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## Research Article

# Occurrence, Antimicrobial Resistance Profiles, and One Health Implications of *Klebsiella* Species in Commercial Layer Chickens and Poultry Farm Environments in Maiduguri, Northeastern Nigeria

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## ABSTRACT

Antimicrobial-resistant *Klebsiella* species in poultry pose a One Health threat, yet surveillance data from Northeastern Nigeria are scarce. This cross-sectional study (July 2025–May 2026) investigated the occurrence, species distribution, and antimicrobial resistance (AMR) profiles of *Klebsiella* species in commercial layers and farm environments in Maiduguri, Nigeria, and assessed associated farmer practices. A total of 125 samples (75 cloacal swabs, 25 surface swabs, 13 feed, and 12 water) were collected from poultry farms and processed using standard microbiological and biochemical methods. Antimicrobial susceptibility was determined using Kirby–Bauer disk diffusion method. Structured questionnaire assessed antimicrobial use and biosecurity practices among 75 respondents from purposively selected commercial poultry farms. The overall prevalence of *Klebsiella* species was 21.6% (27/125), comprising *K. pneumoniae* (70.4%), *K. oxytoca* (22.2%), and *K. variicola* (7.4%). Deep litter management was significantly associated with higher colonization (42.9% against 7.5% in battery cages;  $p = 0.0003$ ). High resistance rates were observed against ampicillin (70.4%), tetracycline (66.7%), and trimethoprim-sulfamethoxazole (59.3%); 44.4% of isolates were multidrug-resistant (MDR), including one extensively drug-resistant isolate. The mean multiple antibiotic resistance index was  $0.38 \pm 0.22$ , with 44.4% of isolates exceeding the 0.21 high-risk threshold. Non-adherence to dosage and absence of veterinary consultation were significantly associated with MDR ( $p = 0.0433$  each), while tertiary education was associated with better biosecurity and stewardship practices ( $p < 0.05$ ). These findings demonstrate substantial environmental and avian reservoirs of antimicrobial-resistant *Klebsiella* in Maiduguri, indicating urgent need for integrated One Health surveillance, antimicrobial stewardship, and improved biosecurity in poultry production.

**Keywords:** Antimicrobial resistance; Biosecurity; Commercial layer chickens; *Klebsiella* species; Multidrug resistance; One Health; Poultry farm environment

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## INTRODUCTION

The poultry industry is among the fastest-growing livestock subsectors globally, contributing to food security, employment, and affordable animal protein (Osei-Akoto *et al.*, 2026). In Nigeria, commercial layer production has expanded substantially over the past two decades; however, its sustainability is threatened by infectious diseases, indiscriminate antimicrobial use, and inadequate biosecurity (Bamidele *et al.*, 2022).

*Klebsiella* species are Gram-negative, encapsulated, facultatively anaerobic bacilli of the family Enterobacteriaceae, widely distributed in soil, water, feed, litter, and the gastrointestinal tracts of humans and animals (Kahin *et al.*, 2024). Although typically commensals, they possess virulence factors—including polysaccharide capsules, lipopolysaccharides, fimbrial adhesins, siderophores, and biofilm-forming capacity—that enable opportunistic infections (Biradar *et al.*, 2026). *Klebsiella pneumoniae* is the most clinically important species, followed by *K. oxytoca*, *K. variicola*, and *K. quasipneumoniae* (Krūmiņa *et al.*, 2026). In poultry, *Klebsiella* species cause septicaemia, respiratory infections, airsacculitis, omphalitis, salpingitis, and reproductive tract infections, and may colonise birds through contaminated feed, water, litter, equipment, and environmental sources (Franklin-Alming *et al.*, 2021). Once introduced, these organisms persist in poultry houses through contaminated dust, manure, wastewater, and litter, facilitating continuous circulation among birds and environmental dissemination (El Baz *et al.*, 2026).

A major concern is the extraordinary genetic plasticity of *Klebsiella* species, which facilitates the acquisition and dissemination of antimicrobial resistance genes through plasmids, transposons, integrons, and other mobile genetic elements (Zdarska *et al.*, 2026). Multidrug-resistant *K. pneumoniae* is recognised as one of the most important antimicrobial-resistant pathogens globally, causing severe infections, treatment failures, and high mortality (Li *et al.*, 2023; Song *et al.*, 2025). Resistance has been reported against  $\beta$ -lactams, cephalosporins, aminoglycosides, tetracyclines, sulfonamides, fluoroquinolones, and chloramphenicol (Yang *et al.*, 2023; Aldali, 2025). Of particular concern is the emergence of extended-

spectrum  $\beta$ -lactamase (ESBL)-producing and carbapenem-resistant strains, which severely limit therapeutic options (Li *et al.*, 2025a). Widespread misuse of antimicrobials in poultry production—often without veterinary prescription, with inappropriate dosing, and without observance of withdrawal periods—exerts selective pressure favouring resistant strains (Abreu *et al.*, 2023; Habiba *et al.*, 2023). Commercial layers are particularly exposed due to their relatively long production cycle, during which they may receive multiple antimicrobial treatments (Ben Ali *et al.*, 2025).

The One Health concept recognises the interconnectedness of human, animal, and environmental health. Antimicrobial-resistant bacteria from poultry systems may be transmitted directly through occupational exposure of farmers, veterinarians, and handlers, or indirectly through contaminated food products, environmental pollution, and water sources (Ali *et al.*, 2026). Biosecurity is among the most effective preventive measures for reducing disease introduction and persistence, and effective biosecurity reduces the need for antimicrobial usage, thereby contributing to antimicrobial stewardship (Al-Khalaifah *et al.*, 2026). In Maiduguri, Borno State, commercial poultry farming is an important livestock enterprise; however, increasing intensification, widespread antimicrobial usage, and limited antimicrobial resistance surveillance may favour the emergence and dissemination of antimicrobial-resistant *Klebsiella* species (Jibril *et al.*, 2021; Sana *et al.*, 2025). Despite increasing recognition of antimicrobial resistance as a global health challenge, information regarding the occurrence, resistance patterns, environmental distribution, and One Health implications of *Klebsiella* species in commercial layer farms in Maiduguri remains limited. This study was therefore designed to investigate the occurrence and species distribution of *Klebsiella* species in commercial layer chickens and farm environments in Maiduguri, determine the antimicrobial resistance profiles of recovered isolates, assess antimicrobial use and biosecurity practices among poultry farmers, and evaluate associations between management practices and the occurrence of antimicrobial-resistant *Klebsiella* species.

