Antibiogram and risk factors for carriage of some antimicrobial-resistant bacteria species in chickens (*Gallus domesticus*) in Maiduguri, Northeast Nigeria

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Abstract

The misuse of antibiotics in poultry farming is a growing concern due to the emergence and spread of antimicrobial-resistant bacteria. This study investigated the prevalence and antibiotic resistance profiles of some bacterial species isolated from broiler and layer chickens in Maiduguri, Borno State, Nigeria. A total of 600 chickens from backyard and commercial poultry farms were sampled. Standard bacteriological methods were used for isolation and identification of *Escherichia coli*, *Salmonella* spp., *Staphylococcus* spp., *Streptococcus* spp. and *Enterococcus* spp. The antibiotic susceptibility of these isolates was assessed using the disk diffusion method. Results showed that 80.3% of the chickens harbored at least one of the targeted bacterial species. *E. coli* was the most prevalent (45.8%), followed by *Salmonella* spp. (37.3%), *Staphylococcus* spp. (15.3%), *Streptococcus* spp. (13.5%), and *Enterococcus* spp. (8.7%). Antibiotic resistance testing revealed high levels of resistance across all isolates, with multi-drug resistance recorded in a significant number of cases. *E. coli* and *Salmonella* spp. exhibited resistance rates exceeding 50% to ampicillin, amoxicillin, and penicillin G. A survey of 150 poultry farmers highlighted that 78.7% use antibiotics prophylactically, often based on advice from non-veterinarians, which is a statistically significant (p < 0.001) risk factor for the emergence of resistant bacteria. Only 16.7% of farmers obtained antibiotics based on veterinary prescriptions, and awareness of antimicrobial resistance among farmers was relatively low (42.0%). The outcome of the study underscores the urgent need for improved antibiotic stewardship and farmer education to mitigate the risk of antibiotic resistance in poultry farming.

Keywords: Anti-microbial resistance; Poultry; Farmers; Enterobacteria; Chickens; Microbiota; Gram-positive organisms; Stewardship; Maiduguri Nigeria.

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Introduction

Poultry farming serves as a critical source of protein for an expanding global populace. However, the widespread use of antibiotics in this industry has sparked concerns with regards to the proliferation of antibiotic-resistant pathogens. Globally, antibiotic resistance poses a significant threat to both human and animal health, stemming from the excessive and often inappropriate utilization of antibiotics across various sectors, including agriculture and veterinary medicine (Larsson and Flach, 2022; Caneschi *et al.*, 2023; Salam *et al.*, 2023).

Antibiotic resistance occurs as microorganisms evolve to withstand antibiotic treatments, leading to the ineffectiveness of these drugs. The consequences of antibiotic resistance extend across public health, healthcare systems, economies and food security. It undermines the efficacy of antibiotics, resulting in prolonged illnesses, escalated healthcare costs, treatment failures and complications in medical procedures (Serwecińska, 2020; Uddin *et al.*, 2021; Mo *et al.*, 2023).

In agriculture, including poultry farming, antibiotics are commonly used for disease prevention and growth promotion (Galadima *et al.*, 2018; Andrew *et al.*, 2020; Xu *et al.*, 2020). Antibiotic-resistant pathogens in poultry can contaminate food products, posing risks to human health through the food chain (Murugaiyan *et al.*, 2022). Moreover, antibiotic resistance in poultry directly impacts human and animal health (Okon *et al.*, 2022). Misuse of antibiotics in poultry farming can constrain treatment options for common infectious pathogens, leading to increased mortality rates (Pulingam *et al.*, 2022; Habiba *et al.*, 2023). It also jeopardizes animal welfare, livestock industry sustainability, and inflicts economic losses on farmers (Ma *et al.*, 2021; Samreen *et al.*, 2021; Salam *et al.*, 2023). Furthermore, concerns arise regarding zoonotic diseases, as resistant bacteria in poultry can potentially transfer to humans, exacerbating the overall antibiotic resistance dilemma. Despite the implementation of stringent regulations governing antimicrobial use in many countries, enforcement gaps persist, contributing to the unchecked utilization of antimicrobials. Nigeria, being one of Africa's largest poultry producers in Africa, confronts the pressing challenge of regulating antibiotic use and addressing its repercussions in this sector (Andrew *et al.*, 2020; Achi *et al.*, 2021).

In the northeastern region of Nigeria, particularly in Maiduguri, the capital city of Borno State, poultry farming serves as one of the cornerstone of livelihoods and sustenance for many small holder poultry farmers and households. However, the prevalence of antimicrobial resistance in poultry within this region has not been adequately investigated/reported, despite its critical implications for both animal and human health. The present study investigated the antibiogram and identified the risk factors associated with the carriage of antimicrobial-resistant Enterobacteria species in chickens (*Gallus domesticus*) in Maiduguri, Northeast Nigeria.

Materials and Methods

Study area: The study area is Maiduguri, which is the capital and largest city of Borno State, located within the Sahel Savannah zone of the Northeastern region of Nigeria. It lies approximately between 11° 5' and 11.83° N latitude and 13° 09' and 13.50° E longitude. The agricultural landscape in Maiduguri, Borno State, includes a substantial presence of livestock farming, with a particular emphasis on poultry (Makinta *et al.*, 2019). The poultry industry is a vital component of the local economy, contributing significantly to both food production and economic livelihoods.

Study Scope: The study focused on layers and broiler chickens from backyard and commercial poultry farms situated within the study area.

Study Design: A longitudinal observational study was conducted from February to November, 2023 in backyard and commercial poultry farms that keep only chickens within Maiduguri metropolis. Chickens in a total of 150 poultry farms were sampled, of which only 25 were registered with the Poultry Clinic of the University of Maiduguri Veterinary Teaching Hospital (UMVTH). Farms of varying sizes were included in the study to capture a representative sample, from small-scale to large-scale operations.

Informed Consent from Farm Caretakers: Samples were collected from farms whose owners/managers have given their informed consent to participate in the study.

Sample collection, bacterial isolation, and identification: A total of 600 chickens were sampled, from which 300 oral swabs and 300 cloacal swabs were collected, randomly. The swab samples were transported on ice to the Department of Veterinary Medicine Research and Teaching Laboratory, University of Maiduguri, Nigeria and processed immediately on arrival for *Salmonella*, *Escherichia coli*, *Staphylococcus*, and *Enterococcus* isolation. The protocols involved in the isolation and identification of the Enterobacteria were carried out according to standard procedure as previously described by Şahan *et al.* (2022) and Ahmed and Gulhan (2024)

Bacteriological (Microbiology) Analysis of Samples: Cell Morphology, Staining Characteristics, and Bacteria Identification: Samples collected underwent a pre-enrichment process through inoculation into tryptic soy broth (TSB), a non-selective medium following standard microbiological procedures. The samples were then incubated at 37°C for 24 hours to foster the growth of a broad spectrum of bacteria. A fraction of the

pre-enriched sample was transferred onto various agar plates containing selective enrichment media, including Mitis Salivarius Agar, Baird-Parker agar with egg yolk tellurite, Eosin Methylene Blue (EMB) agar, MacConkey agar, malachite green-based Rappaport-Vassilliadis (UK) medium with soya (RVS) broth, Selenite F broth (Himedia), and Bile Esculin Agar, specific for presumptive bacteria. All inoculated plates were then incubated at 37°C for 24 hours, and the selective plates were observed for colony growth. All processes were carried out according to standard procedure as previously described by Şahan *et al*. (2022) and Ahmed and Gulhan (2024).

