibiotics across all locations, with no single antimicrobial agent demonstrating uniform effectiveness across the study LGAs. This variability suggests differing selective pressures and likely reflects widespread and possibly indiscriminate antimicrobial use within the production environments. Overall, fluoroquinolones showed inconsistent activity: ciprofloxacin, although widely regarded as highly effective against Gram-negative pathogens including *Salmonella* (Kloskowski *et al.*, 2010; Thai *et al.*, 2023), exhibited a predominantly intermediate response pattern across both single-species and mixed farms, particularly in Umuahia North and South. This intermediate susceptibility pattern, interpreted according to CLSI breakpoints (22–25 mm), indicates a reduction in bacterial susceptibility and serves as an early warning of declining fluoroquinolone efficacy. Ofloxacin and pefloxacin showed slightly better but still variable performance, with occasional susceptibility

observed, particularly in single-species farms, though the overall pattern remained inconsistent, suggesting instability in fluoroquinolone effectiveness across farming systems. In contrast, β -lactam antibiotics, especially cefuroxime, consistently demonstrated the poorest activity, with high levels of resistance across both single-species and multi-species farms, indicating strong resistance pressure likely driven by prolonged and inappropriate use.

A clearer distinction emerged when comparing single-species and multi-species farm systems. In single-species farms, isolates exhibited relatively higher susceptibility to gentamicin, particularly in Umuahia North and South, where it remained the most consistently effective agent, while other antibiotics such as ciprofloxacin and ofloxacin showed mixed intermediate and resistant responses. However, in multi-species farms, susceptibility patterns were more concerning, with a broader shift toward intermediate and resistant phenotypes across nearly all drug classes, including fluoroquinolones and β -lactams. Gentamicin and trimethoprim retained partial effectiveness in some locations within the mixed farming system, but overall susceptibility was lower compared to single-species farms, suggesting higher antimicrobial pressure and potential cross-species transmission of resistant strains. Notably, cephalosporins (cefuroxime, ceftazidime, and cefepime) were largely ineffective in both systems, with resistance being especially pronounced in multi-species farms where complete resistance was frequently observed. These findings are consistent with previous reports that link intensive livestock production and mixed-species farming systems with increased selection and dissemination of multidrug-resistant *Salmonella* due to extensive antimicrobial exposure (Antunes *et al.*, 2016; Castro *et al.*, 2020; Oh *et al.*, 2025). Overall, the data indicate that resistance is no longer confined to specific antibiotic classes but reflects a broader multidrug resistance trend, with multi-species farms potentially serving as higher-risk reservoirs for resistant *Salmonella* populations.

Overall, the findings highlight a concerning pattern of reduced antimicrobial susceptibility among *Salmonella* isolates, characterized by limited full susceptibility, frequent intermediate responses, and emerging resistance across key antibiotic classes. This underscores the need for improved antimicrobial stewardship and continuous surveillance to prevent further escalation of resistance in Umuahia Agricultural zone.

Statistical analysis of inhibition zones showed that most antibiotics did not differ significantly between isolates from single-species and integrated farming systems ($p > 0.05$), indicating broadly comparable antimicrobial susceptibility profiles across both systems. However, trimethoprim (TRX) was the only antibiotic that demonstrated a statistically

significant difference ($p \leq 0.05$) between the two farming systems, with higher inhibition zones observed in isolates from integrated farms. This suggests a potential variation in susceptibility to trimethoprim that may be associated with differences in antimicrobial exposure patterns or ecological conditions between farming systems. Although the exact mechanism cannot be inferred from the present data, factors such as antimicrobial usage practices, waste management strategies, and microbial competition in integrated farm environments have been previously suggested to influence resistance dynamics (Jeon *et al.*, 2019). Overall, the findings indicate largely similar susceptibility patterns between farming systems, with a notable exception observed for trimethoprim.

The small sample size of nine farm operators represents a limitation of this study, as it may not adequately reflect the broader farming population within the study area, thereby reducing the generalizability of the findings. Consequently, interpretations drawn from the questionnaire data should be made with caution.