## MATERIALS AND METHODS

### Study Area

The study was conducted in Maiduguri, the capital city of Borno State, North-Eastern Nigeria. Maiduguri lies between latitude 11°50'N and 11°55'N and longitude 13°05'E and 13°15'E, with an average elevation of approximately 354 m above sea level. The city falls within the Sudan Savannah ecological zone and is characterised by a semi-arid climate with distinct dry and rainy seasons. The dry season extends from approximately October to May, while the rainy season occurs between June and September. Annual rainfall ranges from approximately 500–700 mm, with mean annual temperatures ranging from 28°C to 35°C, although temperatures may exceed 40°C during the peak of the dry season (Ngadda *et al.*, 2025). The study area encompasses five locations within the Maiduguri metropolis where commercial layer poultry farms were selected for sampling: GRA (Government Residential Area), Mairi Quwait, University of Maiduguri Department of Animal Science Poultry Farm, MMC (Maiduguri Metropolitan Council), and Dalori community. These locations were purposively selected to represent diverse poultry production systems and environmental conditions within the metropolitan area.

### Study Design

A cross-sectional study design was employed to investigate the occurrence, antimicrobial resistance patterns, and One Health significance of *Klebsiella* species in commercial layer chickens and poultry farm environments. The study also incorporated a descriptive survey component to assess antimicrobial use practices and biosecurity measures. This design enabled the simultaneous collection of microbiological samples and questionnaire data from the study population. A total of 15 commercial poultry farms were purposively selected. From each farm, five personnel directly involved in poultry handling and farm operations were purposively selected for questionnaire administration, yielding 75 respondents. Respondents included farm owners, farm managers, veterinary/technical consultants (where available), farm workers, and cleaners with direct contact with the birds. Including multiple personnel per farm captured varied perspectives on antimicrobial use and biosecurity practices within each farm. The questionnaire was pre-tested on 10 farmers from non-participating farms, and these responses were excluded from the main study. The study was conducted from July 2025 to May 2026.

### Determination of Sample Size

The sample size for microbiological sampling was calculated using the formula for cross-sectional studies, assuming an expected prevalence of *Klebsiella* species in poultry of 8% based on previous studies (Utami *et al.*, 2025). The sample size was determined as follows:

$$n = (Z^2 \times p \times q) / d^2$$

Where:

n = minimum sample size; Z = standard normal deviate at 95% confidence interval (1.96); p = expected prevalence (0.08); q = 1 - p (0.92); d = precision (0.05)

$$n = (1.96)^2 \times 0.08 \times 0.92 / (0.05)^2$$

$$n = 3.8416 \times 0.0736 / 0.0025$$

$$n = 0.2827 / 0.0025$$

$$n = 113.1$$

The calculated sample size was 113 samples. To account for potential sample loss and contamination, the sample size was increased by 10%, giving a total of 125 samples. These comprised 75 cloacal swabs from commercial layer chickens and 50 environmental samples (25 from poultry house surfaces and 25 from feed and water sources comprising of Feed samples (n = 13) and water samples (n = 12) sources).

### Collection of Samples

#### Cloacal Swab Collection from Commercial Layer Chickens

A total of 75 cloacal swab samples were collected from commercial layer chickens across the five selected poultry farms (15 samples per farm). Birds were randomly selected from each flock using a systematic random sampling technique. Each selected bird was physically restrained, and a sterile cotton-tipped applicator was gently inserted into the cloaca, rotated to ensure adequate sample collection, and withdrawn. The swab was immediately placed into a sterile test tube containing 5 mL of Buffered Peptone Water (BPW) as a transport medium. Samples were labelled with unique identification codes indicating the farm of origin, date of collection, and bird identification number. The samples were transported on ice packs in a cool box to the laboratory.

#### Environmental Sample Collection

Environmental samples (n = 50) were collected from the same poultry farms. These included:

**Poultry House Surface Swabs (n = 25):** Sterile swabs moistened with sterile physiological saline were used to swab surfaces including floors, walls, feeding troughs, drinkers, and cages. A 10 cm × 10 cm area of each surface was swabbed using a standardised

zigzag pattern. Swabs were placed in sterile test tubes containing 5 mL of BPW.

**Feed and Water Samples (n = 25):** Approximately 50 g of poultry feed was aseptically collected from feeding troughs using sterile scoops and placed into sterile plastic bags. Water samples (50 mL) were collected from drinkers using sterile screw-capped bottles. All samples were labelled, placed in a cool box with ice packs, and transported to the laboratory for processing.

#### Microbiological Analyses

All microbiological analyses were carried out at the Department of Microbiology Research Laboratory, Faculty of Life Sciences, University of Maiduguri, following standard microbiological procedures.

#### Isolation and Identification of *Klebsiella* Species

**Pre-enrichment:** Swab samples in BPW were vortexed for 1 min. Feed samples (~25 g) were homogenised in 225 mL sterile BPW (1:10 w/v) using a stomacher for 2 min; water samples (25 mL) were mixed with 225 mL sterile BPW (1:10 v/v). All samples were incubated at 37°C for 4–6 h for non-selective resuscitation of stressed cells (MedVetKlebs Consortium, 2020; Utami *et al.*, 2025; Karacan Sever *et al.*, 2026).

**Enrichment and isolation:** Aliquots (1 mL) were transferred to 9 mL MacConkey Broth and incubated at 37°C for 18–24 h. A 10-μL loopful was then streaked onto MacConkey Agar (MCA) by quadrant streaking and incubated aerobically at 37°C for 24 h. Presumptive *Klebsiella* colonies (large, 2–4 mm, pink-to-red, mucoid, lactose-fermenting) were sub-cultured on Nutrient Agar at 37°C for 18–24 h to obtain pure cultures, which were maintained on Nutrient Agar slants at 4°C (Khan *et al.*, 2024; Utami *et al.*, 2025).

**Gram staining:** Heat-fixed smears were stained with crystal violet (1 min), Gram's iodine (1 min), decolourised with 95% ethanol (10–15 s), and counterstained with safranin (1 min). Gram-negative, short, plump rods arranged singly, in pairs, or in short chains were considered characteristic of *Klebsiella* spp. (Utami *et al.*, 2025).

**Biochemical identification:** Isolates were tested as follows: catalase (3% H<sub>2</sub>O<sub>2</sub>; effervescence within 10–20 s); oxidase (tetramethyl-p-phenylenediamine dihydrochloride; dark purple within 30 s; *Klebsiella* is oxidase-negative); indole (Tryptone Water, 37°C, 24–48 h, Kovac's reagent; red ring within 1–2 min; *K. pneumoniae* negative, *K. oxytoca* positive); methyl red (MR) and Voges–Proskauer (VP) in MR-VP broth (37°C, 48–72 h; red with methyl red = mixed-acid fermentation; pink-red within 15 min with Barritt's

reagents A and B = 2,3-butanediol fermentation; *Klebsiella* is typically MR-negative, VP-positive); citrate utilisation on Simmons Citrate Agar (37°C, 24–48 h; green to blue); Triple Sugar Iron Agar (stabbed butt, streaked slant, 37°C, 24 h; acid slant/acid butt with gas, no H<sub>2</sub>S); urease on Christensen's urea agar (37°C, 24–48 h; yellow to pink-red; strongly positive); motility in semi-solid medium (37°C, 24–48 h; non-motile, growth along stab line only); and carbohydrate fermentation in phenol red broth with 1% filter-sterilised glucose, lactose, sucrose, or mannitol and inverted Durham tubes (37°C, 24–48 h; red to yellow = acid; trapped bubbles = gas) (Khan *et al.*, 2024; Utami *et al.*, 2025).