Observations included the colony morphology of *Streptococcus* species, *Escherichia coli*, *Staphylococcus* species, *Salmonella* species, and *Enterococcus* species. Pure cultures of each bacterial type were obtained following standard procedures as previously described by OIE (2000). Gram staining, carried out according to standard procedures, was used to identify Gram-positive and Gram-negative bacteria. Bacteriological examinations were conducted using the standard method for facultative anaerobic bacteria, following the procedures outlined by Waite and Taylor (2014).

Among the various characteristics examined, cell morphology traits such as shape (bacilli, spiral, filamentous) and the arrangement of bacterial cells, appearing in chains and clusters, were observed under a compound microscope after staining.

The isolated organisms underwent a series of biochemical tests, including the lactose fermentation test, oxidase test, catalase test, indole test, methyl red test, Voges-Proskauer test, citrate (IMViC), motility, and urease test. These tests were conducted following the standard procedures outlined by Cheesbrough (1985).

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Antimicrobial Susceptibility Testing: Antimicrobial susceptibility assays for all bacterial isolates were conducted using the disk diffusion method on Mueller-Hinton agar (Oxoid Ltd, Hampshire, UK), according to the guidelines of Clinical and Laboratory Standards Institute (CLSI, 2022).

The isolates were evaluated for susceptibility and resistance by impregnation with the following 19 commercially available antimicrobial agents used in veterinary and human applications belonging to eight classes: (i). Beta-lactamase inhibitors – Amoxicillin-Clavulanic acid (AMC, 30 µg), Ampicillin (AMP 10 µg), Amoxicillin (AMX 10 µg), Penicillin G (PEN 10 units), and Ceftazidime (CET 30 µg); (ii). Aminoglycosides – Streptomycin (STP 10 µg), Gentamicin (GEN 10 µg), and Neomycin (NEO 30 µg); (iii). Fluoroquinolones – Ciprofloxacin (CIP 10 µg), Ofloxacin (OFX 5 µg), Enrofloxacin (ENR 10 µg), and Levofloxacin (LEV 5 µg); (iv). Cephalosporin – Ceftriaxone (CEF 30 µg); (v). Nitrofuran derivative – Nitrofurantoin (NIT 300 µg); (vi). Diaminopyrimidine with sulfonamide – Trimethoprim/Sulfamethoxazole (Cotrimoxazole) (TRI/SUL 30 µg); (vii). Macrolides – Tylosin (TLY 10 µg) and Erythromycin (ERY 15 µg); and (viii). Tetracyclines – Oxytetracycline (OXY 10 µg) and Doxycycline (30 µg).

Diameter of the zone of inhibition around the antibiotic disc for each isolated bacterial strain was measured and recorded according to the CLSI guidelines (CLSI, 2022) as sensitive, intermediate or resistant. Intermediate results were considered as resistant (Ema *et al.*, 2022). Multiple antibiotic-resistant (MAR) phenotypes were recorded for isolates showing resistance to three or more antimicrobial agents (Peruzy *et al.*, 2020).

Questionnaire Survey: The questionnaire for this study was carefully crafted to investigate the use/misuse of antimicrobial agents among commercial poultry farmers in Maiduguri,

Borno State, Nigeria. A thorough review of relevant literature on antibiotic resistance in poultry farming guided the formulation of questions covering different factors contributing to antimicrobial resistance in bacterial microbiota among commercial broilers and laying chickens in the region.

To ensure the questionnaire's reliability and validity, a panel of experts in poultry farming, veterinary medicine, and public health scrutinized the questions and provided feedback for clarity, comprehensiveness, and relevance. A pilot study with a small group of poultry farmers from Maiduguri helped further refine the questionnaire based on feedback.

Before the main data collection, the questionnaire underwent pre-testing to assess its effectiveness. A sample of poultry farmers representative of the target population received the questionnaire, aiming to evaluate the instructions' clarity, question comprehensibility, relevance of response options, and overall flow. Feedback from pretest participants guided adjustments to ensure the questionnaire's suitability.

The finalized questionnaire included sections addressing critical aspects related to antimicrobial use and resistance in poultry farming. These sections covered inquiries about the reasons for antimicrobial administration, the individuals prescribing antimicrobials, frequency and types of antimicrobials used, and poultry farmers' awareness and knowledge of antimicrobial resistance pathogens.

Data Analysis: Data analysis was performed using GraphPad Prism software (GraphPad Inc., San Diego, CA). Data obtained from the study were presented as percentages. The variances in the occurrence of bacterial microbiota isolates in the samples gathered from both broilers and layer chickens were examined using the Chi-square test, differences were considered significant for p-

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values equal to or less than 0.05. Analysis for the relative risks and odds ratios was estimated using the GraphPad Prism software. Calculation of the lower and upper limits of the 95% confidence interval (CI) for a proportion was done according to the methods described by Newcombe (1998).

Results

Prevalence of Bacterial Organisms in Oral and Cloacal Swabs of Broilers and Layers: A summary of the prevalence of bacterial microbiota species isolated from chickens in Maiduguri, Borno State, Nigeria, is presented in Table 1. Out of the 600 chickens sampled, bacterial microbiota of five different species, namely *Escherichia coli*, *Salmonella* species, *Staphylococcus* species, *Streptococcus* species and *Enterococcus* species were isolated in pure cultures from 482 (80.3%) chickens. Among the bacterial microbiota isolates, *Escherichia coli* was identified as the most prevalent organism: *E. coli* was isolated from 275 chickens, with a prevalence of 45.8% (95% CI: 41.9% – 49.8%) followed in descending order, by *Salmonella* species (15.7%; 95% CI: 13.0% – 18.8%), *Staphylococcus* (8.0%; 95% CI: 6.1% – 10.5%), *Streptococcus* species (6.3%; 95% CI: 4.7% – 8.6%), and *Enterococcus* species (4.5%; 95% CI: 3.1% – 6.5%) [Table 1].