Despite this limitation, the questionnaire survey provided valuable insights into farm management practices that may influence pathogen prevalence and antimicrobial resistance. Although most farmers reported vaccinating their animals, only a proportion sought veterinary consultation during disease outbreaks. Furthermore, some respondents indicated the use of animal droppings as feed inputs in integrated farming systems. Such practices may increase the risk of pathogen transmission and environmental contamination, particularly where adequate treatment or biosecurity measures are lacking. Poor antibiotic stewardship and limited veterinary oversight have been widely recognized as key drivers of antimicrobial resistance in livestock production systems globally (Matheou *et al.*, 2025).

Overall, the findings of this study highlight the potential role of integrated farming systems in the persistence and spread of *Salmonella* and antimicrobial-resistant strains within livestock production environments. The interaction between poultry, fish, and environmental components in these systems creates opportunities for pathogen circulation and the exchange of resistance genes among microbial populations (Cabello, 2006; Antunes *et al.*, 2016).

Therefore, improved biosecurity practices, rational use of antibiotics, and regular surveillance of antimicrobial resistance are essential to reduce the risk of zoonotic transmission and protect both animal and public health. Further studies incorporating molecular characterization of *Salmonella* isolates and resistance genes would provide deeper insights into the epidemiology and transmission dynamics of antimicrobial-resistant pathogens in integrated farming systems.

CONCLUSION

This study demonstrates that *Salmonella* isolates from both single-species and multi-species farms exhibit widespread and concerning patterns of antimicrobial resistance, with marked variability in susceptibility across antibiotics and locations. Overall, ceftazidime and other third-generation cephalosporins showed consistently poor efficacy, while ciprofloxacin and other fluoroquinolones largely produced intermediate responses, indicating declining susceptibility rather than full effectiveness. Gentamicin and trimethoprim/sulfamethoxazole retained comparatively better activity in some settings, although their effectiveness was not uniform across all study areas. Importantly, multi-species farms generally exhibited broader resistance profiles compared to single-species farms, suggesting a higher risk of antimicrobial resistance dissemination in mixed farming systems. These findings underscore the persistence of multidrug-resistant *Salmonella* in livestock environments and highlight the urgent need for strengthened antimicrobial stewardship, improved farm biosecurity practices, and sustained surveillance programs to limit further emergence and spread of resistance.

RECOMMENDATIONS

Based on the findings of this study, the following recommendations are proposed:

1. Strengthening biosecurity practices: Biosecurity measures should be enhanced in both single- and mixed-species farms. This includes strict sanitation routines, controlled access to farm areas, and management strategies such as the use of footbaths and dedicated equipment for fish and poultry to minimize direct and indirect contact between both animal species and their waste.
2. Treatment of poultry waste before use in aquaculture: The application of untreated poultry manure in fish ponds should be discouraged. Farmers should adopt appropriate waste treatment methods, such as composting or other controlled processing techniques, to reduce microbial load and prevent the introduction of pathogenic organisms into aquatic environments.
3. Promotion of Antimicrobial Stewardship: Antibiotic use in livestock production must be strictly regulated and supervised by qualified veterinary professionals. Emphasis should be placed on rational drug selection, adherence to recommended dosages, and observance of withdrawal periods to minimize the selective pressure driving antimicrobial resistance.
4. Capacity Building and Farmer Education: Targeted training programs should be implemented to

increase farmers' awareness of zoonotic diseases, antimicrobial resistance, and good agricultural practices. Such education will promote better compliance with biosecurity and prudent antibiotic-use guidelines.

5. Routine Surveillance and Monitoring: Continuous monitoring of *Salmonella* prevalence and antimicrobial resistance patterns in integrated farming systems is essential. Regular surveillance will facilitate early detection of emerging resistant strains and guide evidence-based control interventions.
6. Strengthening Veterinary and Diagnostic Services: Improved access to quality veterinary care and diagnostic facilities is required to enable timely disease detection, accurate diagnosis, and effective farm health management.
7. Further Research: Future studies should include molecular characterization of *Salmonella* isolates to better understand resistance mechanisms and transmission dynamics within mixed-species poultry–fish farming systems.

The implementation of these recommendations is crucial for safeguarding food safety, curbing the spread of antimicrobial resistance, and supporting sustainable and responsible livestock and aquaculture production practices in the region.

ACKNOWLEDGEMENTS

We thank Mrs Laurretta Uwadia and Mrs Chinenye Okoro for their technical assistance.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest that could influence the work reported in this paper.