#### Species Identification of *Klebsiella* Isolates

Identification to species level was based on the biochemical profiles obtained from the battery of tests described above, with carbohydrate fermentation patterns, indole production, and urease activity serving as the key differentiating characteristics, as described by Bergey's Manual of Systematic Bacteriology and standard diagnostic microbiology references (Alves *et al.*, 2006). *Klebsiella pneumoniae* was characterised as indole negative, methyl red (MR) negative, Voges-Proskauer (VP) positive, citrate positive, urease positive, and non-motile, with acid and gas production from glucose, lactose, sucrose, and mannitol, a positive lysine decarboxylase reaction, and a negative ornithine decarboxylase reaction (Alves *et al.*, 2006; Utami *et al.*, 2025). *Klebsiella oxytoca* was distinguished from *K. pneumoniae* primarily by its positive indole test due to tryptophanase production, while sharing MR-negative, VP-positive, citrate-positive, urease-positive, and non-motile characteristics, and fermenting glucose, lactose, sucrose, and mannitol with acid and gas production; it is also lysine decarboxylase positive and ornithine decarboxylase negative (Maslow *et al.*, 1993). *Klebsiella variicola*, a recently described member of the *K. pneumoniae* species complex, is biochemically similar to *K. pneumoniae* but can be differentiated by its variable indole reaction, variable urease activity, and non-motile nature, while being VP positive and citrate positive and fermenting glucose, lactose, sucrose, and mannitol with acid and gas production; it can further be distinguished from *K. pneumoniae* by its ability to utilise D-melezitose and 3-hydroxybutyrate, although molecular methods such as rpoB or 16S rRNA gene sequencing are required for definitive identification (Rodríguez-Medina *et al.*, 2019). Isolates that could not be clearly assigned to a specific species based on biochemical profile were recorded as *Klebsiella*

species unidentified/unclassified, and all identifications were cross-checked against standard reference tables for Enterobacteriaceae (Kowalczyk *et al.*, 2022).

#### **Antimicrobial Susceptibility Testing**

Antimicrobial susceptibility testing was performed on all confirmed *Klebsiella* isolates using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar (MHA) according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2024, 2026). Pure cultures were grown in nutrient broth at 37°C for 18–24 h, and suspensions were adjusted to the 0.5 McFarland standard ( $\sim 1.5 \times 10^8$  CFU/mL) in sterile 0.85% NaCl and used within 15 min. MHA plates were inoculated evenly in three directions with a sterile cotton swab, dried for 3–5 min, and impregnated with antimicrobial disks (Oxoid, UK;  $\leq 6$  disks per 90-mm plate). Agents were selected for their clinical and veterinary relevance to *Klebsiella* infections, including critically important antimicrobials for human medicine, to assess One Health implications of resistance in poultry. Plates were incubated aerobically at 35–37°C for 16–18 h, and inhibition zones (including disk diameter) were measured in millimetres and interpreted as susceptible, intermediate, or resistant using CLSI M100, 36th edition (2026) breakpoints for *Enterobacterales*. Erythromycin has no disk-diffusion breakpoint, and colistin susceptibility requires confirmation by a validated MIC method (CLSI, 2026). *Escherichia coli* ATCC 25922 served as the primary quality control strain, with *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 as additional controls; results were valid only when zone diameters fell within CLSI acceptable ranges (CLSI, 2024, 2026). Isolates resistant to  $\geq 1$  agent in  $\geq 3$  antimicrobial classes were classified as multidrug-resistant (MDR), those resistant to  $\geq 1$  agent in all but  $\leq 2$  classes ( $\geq 6$  of 8 classes tested) as extensively drug-resistant (XDR), and those resistant to all agents tested as pan-drug-resistant (PDR), per ECDC/CDC international definitions (Khan *et al.*, 2024). The eight classes evaluated were penicillins (ampicillin), penicillin/ $\beta$ -lactamase inhibitor combinations (amoxicillin–clavulanic acid), cephalosporins (ceftriaxone, cefotaxime, ceftazidime), carbapenems (meropenem), aminoglycosides (gentamicin, amikacin), fluoroquinolones (ciprofloxacin, levofloxacin), tetracyclines (tetracycline), and sulfonamides (trimethoprim–sulfamethoxazole), with colistin (polymyxins) considered separately as a last-resort agent.

**MAR index threshold:** MAR index was calculated as the ratio of the number of antimicrobials to which an isolate was resistant to the total number of antimicrobials tested. A MAR index  $>0.2$  was considered high risk, as previously described (Muthu *et al.*, 2022; Isaiah *et al.*, 2025).

#### **Questionnaire Administration for Assessment of Antimicrobial Use and Biosecurity Practices**

A structured, pre-tested questionnaire was administered to 75 respondents across 15 commercial poultry farms to assess knowledge, attitudes, and practices regarding antimicrobial use and biosecurity. On each farm, five personnel with direct involvement in poultry management and bird handling were interviewed, including the farm owner or manager, technical/veterinary consultant (if available), farm workers, and cleaners. The questionnaire was adapted from previous studies in other parts of Nigeria, with modifications to suit the study objectives (Ezeh *et al.*, 2024; Ariom *et al.*, 2026). It was developed in English and translated into Hausa and Kanuri to ensure respondent comprehension.

The questionnaire comprised four sections: (A) socio-demographic characteristics (age, sex, education, farming experience, flock size, production system); (B) antimicrobial use practices (types, sources, reasons for use, frequency, route, dosage, treatment duration, withdrawal period compliance, awareness of antimicrobial resistance, and information sources); (C) biosecurity measures (farm location, housing type, cleaning and disinfection, visitor access, personal protective equipment, vaccination, dead bird disposal, waste management, presence of other animals, and rodent/pest control); and (D) knowledge and awareness of antimicrobial resistance, One Health, prudent antimicrobial use, antimicrobial residues, and relevant regulations. The questionnaire was pre-tested on 10 commercial poultry farmers ( $\sim 10\%$  of the target sample) not included in the main study, and ambiguous or irrelevant items were revised or removed.