A comparative analysis of the prevalence of the bacterial microbiota in oral swabs from broilers and layer chickens in Maiduguri, Borno State, Nigeria is summarized in Table 2. Out of the 300 broilers and 300 layer chickens sampled, *Escherichia coli* were isolated from 89 (29.7%) broilers and 14 (4.7%) layer chickens. The prevalence of *Escherichia coli* was significantly (p < 0.0001) higher in broilers when compared to layers. Moreover, out of the 300 broilers and 300 layer chickens sampled, *Staphylococcus* species were isolated from 26 (8.7%) broilers and 22 (7.3%) layers, and there was no significant difference (p = 0.5472) the in the prevalence of *Staphylococcus* species in broilers and layers. *Streptococcus* species were isolated from 23 (7.7%) broilers and 15 (5.0%) layer chickens, and the prevalence of Streptococcus did not significantly (p = 0.1799) differ between broilers and layers.

Table 1. Prevalence of bacterial microbiota species in oral and cloacal swabs of 600 chickens in Maiduguri, Borno State, Nigeria

Bacteria	Number (%)	95% CI
Escherichia coli	275 (45.8%)	41.9 – 49.8%
Salmonella species	94 (15.7%)	13.0 – 18.8%
Staphylococcus species	48 (8.0%)	6.1 – 10.5%
Streptococcus species	38 (6.3%)	4.7 – 8.6%
Enterococcus species	27 (4.5%)	3.1 – 6.5%
Overall	482 (80.3%)	77.0 – 83.3%

Key: N = Number of chickens sampled; CI = Confidence Interval.

Table 2. Comparative analysis of the prevalence of bacterial microbiota in oral swabs from broilers (300) and layers (300) chickens in Maiduguri, Borno State, Nigeria.

Microbiota species isolated	Type of chicken	Number of chicken with the microbe	Prevalence (%)	*P*-value
Staphylococcus spp.	Broilers	26	8.7%	
	Layers	22	7.3%	0.5472
Total		48	8.0%	
Streptococcus spp.	Broilers	23	7.7%	
	Layers	15	5%	0.1799
Total		38	6.3%	
Escherichia coli	Broilers	89	29.7%	
	Layers	14	4.7%	< 0.0001
Total		103	17.2%	

Comparison of the prevalence of bacterial microbiota obtained from the cloacal swabs from broilers and layers sampled is summarized in Table 3. *Escherichia coli* were isolated from 62 (20.7%) broilers and 110 (36.7%) layers chickens, and the difference between the prevalence in broilers and layers was statistically significant (p < 0.0001). *Salmonella* species were isolated from 27 (9.0%) broilers and 67 (22.3%) layers. The prevalence of *Salmonella* species was significantly (p < 0.0001) lower in broilers when compared to layers. *Enterococcus* species were isolated from 6 (2.0%) broilers and 21 (7.0%) layers chickens. The prevalence of *Enterococcus* species was also significantly (p = 0.0031) lower in broilers when compared to layers.

Table 3. Comparative analysis of the prevalence of bacterial microbiota in cloacal swabs from broilers (300) and layers (300) chickens in Maiduguri, Borno State, Nigeria.

Microbiota species isolated	Type of chicken	Number of chicken with the microbe	Prevalence (%) 95% CI	*P* -value
Escherichia coli	Broilers	62	20.7%	
	Layers	110	36.7%	< 0.0001
	Total	172	28.6%	
Salmonella species	Broilers	27	9.0%	
	Layers	67	22.3%	< 0.0001
	Total	94	15.7%	
Enterococcus species	Broilers	6	2.0%	
	Layers	21	7.0%	0.0031
	Total	27	4.5%	

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Antimicrobial Resistance Profile of Bacterial Isolates: The overall prevalence of antimicrobial susceptibility and resistance among the 48 *Staphylococcus* species isolates from broilers and layers chickens in Maiduguri, Borno State, Nigeria, is summarized in Table 4. The results reveal a higher prevalence of *Staphylococcus* species isolates exhibiting resistance greater than 50% to the following antibiotics: Ampicillin, Amoxicillin, and Penicillin G. Additionally, there was significant resistance to Streptomycin, Tylosin, Oxytetracycline, and Doxycycline. Furthermore, the isolates exhibited high resistance to Ceftazidime, Nitrofurantoin, and Trimethoprim/Sulfamethoxazole. However, these isolates displayed varying levels of susceptibility to other tested antimicrobials, such as Amoxicillin-Clavulanic acid, Gentamicin, Neomycin, Ciprofloxacin, Enrofloxacin, Ofloxacin, Levofloxacin, Erythromycin, and Ceftriaxone. Among the isolates exhibiting resistance to the antimicrobial agents, 34 (70.8%) isolates showed multidrug resistance to some of the tested drugs, while 14 (29.2%) show multidrug susceptibility.

The prevalence of antimicrobial susceptibility and resistance among the 275 *Escherichia coli* isolates from the broilers and layer chickens sampled is presented in Table 4. A high prevalence of antimicrobial resistance (exceeding 50%) in *Escherichia coli* isolates to the following antibiotics was recorded: Ampicillin, Amoxicillin, Penicillin G, and Amoxicillin-Clavulanic acid. Additionally, there is elevated resistance to Erythromycin and Tylosin, and Oxytetracycline and Doxycycline. The isolates also exhibited high resistance to Ceftriaxone and Ceftazidime. Furthermore, the isolates demonstrated increased resistance to Nitrofurantoin, and Trimethoprim/Sulfamethoxazole. However, *Escherichia coli* isolates displayed varying levels of susceptibility to drugs within the Aminoglycosides and Quinolones families.

Notably, all *Escherichia coli* isolates exhibited multidrug resistance to the tested antimicrobial drugs.

Antimicrobial susceptibility and resistance prevalence among the 94 *Salmonella* species isolates from the broilers and layer chickens sampled is summarized in Table 4. There was a high prevalence of antimicrobial resistance in *Salmonella* species isolates (exceeding 50%) for all tested antimicrobial agents. However, *Salmonella* species isolates exhibited higher levels of susceptibility (greater than 50%) to drugs under the Quinolones family. Among the *Salmonella* species isolates resistant to the antimicrobial tests, 55 (58.5%) isolates demonstrated multidrug resistance to some of the tested antimicrobials, while 39 (41.5%) showed multidrug susceptibility

The prevalence of antimicrobial susceptibility and resistance among the 38 *Streptococcus* species isolates from the broilers and layers chickens sampled is presented in Table 4. There was a high prevalence of antimicrobial resistance (reaching 100%) among *Streptococcus* species isolates to antibiotics such as Ampicillin, Amoxicillin, and Penicillin G. Additionally, resistance to Gentamicin and Neomycin was recorded, as well as to all tested antimicrobials in the Macrolide, Tetracyclines, and Nitrofuran derivative families. However, *Streptococcus* species isolates demonstrated higher levels of susceptibility (greater than 50%) to drugs such as Amoxicillin-Clavulanic acid, Streptomycin, and all antimicrobial agents tested. Among the *Streptococcus* species isolates exhibiting resistance to the antimicrobial tests, 81.6% of the isolates showed multidrug resistance, while 18.4% showed multidrug susceptibility.