REFERENCES

- Abdugheni, R., Li, L., Yang, Z. N., Huang, Y., Fang, B. Z., Shurigin, V. & Li, W. J. (2023). Microbial risks caused by livestock excrement: Current research status and prospects. *Microorganisms*, 11(8), 1897
- Adane, P., Tadesse, T., Alemayehu, H. & Eguale, T. (2025). Prevalence, risk factors and antimicrobial susceptibility profile of *Salmonella* in raw chicken eggs sourced from commercial poultry farms and retail shops and in-contact humans in Addis Ababa, Ethiopia. *Journal of Food Protection*, 88(7), 100544
- Adebowale, O., Oyawole, O., Sanni, I., Odunfa, O., Oladejo, O. O., Folorunsho, E. O. & Nelson, G. U. (2025). Cross-sectional study of antimicrobial use on integrated commercial fish–chicken farming in Southwest Nigeria: The link with on-farm biosecurity, farmers' knowledge, and attitudes. *BMC Agriculture*, 1(1), 10.
- Akanmu, A. O., Akol, A. M., Ndolo, D. O., Kutu, F. R. & Babalola, O. O. (2023). Agroecological techniques: Adoption of safe and sustainable agricultural practices among smallholder farmers in Africa. *Frontiers in Sustainable Food Systems*, 7, 1143061.
- Akinbowale, O. L., Peng, H. & Barton, M. D. (2006). Antimicrobial resistance in bacteria isolated from aquaculture sources in Australia. *Journal of applied microbiology*, 100(5), 1103-1113
- Allsopp, M., Santillo, D. & Dorey, C. (2013). Sustainability in aquaculture: Present problems and sustainable solutions. *Ocean Yearbook*, 27(1), 1–22.
- Antunes, P., Mourão, J., Campos, J. & Peixe, L. (2016). Salmonellosis: The role of poultry meat. *Clinical Microbiology and Infection*, 22(2), 110–121
- Ayuti, S. R., Khairullah, A. R., Al-Arif, M. A., Lamid, M., Warsito, S. H., Moses, I. B. & Delima, M. (2024). Tackling salmonellosis: A comprehensive exploration of risk factors, impacts, and solutions. *Open Veterinary Journal*, 14(6), 1313.
- Banerjee, S. & Nur, R. (2014). Study on zooplankton production in ponds under different fish farming systems in West Bengal. *Journal of Krishi Vigyan*, 3(1), 79–83.
- Billah, M. M. & Rahman, M. S. (2024). *Salmonella* in the environment: A review on ecology, antimicrobial resistance, seafood contaminations, and human health implications. *Journal of Hazardous Materials Advances*, 13, 100407.
- Cabello, F. C. (2006). Heavy use of prophylactic antibiotics in aquaculture: A growing problem for human and animal health and for the environment. *Environmental Microbiology*, 8(7), 1137–1144.
- Castro-Vargas, R. E., Herrera-Sánchez, M. P., Rodríguez-Hernández, R. & Rondón-Barragán, I. S. (2020). Antibiotic resistance in *Salmonella* spp. isolated from poultry: A global overview. *Veterinary World*, 13(10), 2070.
- Clinical and Laboratory Standards Institute. (2017). *Performance standards for antimicrobial susceptibility testing* (27th ed., CLSI supplement M100). CLSI.
- Crump, J. A., Sjölund-Karlsson, M., Gordon, M. A. & Parry, C. M. (2015). Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive *Salmonella* infections. *Clinical Microbiology Reviews*, 28(4), 901–937.