It was administered through face-to-face interviews by four trained enumerators fluent in English, Hausa, and Kanuri, with each interview lasting approximately 30–45 minutes; Hausa and Kanuri translations were used where necessary. Informed consent was obtained prior to each interview, and confidentiality and exclusive use of data for research purposes were assured.

#### **Data Analysis**

##### **Microbiological Data**

Data from microbiological analysis were entered into Microsoft Excel (Office 2019), organised by sample

type, farm location, and isolate identification number, and exported to SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to calculate the occurrence of *Klebsiella* species as the percentage of positive samples, with 95% confidence intervals (CI) determined by the Wilson score method, overall and stratified by sample type (cloacal swabs, surface swabs, feed, and water) and farm location; species distribution as the proportion of each *Klebsiella* species (*K. pneumoniae*, *K. oxytoca*, *K. variicola*, and unclassified) among confirmed isolates; antimicrobial resistance rates as the percentage of isolates resistant to each agent, with 95% CI, by antimicrobial class and stratified by sample type and species; and the prevalence of multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR) phenotypes, defined as resistance to  $\geq 1$  agent in  $\geq 3$  classes,  $\geq 1$  agent in all but  $\leq 2$  classes, and all classes tested, respectively (Khan *et al.*, 2024). Comparisons of occurrence and resistance rates between sample types, among farms, and among *Klebsiella* species were performed using the Chi-square ( $\chi^2$ ) test or Fisher's exact test where expected cell frequencies were  $< 5$ , with  $p < 0.05$  considered significant with 95% CI were calculated to quantify associations where applicable. Results were presented in tables.

#### Questionnaire Data

Questionnaire data were coded numerically (e.g., sex: 1 = male, 2 = female; education: 1 = none, 2 = primary, 3 = secondary, 4 = tertiary), entered into Microsoft Excel, and analysed using SPSS version 25.0. Descriptive statistics (frequencies, percentages, means, and standard deviations) were used to summarise respondent demographics and practices, with categorical variables summarised as frequencies and percentages and continuous variables (e.g., age, farming experience) as means  $\pm$  SD or medians with interquartile ranges (IQR) depending on distribution. Associations between farmer characteristics (age, sex, education, farming experience) and practices (antimicrobial use, biosecurity compliance) were explored using Chi-square tests, and multivariable logistic regression was performed to identify independent predictors of good biosecurity practices and responsible antimicrobial use, adjusting for confounders, with variables having  $p < 0.20$  in univariable analysis included in the multivariable model and  $p < 0.05$  considered significant. Internal consistency was assessed using Cronbach's alpha, with  $\geq 0.70$  considered acceptable. Results were presented in frequency and cross-tabulation tables,

with selected verbatim responses from qualitative questions included to provide additional insight.

#### Ethical Considerations

Informed consent was obtained from all poultry farmers prior to questionnaire administration and collection of samples, with the purpose, procedures, potential risks, and benefits of the study explained to each participant in Hausa and Kanuri to ensure full understanding; participation was entirely voluntary, and respondents were informed of their right to withdraw at any time without consequence or penalty. Confidentiality of responses and exclusive use of farm data for research purposes were assured, all personal identifiers were removed, each respondent was assigned a unique identification code, and data were stored securely. Samples were collected from poultry using standard animal handling procedures to minimise stress and discomfort, with birds handled gently and restrained for the shortest possible time during cloacal swab collection; environmental sampling did not interfere with normal farm operations or cause harm to the birds, and no animals were sacrificed for the purpose of this study.

#### RESULTS

A total of 125 samples comprising cloacal swabs ( $n = 75$ ), surface swabs ( $n = 25$ ), feed samples ( $n = 13$ ), and water samples ( $n = 12$ ) were examined for the presence of *Klebsiella* species. The overall prevalence of *Klebsiella* species was 21.6% (27/125; 95% CI: 15.3–29.0%). Among sample categories, cloacal swabs exhibited the highest prevalence at 24.0% (18/75; 95% CI: 15.8–34.8%), followed by surface swabs at 20.0% (5/25; 95% CI: 8.9–39.1%), water samples at 16.7% (2/12; 95% CI: 4.7–44.8%), and feed samples at 15.4% (2/13; 95% CI: 4.3–42.2%). However, there was no statistically significant association between sample category and *Klebsiella* occurrence ( $\chi^2 = 0.7619$ ;  $p = 0.8586$ ) (Table 1).

Stratification by sampling location revealed that Mairi Quwait had the highest prevalence of *Klebsiella* species at 33.3% (9/27; 95% CI: 18.6–52.2%), followed by Dalori Community at 28.0% (7/25; 95% CI: 14.3–47.6%), Maiduguri Metropolitan Council (MMC) at 27.8% (5/18; 95% CI: 12.5–50.9%), and Government Reserved Area (GRA) at 13.3% (4/30; 95% CI: 5.3–29.7%). The University of Maiduguri exhibited the lowest prevalence at 8.0% (2/25; 95% CI: 2.2–23.0%). Despite these variations, the association between sampling location and *Klebsiella* occurrence was not statistically significant ( $\chi^2 = 7.147$ ;  $p = 0.1283$ ) (Table 1).

Analysis of *Klebsiella* occurrence in commercial layer chickens by age group revealed a progressive increase in prevalence with advancing age. Adult layers (>20 weeks) exhibited the highest prevalence at 36.0% (9/25; 95% CI: 19.1–56.6%), followed by point-of-lay pullets (18–20 weeks) at 21.4% (6/28; 95% CI: 9.0–40.7%), and growing pullets (<18 weeks) at 13.6% (3/22; 95% CI: 3.6–33.4%). However, this age-related trend was not statistically significant ( $\chi^2 = 3.371$ ;  $p = 0.1854$ ) (Table 2).

Management system was identified as a significant determinant of *Klebsiella* occurrence. Chickens reared under the deep litter system demonstrated a markedly higher prevalence of 42.9% (15/35; 95% CI: 28.0–59.2%) compared to those maintained in battery cages at 7.5% (3/40; 95% CI: 2.6–19.9%). This difference was statistically significant ( $\chi^2 = 12.79$ ;  $p = 0.0003$ ), indicating that the deep litter management system constitutes a significant risk factor for *Klebsiella* colonization in commercial layer chickens (Table 2).

Speciation of the 27 *Klebsiella* isolates revealed that *K. pneumoniae* was the predominant species, accounting for 70.4% (19/27) of all isolates, followed by *K. oxytoca* at 22.2% (6/27), and *K. variicola* at 7.4% (2/27). Cloacal swabs yielded the highest number of isolates for both *K. pneumoniae* (68.4%; 13/19) and *K. oxytoca* (83.3%; 5/6). Surface swabs contributed 21.1% (4/19) of *K. pneumoniae* and 16.7% (1/6) of *K. oxytoca* isolates. Notably, *K. variicola* was exclusively recovered from feed samples (50.0%; 1/2) and water samples (50.0%; 1/2), with no isolation from cloacal or surface swabs. Feed and water samples each contributed 5.3% (1/19) of *K. pneumoniae* isolates, while no *K. oxytoca* was recovered from these environmental sources (Table 3).