Antimicrobial susceptibility and resistance prevalence among the 27 *Enterococcus* species isolates from the broilers and layers chickens sampled is summarized in Table 4. There was a 100% prevalence of antimicrobial resistance among *Enterococcus* species isolates to

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antibiotics such as Ampicillin, Amoxicillin, Penicillin G, and Amoxicillin-Clavulanic acid. Resistance to Gentamicin, Streptomycin, and Neomycin was recorded, as well as to Erythromycin and Tylosin. Resistance was also recorded to all drugs tested in the Tetracyclines, Nitrofuran derivative, and Diaminopyrimidine with sulfonamide families. *Enterococcus* species isolates exhibited higher levels of susceptibility (greater than 50%) to all drugs tested in the Quinolones and Cephalosporin families. All *Enterococcus* species isolates demonstrated 100% multidrug resistance.

Table 4. Antimicrobial resistance profile of *Staphylococcus*, *Escherichia coli*, *Salmonella, Streptococcus*, *Enterococcus* species isolates from chicken in Maiduguri, Borno State, Nigeria.

Antimicrobials	Resistance profile (%)				
	Staphylococcus	*Escherichia coli*	*Salmonella*	*Streptococcus*	*Enterococcus*
AMP 10 µg	40 (83.3%)	271 (98.5%)	54 (57.4%)	38 (100%)	27 (100%)
AMO 10 µg	36 (75.0%)	253 (92.0%)	57 (60.6%)	38 (100%)	27 (100%)
AMO/CLA 30 µg	14 (29.2%)	158 (57.5%)	69 (73.4%)	10 (26.3%)	27 (100%)
PEN 10 units	30 (62.5%)	275 (100%)	78 (83.0%)	38 (100%)	27 (100%)
GEN 10 µg	12 (25.0%)	135 (49.1%)	72 (76.6%)	38 (100%)	27 (100%)
STP 10 µg	44 (91.7%)	101 (36.7%)	79 (84.0%)	15 (39.5%)	27 (100%)
NEO 30 µg	8 (16.7%)	117 (42.5%)	71 (75.5%)	38 (100%)	27 (100%)
CIP 10 µg	2 (4.2%)	36 (13.1%)	8 (8.5%)	6 (15.8%)	22 (81.5%)
ENR 10 µg	11 (22.9%)	73 (26.5%)	11 (11.7%)	16 (42.1%)	18 (66.7%)
OFX 5 µg	1 (2.1%)	13 (4.7%)	4 (4.3%)	2 (5.3%)	24 (88.9%)
LVX 5 µg	23 (47.9%)	37 (13.5%)	8 (8.5%)	4 (10.5%)	21 (77.8%)
ERY 15 µg	6 (12.5%)	275 (100%)	81 (86.2%)	38 (100%)	27 (100%)
TLY 10 µg	38 (79.2%)	275 (100%)	94 (100%)	38 (100%)	27 (100%)
OXY 10 µg	36 (75.0%)	231 (84.0%)	56 (59.6%)	38 (100%)	27 (100%)
DOX 30 µg	41 (85.4%)	201 (73.1%)	59 (62.8%)	38 (100%)	27 (100%)
CEF 30 µg	6 (12.5%)	263 (95.6%)	94 (100%)	18 (47.5%)	18 (67.7%)
CET 30 µg	47 (97.9%)	255 (92.7%)	94 (100%)	14 (36.8%)	22 (81.5%)
NIT 300 µg	27 (56.3%)	201 (73.1%)	81 (86.2%)	38 (100%)	27 (100%)
TRI/SUL 30 µg	42 (87.5%)	190 (69.1%)	69 (73.4%)	10 (26.3%)	27 (100%)
Multidrug resistance	34 (70.8%)	275 (100%)	55 (58.5%)	31 (81.6%)	27 (100%)

Key: Ampicillin (AMP 10 µg), Amoxicillin (AMO 10 µg), Amoxicillin-Clavulinate acid (AMO/CLA 30 µg) (Augmentin), Penicillin G (PEN 10 units), Gentamicin (GEN 10 µg), Streptomycin (STP 10 µg), Neomycin (NEO 30 µg), Ciprofloxacin (CIP 10 µg), Enrofloxacin (ENR 10 µg), Ofloxacin (OFX 5 µg), Levofloxacin (LVX 5 µg), Erythromycin (ERY 15 µg), Tylosin (TLY 10 µg), Oxytetracycline (OXY 10 µg), Doxycycline (DOX 30 µg), Cepftriaxone (CEF 30 µg), Ceftazidime (CET 30 µg), Nitrofurantoin (NIT 300 µg), Trimetoprim/Sulfamethoxazole (Cotrimoxazole) (TRI/SUL 30 µg).

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Results of Questionnaire Survey: The responses of poultry farmers regarding various factors influencing antimicrobial misuse among commercial poultry farmers in Maiduguri, Borno State, Nigeria, are summarized in Table 5. Out of 150 respondents, 32 (21.3%) responded that they administer antimicrobials to their birds therapeutically to treat illnesses, while 118 (78.7%) stated that they use antimicrobials prophylactically to prevent their birds from becoming ill due to infections. Poultry farmers who administer antimicrobials prophylactically to prevent infection were significantly (p < 0.001) than those that administer it therapeutically.

Twenty five out of 150 respondents (16.7%) indicated that the prescriptions and directions

regarding which brand of antimicrobials to administer and how to administer them to birds were given by registered veterinarians, while 125 (83.3%) stated that such prescriptions and directions are given by non-veterinarians, primarily fellow poultry farmers and drug sellers. Significantly higher (p < 0.001) number of poultry farmers get their prescriptions from non-veterinarians.

Regarding the knowledge of antimicrobial resistant pathogens among poultry farmers in the study area, out of 150 respondents, 63 (42.0%) stated that they are aware of antimicrobial resistant pathogens among birds, while 87 (58.0%) responded that they are unaware of such resistant pathogens.

Table 5. Responses of commercial poultry farmers in Maiduguri, Borno State, Nigeria with regards to antimicrobial use/misuse.