- Djeffal, S., Mamache, B., Elgroud, R., Hireche, S. & Bouaziz, O. (2018). Prevalence and risk factors for *Salmonella* spp. contamination in broiler chicken farms and slaughterhouses in northeast Algeria. *Veterinary World*, 11(8), 1102.
- Edwards, P. (2015). Aquaculture environment interactions: past, present and likely future trends. *Aquaculture*, 447, 2-14.
- Elsaidy, N., Abouelenien, F. & Kirrella, G. A. (2015). Impact of using raw or fermented manure as fish feed on microbial quality of water and fish. *Egyptian Journal of Aquatic Research*, 41(1), 93–100.
- Enshaie, E., Nigam, S., Patel, S. & Rai, V. (2025). Livestock antibiotics use and antimicrobial resistance. *Antibiotics*, 14(6), 621.
- Gabriel, U., Akinrotimi, O., Bekibele, D., Anyanwu, P. & Onunkwo, D. (2007). Economic benefit and ecological efficiency of integrated fish farming in Nigeria. *Scientific Research and Essays*, 2, 302–308.
- Gawish, M. F., Ahmed, A. M., Torky, H. A. & Shimamoto, T. (2021). Prevalence of extended- spectrum β -lactamase (ESBL)-producing *Salmonella enterica* from retail fishes in Egypt: A major threat to public health. *International Journal of Food Microbiology*, 351, 109268
- IBM Corp. (2020). *IBM SPSS Statistics for Windows* (Version 27.0) [Computer software]. IBM Corp.
- Jeon, H. Y., Kim, Y. B., Lim, S. K., Lee, Y. J. & Seo, K. W. (2019). Characteristics of cephalosporin-resistant *Salmonella* isolates from poultry in Korea, 2010–2017. *Poultry Science*, 98(2), 957–965.
- Jie-yi, D., Xianzhen, G., Xiu-zhen, F., Meizhen, L. & Wenyou, Z. (1988). *Preliminary studies on the effect of livestock manure application on bacterial fish disease and human hygiene*.
- Jones, B. A., Grace, D., Kock, R., Alonso, S., Rushton, J., Said, M. Y. & Pfeiffer, D. U. (2013). Zoonosis emergence linked to agricultural intensification and environmental change. *Proceedings of the National Academy of Sciences*, 110(21), 8399–8404.
- Kaur, K., Singh, S. & Kaur, R. (2024). Impact of antibiotic usage in food-producing animals on food safety and possible antibiotic alternatives. *The Microbe*, 4, 100097.
- Kloskowski, T., Gurtowska, N. & Drewa, T. (2010). Does ciprofloxacin have an obverse and a reverse? *Pulmonary Pharmacology & Therapeutics*, 23(5), 373–375.
- Little, D. & Edwards, P. (2003). Integrated livestock-fish farming systems. *Food & Agriculture Organization*.
- Matheou, A., Abousetta, A., Pascoe, A. P., Papakostopoulos, D., Charalambous, L., Panagi, S. & Johnson, E. O. (2025). Antibiotic use in livestock farming: A driver of multidrug resistance? *Microorganisms*, 13(4), 779.
- Oh, H., Choi, Y. & Lee, J. (2025). Antibiotic-resistant *Salmonella* in animal products jeopardize human health. *Food Science of Animal Resources*, 45(2), 409.
- Okocha, R. C., Olatoye, I. O. & Adediji, O. B. (2018). Food safety impacts of antimicrobial use and their residues in aquaculture. *Public Health Reviews*, 39(1), 21.
- Petersen, A., Andersen, J. S., Kaewmak, T., Somsiri, T. & Dalsgaard, A. (2002). Impact of integrated fish farming on antimicrobial resistance in a pond environment. *Applied and Environmental Microbiology*, 68(12), 6036–6042.
- Pulido-Villamarín, A. P., Santamaría-Durán, A. N., Castañeda-Salazar, R., Chamorro-Tobar, I., Carrascal-Camacho, A. K., Aranda-Silva, M. & Zambrano-Moreno, C. (2020). Assessment of antibodies against three zoonotic bacteria and associated risk factors in pig farms in Colombia. *Revue Scientifique et Technique (International Office of Epizootics)*, 39(3), 923–945.
- Ramirez-Plascencia, H. H. F., Colima-Fausto, A. G., Licona Lasteros, K. C., Díaz-Zaragoza, M., Cazarez-Navarro, G., Macias-Barragan, J. G. & Rodriguez-Preciado, S. Y. (2025). Presence of microorganisms in the environment: One Health approach. *Microorganisms*, 13(11), 2435.
- Ruzauskas, M., Klimienė, I., Armalyte, J., Bartkiene, E., Šiugždiniene, R., Skerniskyte, J., Krasauskas, R. & Suziedeliene, E. (2018). Composition and antimicrobial resistance profile of Gram-negative microbiota prevalent in aquacultured fish. *Journal of Food Safety*, 38(3), e12447
- Thai, T., Salisbury, B. H. & Zito, P. M. (2023). Ciprofloxacin. In *StatPearls*. StatPearls Publishing.
- Ukwueze, F. (2019). Evaluation of food safety and quality regulations in Nigeria. *Journal of Law, Policy and Globalization*, 92, 149–155.