The antimicrobial susceptibility profile of the 27 *Klebsiella* isolates revealed substantial resistance across multiple antimicrobial classes. The highest resistance rates were observed against ampicillin (70.4%; 19/27) and tetracycline (66.7%; 18/27), followed by trimethoprim-sulfamethoxazole (59.3%; 16/27), ceftriaxone (48.1%; 13/27), amoxicillin-clavulanic acid (40.7%; 11/27), cefotaxime (40.7%; 11/27), and ceftazidime (40.7%; 11/27). Chloramphenicol exhibited a resistance rate of 29.6% (8/27), while ciprofloxacin and levofloxacin each demonstrated 22.2% (6/27) resistance. Lower resistance rates were observed for meropenem (18.5%; 5/27), gentamicin (18.5%; 5/27), and amikacin (18.5%; 5/27) (Table 4).

Susceptibility rates were highest for meropenem (70.4%; 19/27), amikacin (59.3%; 16/27), and

chloramphenicol (51.9%; 14/27). Intermediate susceptibility was most frequently observed for gentamicin (33.3%; 9/27), ciprofloxacin (29.6%; 8/27), levofloxacin (29.6%; 8/27), and amikacin (22.2%; 6/27). Colistin and erythromycin were not tested using disk diffusion due to the absence of Clinical and Laboratory Standards Institute (CLSI) disk diffusion breakpoints for Enterobacterales (Table 4).

Analysis of multidrug resistance (MDR) patterns revealed that 37.0% (10/27) of *Klebsiella* isolates were MDR, defined as resistance to at least one agent in three to five antimicrobial classes. Among MDR isolates, resistance to four antimicrobial classes was most common (14.8%; 4/27), followed by resistance to three classes (11.1%; 3/27) and five classes (11.1%; 3/27). Two isolates (7.4%) met the criteria for extensively drug-resistant (XDR) phenotype, defined as resistance to at least one agent in all but  $\leq 2$  of the 8 antimicrobial classes tested (i.e., resistant to  $\geq 6$  classes). No pan-drug-resistant (PDR) isolates were identified. Non-MDR isolates, defined as resistance to one or two antimicrobial classes, constituted 55.6% (15/27) of the total isolates. No pan-susceptible isolates were recovered. Overall, 44.4% (12/27) of isolates were resistant to  $\geq 3$  antimicrobial classes (MDR + XDR) (Table 5, Panel A).

The multiple antibiotic resistance (MAR) index distribution ranged from 0.00 to 1.00, with a mean MAR index of  $0.38 \pm 0.22$ . The majority of isolates (55.6%; 15/27) exhibited MAR indices between 0.01 and 0.20, categorized as low risk. High-risk MAR indices were observed in 44.4% (12/27) of isolates, distributed as follows: 0.21–0.40 (25.9%; 7/27), 0.41–0.60 (14.8%; 4/27), and 0.61–1.00 (3.7%; 1/27). No isolate had a MAR index of 0.00 (Table 5, Panel B).

The socio-demographic profile of the 75 respondents surveyed across 15 commercial poultry farms revealed that the majority were within the 31–40 years age bracket (38.7%; 29/75), followed by those  $\leq 30$  years (24.0%; 18/75), 41–50 years (22.7%; 17/75), 51–60 years (10.7%; 8/75), and  $>60$  years (4.0%; 3/75). Male farmers predominated (68.0%; 51/75) compared to females (32.0%; 24/75). Regarding educational attainment, 36.0% (27/75) had secondary education, 28.0% (21/75) had tertiary education, 25.3% (19/75) had primary education, and 10.7% (8/75) had no formal education. Farming experience was predominantly 5–10 years (42.7%; 32/75), followed by  $<5$  years (28.0%; 21/75), 11–20 years (21.3%; 16/75), and  $>20$  years (8.0%; 6/75). Flock size was most commonly 500–1000 birds

(41.3%; 31/75), followed by <500 birds (29.3%; 22/75), 1001–5000 birds (21.3%; 16/75), and >5000 birds (8.0%; 6/75). The intensive battery cage system was the predominant production system (57.3%; 43/75), followed by intensive deep litter (36.0%; 27/75) and semi-intensive systems (6.7%; 5/75) (Table 6).

Antimicrobial use was widespread among the surveyed farmers, with 90.7% (68/75) reporting antibiotic use in their flocks. The primary sources of antibiotics were agro-veterinary shops (45.6%; 31/68), veterinary pharmacies (32.4%; 22/68), open markets (14.7%; 10/68), and unlicensed vendors (7.4%; 5/68). Notably, self-prescription was the most common source of prescription (47.1%; 32/68), followed by fellow farmers (27.9%; 19/68), veterinarians (16.2%; 11/68), and extension agents (8.8%; 6/68). The primary reasons for antibiotic use were disease prevention (38.2%; 26/68), disease treatment (35.3%; 24/68), and growth promotion (26.5%; 18/68). The frequency of antibiotic use was predominantly every 2–3 months (41.2%; 28/68), followed by occasional use (30.9%; 21/68) and monthly use (27.9%; 19/68). Drinking water was the most common route of administration (61.8%; 42/68), followed by feed incorporation (26.5%; 18/68), injection (8.8%; 6/68), and topical application (2.9%; 2/68). Adherence to dosage was reported as sometimes in 42.6% (29/68) of cases, always in 30.9% (21/68), and never in 26.5% (18/68). The withdrawal period was not observed by 79.4% (54/68) of farmers, while only 20.6% (14/68) complied with withdrawal requirements. Record keeping was practiced by only 13.2% (9/68) of farmers, and 83.8% (57/68) reported using antibiotics without veterinary consultation (Table 7).