Variables	Responses	Number of Respondents	Percentage of respondents	*p*-value
Rationale behind antimicrobial administration	Therapeutic	32	21.3%	< 0.001
	Prophylactic	118	78.7%	
Individuals responsible for prescribing antimicrobials	Veterinarians	25	16.7%	< 0.001
	Non-Veterinarians	125	83.3%	
Level of awareness regarding antimicrobial resistance pathogens	Aware	63	42.0%	> 0.05
	Unaware	87	58.0%	

Discussion

The overall prevalence rate of bacterial microbiota in the sampled chickens was 80.3%, indicating a substantial presence of bacterial infections in the poultry population. The study identified five key bacterial species – *Escherichia coli*, *Salmonella* species, *Staphylococcus* species, *Streptococcus* species, and *Enterococcus* species, with varying prevalence rates of 45.8%, 15.7%, 8.0%, 6.3%,

and 4.5%, respectively. This finding highlights a significant microbial burden in commercial poultry population system in the study area, with potentials of risks to both poultry and public health. The findings of the present study concur with those reported by Pal *et al*. (2022), who conducted a similar investigation on broiler and layer birds in Nepal. However, Pal *et al*. (2022) did not report the isolation of *Staphylococcus* species and *Streptococcus*

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species, though our study found them present. Nonetheless, Pal *et al*. (2022) identified *Enterococcus* species, *Salmonella* species, and *Escherichia coli* in 36%, 39%, and 63% of swabs from broiler birds, respectively. In layer birds, they detected *Enterococcus* species, *Salmonella* species, and *Escherichia coli* in 31%, 48%, and 60% of swabs, respectively. It is thought that the disparity between the two studies may lie in the source of the samples; the earlier cited study was on samples collected from the cloacal orifice, whereas the present study worked with swabs of both oral and cloacal orifices.

Escherichia coli emerged as the most prevalent bacterial species, particularly in broilers, emphasizing its significance in poultry microbiota; this finding is in line with reports by Mandal *et al*. (2022) and Pal *et al*., (2022). The relatively high recovery rates of *Escherichia coli* from both cloacal and oral swabs in layers and broiler chickens sampled from commercial poultry farms in this study can be attributed to various factors. Firstly, *Escherichia coli* is a normal component of the intestinal microbiota in poultry, making its presence in cloacal swabs and the oral cavity common (Ribeiro *et al*., 2023; Islam *et al*., 2023). Fecal contamination, facilitated by the cloaca being the common opening for the digestive, urinary, and reproductive tracts, might contribute to the higher recovery rates of *Escherichia coli* from these orifices (Lee *et al*., 2020; Moffo *et al*., 2021). Poultry environments, including feed, water, and facilities, can be contaminated with *Escherichia coli*, and the poor hygiene practices observed in some of the poultry farms may further exacerbate the issue. In addition, stress conditions, such as overcrowding as observed in some of the poultry farms and environmental changes such as high environmental temperature, might weaken the birds' immune systems, increasing *Escherichia coli* shedding, as reported by Abo Ghanima *et al*. (2020) and Hirakawa *et al*.

(2020). Also, it has been reported that underlying diseases affecting the gastrointestinal tract and the misuse of antibiotics in poultry farming can lead to higher recovery rates of *Escherichia coli* from broilers and layers chickens (Mandal *et al*., 2022).

The higher prevalence of *Escherichia coli* in broilers compared to layers in this study may be attributed to prevailing management practices, as broilers are often intensively raised with higher stocking densities, creating an environment conducive to bacterial transmission. This finding concurs with earlier reports by Halder *et al*. (2021) and Tan *et al*. (2023), which indicated *Escherichia coli* strains as the most prevalent bacterial pathogen isolated from chickens. Gedeno *et al*. (2022) and Pereira *et al*. (2024) have also reported *Escherichia coli* followed by *Salmonella* species as the most prevalent bacterial organism in chickens, even though their studies involved the collection of samples from sick chickens and live bird markets, respectively, compared to the present study where samples were collected from apparently healthy chickens and commercial poultry farms.

The occurrence of *Salmonella* species as part of the microbiota in layers and broilers raised in commercial poultry farms observed in this study is a cause for concern, given that *Salmonella* is a recognized zoonotic pathogen with implications for food safety. It ranks among the most critical foodborne pathogens globally, contributing to severe public health challenges (Ehuwa *et al*., 2021; Galán-Relaño *et al*., 2023). The finding of *Salmonella* species in commercial chickens in this study agrees with earlier reports by Zeng *et al*. (2019), Pal *et al*. (2022) and Tan *et al*. (2023), all of who found this bacterial species in apparently healthy chickens. However, the *Salmonella* isolates recovered in this current study are non-typhoidal strains which constitute part of normal commensal flora of chickens; this is consistent with the findings of Khan *et al*.

(2018). The isolation of *Salmonella* species in cloacal swabs from both layer and broiler chickens in this study may be due to several factors. Primarily, it is thought that inappropriate biosecurity measures and unsanitary management practices in commercial poultry farms from which the samples were collected could facilitate the transmission of non-infectious and infectious pathogens. This aligns with the findings of Wiersema *et al*. (2021) and Gast *et al*. (2022), who observed that dense stocking conditions and birds being in close proximity could enhance the spread of *Salmonella* via fecal-oral pathways. Another important factor contributing to the reasonable presence of *Salmonella* in poultry is the asymptomatic carriage of the bacteria by seemingly healthy birds. It has been reported that both laying hens and broiler chickens can harbor *Salmonella* in their digestive systems without displaying any outward signs of illness, as highlighted by Wang *et al*. (2023). Consequently, these carrier birds can excrete the bacteria in their feces, leading to contamination of the farm surroundings, including feed, water and bedding materials, posing a significant risk of infection to other birds within the flock (Ethèves *et al*., 2021).

The results of this present study showed a notable presence of *Staphylococcus*, *Streptococcus*, and *Enterococcus* species within poultry populations in the study area, highlighting the intricate nature of microbial communities in these birds. A comparative analysis between broilers and layers revealed differing prevalence rates, with broilers exhibiting higher rates of all isolated bacteria in their oral swabs when compared to layers. This suggests potential variations in susceptibility, management practices, or exposure routes to bacterial infections among these chicken types. While previous studies by Kwoji *et al*. (2019) and Abdulrahman *et al*. (2018) reported higher prevalence rates of *Staphylococcus aureus* in village chickens from

the present area, our findings showed lower rates in commercial poultry. Notably, our study is the first to report the isolation of *Streptococcus* species from commercial broilers and layers in this region, a bacterium with known zoonotic potential linked to chicken exposure (Patel et al., 2023). Additionally, the prevalence rates of *Streptococcus* (53.1%) and *Enterococcus* (42.8%) species reported by Abed *et al*. (2021) were higher than those found in the present study, possibly due to differences in diagnostic methods; their study utilized molecular tools, while ours relied on phenotypic identification.

The present study identified a trend of concern among commercial poultry farmers in the study area, as seen in certain regions of Nigeria and some parts of the world, where antimicrobial compounds are used indiscriminately (Imam *et al*., 2020; Chah *et al*., 2022; Ndahi *et al*., 2023). When examining the prevalence of microbiota isolates in chickens from our study area, our findings highlight the significant impact of antibiotic misuse on the emergence and spread of antibiotic-resistant pathogens in poultry. The varying levels of antimicrobial resistance observed in *Staphylococcus*, *Escherichia coli*, *Salmonella*, *Streptococcus*, and *Enterococcus* isolates from apparently healthy chickens in our study highlights the significant consequences of widespread and indiscriminate antibiotic usage in poultry farming. This situation poses serious threats to both poultry health and food safety in the study area, as it potentially reduces the effectiveness of available treatments for veterinary and human infections alike. Additionally, the presence of zoonotic pathogens among these resistant bacteria highlights the potential transmission risks to humans (Bamidele *et al*., 2022; Raut *et al*., 2023). Previous studies from some developing countries including Nigeria has identified various factors contributing to antibiotic misuse in poultry, including suboptimal

dosing, prolonged administration and the utilization of antibiotics for growth promotion rather than therapeutic reasons (Xu *et al*., 2020; Chah *et al*., 2022; Ndahi *et al.*, 2023). These practices exert selection pressure, favoring the proliferation of antibiotic-resistant bacteria within the poultry population (Abreu *et al.*, 2023).

Findings in the present study showed a significantly high prevalence of resistance, exceeding 50%, among *Staphylococcus* species isolates to commonly utilized antibiotics, notably Ampicillin, Amoxicillin, and Penicillin G, all falling under the critical Penicillin (Beta-lactams) category. This aligns with several similar studies conducted in Nigeria (Ezeh *et al*., 2023), indicating a substantial challenge in effectively treating *Staphylococcus* species infections in poultry using these extensively employed antibiotics. The resistance extends beyond specific antibiotic classes, encompassing notable resistance to Streptomycin, Tylosin, Oxytetracycline, and Doxycycline. This wide-ranging resistance pattern suggests a multifaceted issue necessitating meticulous consideration and comprehensive strategies for antibiotic stewardship.

Furthermore, this study highlights the high resistance of *Staphylococcus* species isolates to crucial antibiotics such as Ceftazidime, Nitrofurantoin, and Trimethoprim/Sulfamethoxazole. Particularly worrisome is the resistance to Cephalosporins, given their significance as last-resort treatments against resistant pathogens (Mancuso *et al*., 2021; Uddin *et al*., 2021; Chukwu *et al*., 2022). The presence of multidrug resistance in 70.8% of *Staphylococcus* species isolates emphasizes the intricate nature of antibiotic resistance within the poultry population in the study area, potentially hindering the identification of effective treatment options and raising concerns about the potential spread of resistant strains within and beyond the poultry

sector. Although there is widespread resistance among *Staphylococcus* species isolates to certain antibiotics, the isolates in the present study demonstrated varying levels of susceptibility to other tested antimicrobials, including Amoxicillin-Clavulanic acid, Gentamicin, Neomycin, Ciprofloxacin, Enrofloxacin, Ofloxacin, Levofloxacin, Erythromycin, and Ceftriaxone. This variability highlights the necessity for a nuanced and tailored approach to antibiotic employment in poultry farming. The variability in susceptibility to different antibiotic classes emphasizes the importance of targeted antibiotic therapy based on susceptibility testing. These findings on *Staphylococcus* in the present study, while resembling those of Sonola *et al*. (2021) and Rao *et al*. (2022), who reported resistance of *Staphylococcus* species isolates from poultry to Erythromycin, slightly differ in that the present study observed some level of susceptibility to Erythromycin. Nevertheless, this study aligns with reports of Vitale *et al*. (2019) and Amoako *et al*. (2020), who also documented high resistance of *Staphylococcus* isolates from chickens to Tetracyclines, despite the earlier studies focusing specifically on methicillin-resistant *Staphylococcus aureus*. These differences may stem from variations in study design and the types of antimicrobials tested.

The results from this study regarding the antimicrobial resistance profile of *Escheirichia coli* isolates obtained from both broilers and layer chickens is a cause for concern; it reveals heightened resistance levels exceeding 50% to various antibiotics, particularly those within the Penicillin class, Macrolides, and Tetracyclines. The isolates exhibited notable resistance to commonly employed antimicrobial agents in both veterinary and human medicine, and this concurs with previous studies conducted by Seo and Lee (2021), Kiiti *et al*. (2021), Pal *et al*. (2022), and Xexaki et al. (2023). Of particular concern is the high resistance to antibiotics such as

Ampicillin, Amoxicillin, Penicillin G, and Amoxicillin-Clavulanic acid, all belonging to the Penicillin class, indicating a significant problem likely stemming from the misuse of these antibiotics in commercial poultry farming practices. This misuse may entail improper dosages, prolonged administration, or the unsupervised use of antibiotics. The resistance of *E. coli* against Macrolides (Erythromycin and Tylosin) and Tetracyclines (Oxytetracycline and Doxycycline) further emphasizes the pressing need for improved antibiotic stewardship within the poultry industry of the study area. The high resistance rates of *Escherichia coli* isolates to these antimicrobial classes align closely with the reports of Rahman *et al.* (2020), further emphasizing the severity of the issue. Interestingly, Hess *et al.* (2022) reported a similar pattern of high antimicrobial resistance among *Escherichia coli* isolates from organic laying hens despite the absence of antimicrobial treatments, suggesting a potential for *Escherichia coli* to develop antimicrobial resistance even in the absence of direct antibiotic exposure. This highlights the rapid acquisition of antimicrobial resistance by *Escherichia coli*, a phenomenon well-documented in healthy animals (Rivera-Gomis *et al.*, 2021). Despite less frequent isolation of antibiotic-resistant commensal *Escherichia coli* from poultry raised using non-conventional management systems, the prevalence remains a concern (Pesciaroli *et al.*, 2020). The observed resistance to critical Cephalosporins like Ceftriaxone and Ceftazidime implies a possible transfer of resistance mechanisms from poultry to humans, given the importance of these antibiotics in human medicine. This resistance aligns with reports of Seo *et al.* (2020) and Kiiti *et al.* (2021), emphasizing the contribution of antibiotic usage in poultry to the proliferation of resistant *Escherichia coli* strains.

The high resistance levels of *Escherichia coli* to most antibiotics in this study may be attributed to the accessibility and affordability of these antimicrobial agents in veterinary pharmacies and animal health shops within the study area, as suggested by previous studies from Nigeria, Eastern Turkey, Italy, Bangladesh, and Tanzania (Aworh *et al.*, 2020; Baran *et al.*, 2020; Musa *et al.*, 2020; Parvin *et al.*, 2020, and Kiiti *et al.*, 2021). This interplay between antibiotic usage in animals and its repercussions on human health underscores the necessity for a comprehensive One Health approach to address antibiotic resistance effectively. Moreover, the increasing resistance to Nitrofurantoin and Trimethoprim/Sulfamethoxazole complicates the antibiotic landscape further, as these drugs are commonly used in both veterinary and human medicine (Bielec *et al.*, 2023). The varying levels of susceptibility within the Aminoglycosides and Quinolones families suggest that not all antibiotics are equally affected, which agrees with the findings of Hess *et al.* (2022). Understanding the varying susceptibility within antibiotic families may offer insights into potential alternative antibiotic choices for the poultry industry in the study area. However, the overall observation of multidrug resistance among all *Escherichia coli* isolates is alarming, severely limiting treatment options and posing significant challenges in controlling bacterial infections in both poultry and potentially humans.