Assessment of biosecurity practices revealed substantial deficiencies across multiple parameters. Quarantine of new birds was practiced by only 18.7% (14/75) of farms, while 81.3% (61/75) did not implement this measure. Regular disinfection of equipment was conducted in only 16.0% (12/75) of farms, with occasional disinfection in 37.3% (28/75) and no disinfection in 46.7% (35/75). Visitor control was restricted in 22.7% (17/75) of farms, whereas 77.3% (58/75) had no visitor restrictions. Foot baths were present in only 17.3% (13/75) of farms, and protective clothing was always used in 25.3% (19/75), sometimes in 34.7% (26/75), and never in 40.0% (30/75). Proper disposal of dead birds was practiced in 28.0% (21/75) of farms, while 72.0% (54/75) employed improper disposal methods. Daily cleaning of drinkers was performed in 24.0% (18/75) of farms,

weekly in 38.7% (29/75), and occasionally or never in 37.3% (28/75). Similarly, daily cleaning of feeders was conducted in 20.0% (15/75) of farms, weekly in 36.0% (27/75), and occasionally or never in 44.0% (33/75). Vaccination was regularly practiced in 69.3% (52/75) of farms, irregularly in 24.0% (18/75), and never in 6.7% (5/75). Rodent and pest control measures were implemented in 32.0% (24/75) of farms, while 68.0% (51/75) lacked such measures. Record keeping was maintained in only 14.7% (11/75) of farms, with 85.3% (64/75) not keeping records (Table 8).

Assessment of farmer awareness regarding antimicrobial resistance (AMR) and One Health concepts revealed significant knowledge gaps. Only 25.3% (19/75) of farmers had heard of AMR, while 74.7% (56/75) were unaware. Knowledge of the causes of AMR was good in 8.0% (6/75), fair in 17.3% (13/75), and poor in 74.7% (56/75) of respondents. AMR was recognized as a public health issue by only 20.0% (15/75) of farmers. Training on AMR had been received by just 6.7% (5/75) of respondents. Knowledge of prudent antibiotic use was good in 12.0% (9/75), fair in 24.0% (18/75), and poor in 64.0% (48/75) of farmers. Awareness of the One Health concept was low, with only 14.7% (11/75) having heard of it. The potential for AMR transmission from poultry to humans was recognized by 18.7% (14/75) of farmers. Sources of AMR information included radio/television (10.7%; 8/75), health workers (6.7%; 5/75), veterinarians (5.3%; 4/75), and fellow farmers (2.7%; 2/75), while 74.7% (56/75) had no source of AMR information. Notably, 69.3% (52/75) of farmers believed that antibiotics could be discontinued when birds showed improvement, and 54.7% (41/75) believed that antibiotics could be shared among farmers. Awareness of antimicrobial residues in eggs was low, with only 16.0% (12/75) of farmers reporting awareness (Table 9).

Analysis of the association between biosecurity practices and *Klebsiella* occurrence revealed that farms that never disinfected equipment had a higher proportion of *Klebsiella*-positive farms (20.0%; 7/35) compared to those that disinfected regularly or occasionally (7.5%; 3/40), although this difference was not statistically significant ( $p = 0.1737$ ). Similarly, farms without foot baths had a higher positivity rate (14.5%; 9/62) compared to those with foot baths (7.7%; 1/13;  $p = 1.0000$ ). Farms that did not quarantine new birds had a positivity rate of 14.8% (9/61) compared to 7.1% (1/14) in those that did ( $p = 0.6767$ ). Visitor control was associated with a lower positivity rate (5.9%; 1/17) compared to unrestricted farms (15.5%; 9/58;  $p = 0.4389$ ). Farms without

record keeping had a positivity rate of 15.6% (10/64), while no positive farms were recorded among those with record keeping (0.0%; 0/11;  $p = 0.3409$ ). Protective clothing use was associated with a lower positivity rate (8.9%; 4/45) compared to farms where protective clothing was never used (20.0%; 6/30;  $p = 0.1853$ ). None of these associations reached statistical significance at  $p < 0.05$  (Table 10).

Analysis of the association between antimicrobial use practices and MDR in *Klebsiella* isolates revealed that isolates from farms where prescription was by self or fellow farmers exhibited a higher MDR rate (52.9%; 9/17) compared to those prescribed by veterinarians (16.7%; 1/6) or extension agents (50.0%; 2/4), although this difference was not statistically significant ( $p = 0.2979$ ). Isolates from farms where antibiotics were used for prevention or growth promotion had a higher MDR rate (58.8%; 10/17) compared to those used for treatment (20.0%; 2/10), but this difference was not statistically significant ( $p = 0.1071$ ). Adherence to dosage was significantly associated with MDR, with isolates from farms where dosage was sometimes or never adhered to exhibiting a markedly higher MDR rate (57.9%; 11/19) compared to those where dosage was always adhered to (12.5%; 1/8;  $p = 0.0433$ ). Similarly, veterinary consultation was significantly associated with MDR, with isolates from farms without veterinary consultation having a higher MDR rate (57.9%; 11/19) compared to those with consultation (12.5%; 1/8;  $p = 0.0433$ ). Withdrawal period observance was not significantly associated with MDR, although isolates from farms where withdrawal was not observed had a higher MDR rate (52.4%;

11/21) compared to those where it was observed (16.7%; 1/6;  $p = 0.1819$ ) (Table 11).

Analysis of the association between educational level and antimicrobial use/biosecurity practices revealed that farmers with tertiary education demonstrated significantly better adherence to dosage (57.1%; 12/21) compared to those with secondary education (25.9%; 7/27) and primary/no formal education (7.4%; 2/27;  $\chi^2 = 14.58$ ;  $p = 0.0007$ ). Similarly, withdrawal period observance was significantly higher among farmers with tertiary education (42.9%; 9/21) compared to secondary (14.8%; 4/27) and primary/no formal education (3.7%; 1/27;  $\chi^2 = 12.34$ ;  $p = 0.0021$ ). Record keeping was significantly associated with educational level, with 33.3% (7/21) of tertiary-educated farmers keeping records compared to 7.4% (2/27) of secondary-educated and 0.0% (0/27) of primary/no formal education farmers ( $\chi^2 = 13.27$ ;  $p = 0.0013$ ). Protective clothing use was significantly higher among tertiary-educated farmers (52.4%; 11/21) compared to secondary (22.2%; 6/27) and primary/no formal education (7.4%; 2/27;  $\chi^2 = 12.85$ ;  $p = 0.0016$ ). Equipment disinfection was significantly associated with educational level, with 42.9% (9/21) of tertiary-educated farmers practicing disinfection compared to 11.1% (3/27) of secondary-educated and 0.0% (0/27) of primary/no formal education farmers ( $\chi^2 = 16.89$ ;  $p = 0.0002$ ). Veterinary consultation was not significantly associated with educational level, although a gradient was observed with 38.1% (8/21) of tertiary-educated, 18.5% (5/27) of secondary-educated, and 11.1% (3/27) of primary/no formal education farmers consulting veterinarians ( $\chi^2 = 5.324$ ;  $p = 0.0698$ ) (Table 12).