The prevalence of multidrug-resistant *Escherichia coli* isolates reported in this study, standing at 100.0%, surpasses rates observed in similar studies conducted elsewhere, indicating a critical issue within the region's poultry production system. This prevalence recorded in the present study is notably higher than 86.76% reported in Tanzania (Kiiti *et al.*, 2021), 69.3% and 51.6% in Dar es Salaam by Mgaya *et al.* (2021) and Kimera *et al.* (2021) respectively, 63.4% in Qatar (Eltai *et al.*, 2020), and 68% in Senegal (Vounba *et al.*, 2019), further emphasizing the gravity of the situation. Despite the high susceptibility levels

observed for Aminoglycosides and Quinolones, which align with the study of Kiiti *et al*. (2021), differences exist with findings from Eastern Turkey (Baran *et al*., 2020), Nigeria (Aworh *et al*., 2020), and Korea (Seo and Lee, 2021), indicating variations in antibiotic susceptibility across different regions and management systems.

The present study also highlights a troubling trend of heightened antimicrobial resistance among *Salmonella* strains isolated from both broilers and laying chickens, which agrees with the findings of Nguyen *et al*. (2021) and Forgaciu *et al*. (2022). The striking 58.5% rate of multidrug resistance observed in *Salmonella* isolates poses a grave threat to poultry health and food safety, with potential implications for human infections. These isolates exhibited resistance to various antimicrobial classes, including Penicillin (Beta-lactams), Aminoglycosides, Macrolides, Tetracyclines, Cephalosporin, Nitrofuran derivative, and Diaminopyrimidine with sulfonamide, indicating an urgent need for intervention strategies to address antibiotic misuse in poultry farming.

Notably, *Salmonella* isolates in this study showed relatively higher susceptibility levels (greater than 50%) to drugs within the Quinolones family. Understanding the factors contributing to this susceptibility could offer insights into potential alternative treatments and assist in designing effective antibiotic stewardship programs. The finding that 58.5% of *Salmonella* isolates in this study exhibited multidrug resistance underscores the challenge in treating infections caused by these strains. These findings align with studies conducted in various countries such as the United States (Velasquez *et al*., 2018), Iran (Bahramianfard *et al*., 2021), and Europe (Cortés *et al*., 2022). They also coincide with previous study conducted by Nguyen *et al*. (2021), which reported critically high resistance among *Salmonella* isolates from

poultry farms to tetracycline, ampicillin, and sulfamethoxazole/trimethoprim.

Previous studies have also documented high resistance among *Salmonella* isolates from poultry to colistin, ciprofloxacin, doxycycline, kanamycin, streptomycin, sulfamethoxazole, and tetracycline (Nelson *et al*., 2020; Wang *et al*., 2020; Karabasanavar *et al*., 2020), consistent with the present findings. Common resistance patterns observed include resistance to amoxicillin, ampicillin, ciprofloxacin, erythromycin, gentamycin, penicillin, sulfamethaxole, and tetracycline, as reported by Nguyen *et al*. (2021).

Similarly, Cortés *et al*. (2022) reported high resistance rates among *Salmonella* isolates from broilers to sulfamethoxazole, gentamicin, ciprofloxacin, nalidixic acid, and tetracycline, with layers showing elevated resistance to sulfamethoxazole and tetracycline. However, our study revealed a 41.5% rate of susceptibility, indicating the presence of strains not yet resistant to a broad spectrum of antibiotics, consistent with the findings of Pławińska-Czarnak *et al*. (2022) and Syed *et al*. (2023).

The widespread prevalence of antibiotic resistance poses a significant economic threat to the commercial poultry sector, with treatment failures and increased mortality rates posing financial risks for poultry farmers (Assoumy *et al*., 2021). Antibiotic-resistant *Salmonella* strains in poultry directly impact food safety, as contaminated poultry products may harbor drug-resistant pathogens, potentially causing foodborne illnesses in consumers (Rafiq *et al*., 2022; Abreu *et al*., 2023). The transmission of multidrug-resistant *Salmonella* from poultry to humans represents a significant public health concern, complicating treatment for human infections and leading to higher morbidity and mortality rates (Ahmad *et al*., 2021; Abreu *et al*., 2023).

The present study also unveiled a notable prevalence of antibiotic resistance among

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Streptococcus species isolates obtained from both broilers and layers. Notably, there is significant resistance observed against crucial antibiotics commonly utilized in poultry farming. The fact that antibiotics like Ampicillin, Amoxicillin, and Penicillin G exhibit a 100% resistance prevalence is deeply concerning, especially given their widespread usage in poultry farming for both disease prevention and treatment purposes. This discovery corroborates with Hermana *et al.* (2021), who similarly noted resistance to penicillin in all *Streptococcus* isolates from chickens. The emergence of resistance to these antibiotics hints at a potential decline in their effectiveness for managing bacterial infections in poultry, posing risks to bird health and welfare. The findings of this study are consistent with Abed *et al.* (2021), who identified significantly higher multidrug resistance in *Streptococcus* species isolates, linking it to resistance-associated genes.

Observations from the present study indicate resistance to antibiotics across various drug classes, encompassing Aminoglycosides, Macrolides, Tetracyclines, and Nitrofuran derivatives, showcasing a complex resistance pattern. This complexity may present challenges in selecting appropriate antibiotics for treating bacterial infections in poultry, potentially resulting in prolonged illness, heightened mortality rates, and economic losses. The substantial percentage (81.6%) of *Streptococcus* species isolates demonstrating multidrug resistance is particularly alarming, complicating the search for effective treatment options. The persistence of multidrug-resistant strains in poultry poses a grave threat to public health, as these strains could transfer resistance genes to human pathogens.

Despite antibiotics in the Quinolones class displaying higher susceptibility levels, the limited number of effective antibiotics raises concerns regarding the sustainability of antibiotic use in poultry production. This

underscores the urgent need for alternative strategies such as enhanced biosecurity measures, vaccination programs, and responsible antibiotic use practices to prevent and manage bacterial infections in poultry.

The present study also showed a complete prevalence of antimicrobial resistance within *Enterococcus* species isolates, indicating the inefficacy of specific antibiotics in addressing *Enterococcus* infections in poultry within the Maiduguri region. This discovery is in line with findings by Mudenda *et al.* (2023), who similarly reported resistance to a range of antibiotics among *Enterococcus* species isolates. The notable resistance of *Enterococcus* to antibiotics documented in present study may be linked to the excessive and inappropriate utilization of medications in poultry farming activities which buttress similar findings by Semedo-Lemsaddek *et al.*, 2021 and Ribeiro *et al.* (2023). The resistance exhibited by *Enterococcus* species is especially alarming as these bacteria can act as reservoirs for antibiotic resistance genes, potentially endangering public health via the food chain (Roy *et al.*, 2022).

The high prevalence of resistance indicates a plausible association with antibiotic misuse within the poultry production system, stressing the urgency for improved antibiotic stewardship practices to mitigate resistance risks. Antibiotic resistance can severely impact poultry production, resulting in heightened rates of illness and death among poultry stocks and financial losses for farmers. Consistent monitoring of antibiotic resistance in poultry and stringent regulations governing antibiotic usage in animal husbandry are imperative to ensure the sustainability of the poultry industry in Maiduguri.

The findings of this study unveil the intricate dynamics surrounding antimicrobial use in poultry farming in Maiduguri, Borno State, Nigeria. The majority of commercial poultry farmers utilize antimicrobials for prophylactic

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purposes, while others use them to treat infections in their flocks. The notable contrast between the small proportion of poultry farmers administering antimicrobials therapeutically and the very high proportion using them prophylactically emphasizes a prevailing trend of preventive rather than curative antimicrobial use in commercial poultry farming. Prophylactic administration of antimicrobials is commonly done as a compensation for substandard farm management conditions, to prevent frequently occurring poultry diseases (Imam *et al*., 2020; Ndahi *et al*., 2023), or due to the absence of vaccinations against poultry diseases. This trend could be attributed to various factors, including but not limited to, the perceived economic benefits of disease prevention, the costs associated with veterinary treatments (Dadgostar, 2019; Smith *et al*., 2019), which might prompt farmers to administer drugs prophylactically to avoid severe clinical events that necessitate costly veterinary interventions. Farmers may also face challenges in accessing veterinary services for disease diagnosis and lack awareness about appropriate antimicrobial use practices.

Laboratory confirmation of livestock diseases, including poultry, in Borno State, Northeastern Nigeria, is predominantly conducted at the University of Maiduguri's Veterinary Teaching Hospitals and Research and Teaching laboratories of the Departments of Veterinary Microbiology, Veterinary Entomology and Parasitology, Veterinary Medicine, and Veterinary Pathology (Lawal *et al*., 2023). These facilities represent only a fraction of the laboratories needed, when compared to the number of poultry farms in the State. Consequently, farmers might be unable to utilize or access these institutional laboratories for disease diagnosis, potentially leading to widespread prophylactic administration of antimicrobials based on farmers' perceptions of disease risk or their own experiences.

The substantial reliance on non-veterinarians, particularly veterinary drugs vendors, retailers, hawkers and fellow poultry farmers, for antimicrobial prescriptions and guidance in the present study area is worthy of concern. This reliance may stem from various factors, including limited access to veterinary services, financial constraints and traditional practices within the local poultry farming community. The absence of veterinary oversight in antimicrobial usage decisions could contribute to inappropriate drug selection, dosing, and duration, thereby exacerbating the emergence and spread of antimicrobial resistance (AMR), as reported by Gray *et al*. (2021). Previous studies have demonstrated that antimicrobial usage on commercial poultry farms is significantly influenced by advice from antimicrobial suppliers, particularly feed and chick traders who collaborate closely with representatives of drug companies to achieve target sales (Imam *et al*., 2020; Masud *et al*., 2020). A large proportion of commercial chicken farmers in the present study area were observed to receive antimicrobials from feed and chick traders at the point of collection of day-old-chicks. This observation aligns with Masud *et al*. (2020), who reported that the use of antimicrobials may be influenced by contractual agreements with feed and chick traders, who supply all production inputs (e.g., day-old chicks and feed) on credit to farmers and subsequently purchase the poultry products from farmers at pre-arranged prices. This practice is more prevalent among broiler farmers than layer farmers, thus likely explaining the observed differences between the two production types in this study.

The misuse of antimicrobials observed in this study, characterized by prophylactic rather than therapeutic use and reliance on non-veterinarians for prescribing decisions, is likely to contribute to the emergence and

dissemination of antimicrobial-resistant pathogens in poultry populations. Such practices foster the selection pressure for resistant strains, compromising the effectiveness of antimicrobial treatments and potentially transmitting resistant bacteria to humans through the food chain (Caneschi *et al.*, 2023).

The findings of this study indicating a significant proportion of poultry farmers being unaware of antimicrobial resistance pathogens among birds underscore a critical gap in knowledge and awareness. This lack of awareness may be attributed to inadequate education and training on AMR-related issues, limited dissemination of information on AMR within the poultry farming community, and cultural factors influencing perceptions of disease and medication, in agreement with Hedman *et al.* (2020) and Mudenda *et al.* (2022). Addressing the challenges associated with antimicrobial misuse in poultry farming requires a multifaceted approach. There is an urgent need for targeted educational programs aimed at raising awareness about AMR, promoting responsible antimicrobial use practices, and enhancing the capacity of poultry farmers to make informed decisions regarding antimicrobial use.

Conclusion: This study highlighted a significant burden of Enterobacteria, which includes *Escherichia coli*, *Salmonella*, *Staphylococcus*, *Streptococcus*, and *Enterococcus* species, and antimicrobial resistance (AMR) in broilers and layers in Maiduguri, Borno State, Nigeria, with notable multidrug resistance across these species. *Escherichia coli* emerged as the most prevalent bacterial species in both broilers and layers followed closely by *Salmonella* species, with substantial resistance to multiple antibiotics, including Ampicillin, Amoxicillin, and Penicillin G. A comparative analysis of bacterial microbiota prevalence between broilers and layer chickens revealed variations in susceptibility patterns, with broilers showing higher prevalence rates of certain

bacterial species, notably *Escherichia coli*, compared to layers. However, the prevalence rates of *Staphylococcus* species and *Streptococcus* species did not significantly differ between broilers and layers. Additionally, the misuse of antimicrobials by poultry farmers, driven by non-veterinary advice, underscores the urgent need for improved veterinary oversight and farmer education on AMR.

The limitations of this study include the regional focus on Maiduguri, which may not be representative of other areas, and the reliance on cross-sectional data, which limits the ability to establish causal relationships. Future research should aim to include longitudinal data and a broader geographic scope to provide a more comprehensive understanding of AMR dynamics in poultry.

Recommendations: The establishment and enforcement of regulations ensuring that antimicrobial prescriptions and administration instructions come exclusively from registered veterinarians is recommended. This step is crucial to curb the misuse of antimicrobials. Extensive educational programs for poultry farmers focusing on the risks of AMR, proper antimicrobial usage, and the importance of seeking veterinary advice is advocated. The development and maintenance of a robust AMR surveillance system to monitor the prevalence and trends of AMR in poultry is further recoomended to enable timely interventions and policy adjustments. Promotion and support of the the use of alternative health management practices, such as improved biosecurity measures, vaccination programs, and probiotics, to reduce reliance on antimicrobials is also advocated. Also, the development and implementation of policies and legislation that support responsible antimicrobial use in poultry farming, including restrictions on over-the-counter sales of antimicrobials is further advocated.

Acknowledgments

Competing Interests

The authors declare that they have no competing interests.

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