**Table 1: Occurrence of *Klebsiella* Species in Commercial Layer Chickens and Poultry Farm Environmental Samples in Maiduguri, Borno State, Nigeria by Sample Category and Sampling Location (n = 125)**

| Variable          | Category                | No. of samples Examined | No. of samples Positive | Prevalence (%) | 95% CI LL – UL | $\chi^2$ | p-value |
|-------------------|-------------------------|-------------------------|-------------------------|----------------|----------------|----------|---------|
| Sample category   | Cloacal swabs           | 75                      | 18                      | 24.0           | 15.8 – 34.8    | 0.7619   | 0.8586  |
|                   | Surface swabs           | 25                      | 5                       | 20.0           | 8.9 – 39.1     |          |         |
|                   | Feed samples            | 13                      | 2                       | 15.4           | 4.3 – 42.2     |          |         |
|                   | Water samples           | 12                      | 2                       | 16.7           | 4.7 – 44.8     |          |         |
| Sampling location | GRA                     | 30                      | 4                       | 13.3           | 5.3 – 29.7     | 7.147    | 0.1283  |
|                   | Mairi Quwait            | 27                      | 9                       | 33.3           | 18.6 – 52.2    |          |         |
|                   | Dalori Community        | 25                      | 7                       | 28.0           | 14.3 – 47.6    |          |         |
|                   | MMC                     | 18                      | 5                       | 27.8           | 12.5 – 50.9    |          |         |
|                   | University of Maiduguri | 25                      | 2                       | 8.0            | 2.2 – 23.0     |          |         |
| Total             |                         | 125                     | 27                      | 21.6           | 15.3 – 29.0    |          |         |

**Keys:**  $\chi^2$  = Chi – Square; CI = Confidence Interval; LL – UL = Lower Limit – Upper Limit;  
No statistically significant associations were observed ( $p > 0.05$ )

**Table 2: Occurrence of *Klebsiella* Species in Commercial Layer Chickens in Maiduguri, Borno State, Nigeria by Age Group and Management System (n = 75)**

| Variable          | Category                     | No. of samples Examined | No. of samples Positive | Prevalence (%) | 95% CI LL – UL     | $\chi^2$ | p-value |
|-------------------|------------------------------|-------------------------|-------------------------|----------------|--------------------|----------|---------|
| Age group (weeks) | Growing pullets (<18)        | 22                      | 3                       | 13.6           | 3.6 – 33.4         | 3.371    | 0.1854  |
|                   | Point-of-lay pullets (18–20) | 28                      | 6                       | 21.4           | 9.0 – 40.7         |          |         |
|                   | Adult layers (>20)           | 25                      | 9                       | 36.0           | 19.1 – 56.6        |          |         |
| Management system | Battery cage                 | 40                      | 3                       | 7.5            | 2.6 – 19.9         | 12.79    | 0.0003* |
|                   | Deep litter                  | 35                      | 15                      | 42.9           | 28.0 – 59.2        |          |         |
| <b>Total</b>      |                              | <b>75</b>               | <b>18</b>               | <b>24.0</b>    | <b>15.8 – 34.8</b> |          |         |

**Keys:**  $\chi^2$  = Chi – Square; CI = Confidence Interval; LL – UL = Lower Limit – Upper Limit;

\*Statistically significant at p < 0.05

**Table 3: Species Distribution of *Klebsiella* Isolates (n = 27) in Commercial Layer Chickens and Poultry Farm Environmental Samples by Sample Source in Maiduguri, Borno State, Nigeria**

| Sample Source | <i>K. pneumoniae</i> n (%) | <i>K. oxytoca</i> n (%) | <i>K. variicola</i> n (%) | Total n (%)       |
|---------------|----------------------------|-------------------------|---------------------------|-------------------|
| Cloacal swabs | 13 (68.4)                  | 5 (83.3)                | 0 (0.0)                   | 18 (66.7)         |
| Surface swabs | 4 (21.1)                   | 1 (16.7)                | 0 (0.0)                   | 5 (18.5)          |
| Feed samples  | 1 (5.3)                    | 0 (0.0)                 | 1 (50.0)                  | 2 (7.4)           |
| Water samples | 1 (5.3)                    | 0 (0.0)                 | 1 (50.0)                  | 2 (7.4)           |
| <b>Total</b>  | <b>19 (100.0)</b>          | <b>6 (100.0)</b>        | <b>2 (100.0)</b>          | <b>27 (100.0)</b> |

**Footnote:** Percentages are column percentages (proportion of each species contributed by each sample source); n = number

**Table 4: Antimicrobial Susceptibility Profile of *Klebsiella* Isolates (n = 27) in Commercial Layer Chickens and Poultry Farm Environmental Samples in Maiduguri, Borno State, Nigeria**

| Antimicrobial Agent           | Class                                    | Susceptible n (%) | Intermediate n (%) | Resistant n (%) |
|-------------------------------|------------------------------------------|-------------------|--------------------|-----------------|
| Ampicillin                    | Penicillin                               | 8 (29.6)          | 0 (0.0)            | 19 (70.4)       |
| Amoxicillin-Clavulanic acid   | Penicillin/ $\beta$ -lactamase Inhibitor | 13 (48.1)         | 3 (11.1)           | 11 (40.7)       |
| Ceftriaxone                   | 3rd Generation Cephalosporin             | 11 (40.7)         | 3 (11.1)           | 13 (48.1)       |
| Cefotaxime                    | 3rd Generation Cephalosporin             | 13 (48.1)         | 3 (11.1)           | 11 (40.7)       |
| Ceftazidime                   | 3rd Generation Cephalosporin             | 13 (48.1)         | 3 (11.1)           | 11 (40.7)       |
| Meropenem                     | Carbapenem                               | 19 (70.4)         | 3 (11.1)           | 5 (18.5)        |
| Gentamicin                    | Aminoglycoside                           | 13 (48.1)         | 9 (33.3)           | 5 (18.5)        |
| Amikacin                      | Aminoglycoside                           | 16 (59.3)         | 6 (22.2)           | 5 (18.5)        |
| Ciprofloxacin                 | Fluoroquinolone                          | 13 (48.1)         | 8 (29.6)           | 6 (22.2)        |
| Levofloxacin                  | Fluoroquinolone                          | 13 (48.1)         | 8 (29.6)           | 6 (22.2)        |
| Tetracycline                  | Tetracycline                             | 6 (22.2)          | 3 (11.1)           | 18 (66.7)       |
| Trimethoprim-Sulfamethoxazole | Sulfonamide                              | 10 (37.0)         | 1 (3.7)            | 16 (59.3)       |
| Chloramphenicol               | Phenicol                                 | 14 (51.9)         | 5 (18.5)           | 8 (29.6)        |

**Footnote:** Erythromycin and colistin were not tested due to absence of CLSI disk diffusion breakpoints for Enterobacterales

**Table 5: Multidrug Resistance Patterns and MAR Index Distribution of *Klebsiella* Isolates (n = 27) in Commercial Layer Chickens and Poultry Farm Environmental Samples in Maiduguri, Borno State, Nigeria**

| <b>Panel A: MDR Patterns (n = 27)</b>           |                                          |                 |                |
|-------------------------------------------------|------------------------------------------|-----------------|----------------|
| Resistance Category                             | Classes Resisted                         | No. of Isolates | Percentage (%) |
| Pansusceptible (PDS)                            | 0                                        | 0               | 0.0            |
| Non-MDR                                         | 1–2                                      | 15              | 55.6           |
| MDR                                             | 3                                        | 3               | 11.1           |
| MDR                                             | 4                                        | 4               | 14.8           |
| MDR                                             | 5                                        | 3               | 11.1           |
| XDR                                             | $\geq 6$ (all but $\leq 2$ of 8 classes) | 2               | 7.4            |
| PDR                                             | All 8 classes                            | 0               | 0.0            |
| <b>Total</b>                                    |                                          | <b>27</b>       | <b>100.0</b>   |
| Total MDR (3–5 classes)                         | 3–5                                      | 10              | 37.0           |
| Total XDR ( $\geq 6$ classes)                   | $\geq 6$                                 | 2               | 7.4            |
| Total MDR + XDR ( $\geq 3$ classes)             | $\geq 3$                                 | 12              | 44.4           |
| <b>Panel B: MAR Index Distribution (n = 27)</b> |                                          |                 |                |
| MAR Index Range                                 | No. of Isolates                          | Percentage (%)  | Risk Category  |
| 0.00                                            | 0                                        | 0.0             | No risk        |
| 0.01–0.20                                       | 15                                       | 55.6            | Low risk       |

|                       |           |                    |           |
|-----------------------|-----------|--------------------|-----------|
| 0.21–0.40             | 7         | 25.9               | High risk |
| 0.41–0.60             | 4         | 14.8               | High risk |
| 0.61–1.00             | 1         | 3.7                | High risk |
| <b>Total</b>          | <b>27</b> | <b>100.0</b>       |           |
| <b>Mean MAR Index</b> |           | <b>0.38 ± 0.22</b> |           |

**Keys:** MDR = multidrug-resistant; XDR = extensively drug-resistant; PDR = pan-drug-resistant; MAR = multiple antibiotic resistance.

**Footnotes:** **MDR:** Resistance to at least one agent in 3–5 antimicrobial classes; **XDR:** Resistance to at least one agent in all but ≤2 of the 8 antimicrobial classes tested (i.e., resistant to ≥6 classes), with the isolate remaining susceptible to only one or two classes; **PDR:** Resistance to all 8 antimicrobial classes tested.

MAR index >0.2 was considered high risk, indicating that the isolate originated from an environment with high antimicrobial selection pressure

**Antimicrobial classes tested (8 total):** penicillins, penicillin/β-lactamase inhibitor combinations, cephalosporins, carbapenems, aminoglycosides, fluoroquinolones, tetracyclines, and sulfonamides. Colistin (polymyxins) was evaluated separately and was **not** included in the class count for MDR/XDR/PDR classification.

**Table 6: Socio-demographic Characteristics of Poultry Farmers and Farm Characteristics (n = 75) in Maiduguri, Borno State, Nigeria**

| Variable           | Category                 | Frequency (n) | Percentage (%) |
|--------------------|--------------------------|---------------|----------------|
| Age (years)        | ≤30                      | 18            | 24.0           |
|                    | 31–40                    | 29            | 38.7           |
|                    | 41–50                    | 17            | 22.7           |
|                    | 51–60                    | 8             | 10.7           |
|                    | >60                      | 3             | 4.0            |
| Sex                | Male                     | 51            | 68.0           |
|                    | Female                   | 24            | 32.0           |
| Educational level  | No formal education      | 8             | 10.7           |
|                    | Primary                  | 19            | 25.3           |
|                    | Secondary                | 27            | 36.0           |
|                    | Tertiary                 | 21            | 28.0           |
| Farming experience | <5 years                 | 21            | 28.0           |
|                    | 5–10 years               | 32            | 42.7           |
|                    | 11–20 years              | 16            | 21.3           |
|                    | >20 years                | 6             | 8.0            |
| Flock size         | <500 birds               | 22            | 29.3           |
|                    | 500–1000 birds           | 31            | 41.3           |
|                    | 1001–5000 birds          | 16            | 21.3           |
|                    | >5000 birds              | 6             | 8.0            |
| Production system  | Intensive (battery cage) | 43            | 57.3           |
|                    | Intensive (deep litter)  | 27            | 36.0           |
|                    | Semi-intensive           | 5             | 6.7            |

**Table 7: Antimicrobial Use Practices Among Poultry Farmers (n = 75) in Maiduguri, Borno State, Nigeria**

| Practice                | Category              | Frequency (n) | Percentage (%) |
|-------------------------|-----------------------|---------------|----------------|
| Use of antibiotics      | Yes                   | 68            | 90.7           |
|                         | No                    | 7             | 9.3            |
| Source of antibiotics   | Veterinary pharmacy   | 22            | 32.4           |
|                         | Agro-veterinary shop  | 31            | 45.6           |
|                         | Open market           | 10            | 14.7           |
|                         | Unlicensed vendor     | 5             | 7.4            |
| Source of prescription  | Veterinarian          | 11            | 16.2           |
|                         | Extension agent       | 6             | 8.8            |
|                         | Self-prescription     | 32            | 47.1           |
|                         | Fellow farmers        | 19            | 27.9           |
| Reason for use          | Treatment of disease  | 24            | 35.3           |
|                         | Prevention of disease | 26            | 38.2           |
|                         | Growth promotion      | 18            | 26.5           |
| Frequency of use        | Monthly               | 19            | 27.9           |
|                         | Every 2–3 months      | 28            | 41.2           |
|                         | Occasionally          | 21            | 30.9           |
| Route of administration | Drinking water        | 42            | 61.8           |
|                         | Feed incorporation    | 18            | 26.5           |
|                         | Injection             | 6             | 8.8            |
|                         | Topical               | 2             | 2.9            |
| Adherence to dosage     | Always                | 21            | 30.9           |
|                         | Sometimes             | 29            | 42.6           |
|                         | Never                 | 18            | 26.5           |
| Withdrawal period       | Observed              | 14            | 20.6           |
|                         | Not observed          | 54            | 79.4           |

|                                     |     |    |      |
|-------------------------------------|-----|----|------|
| Record keeping of Antibiotic use    | Yes | 9  | 13.2 |
|                                     | No  | 59 | 86.8 |
| Use without veterinary consultation | Yes | 57 | 83.8 |
|                                     | No  | 11 | 16.2 |

**Footnote:** Percentages for antibiotic use practices are based on the 68 farmers who reported using antibiotics

**Table 8: Biosecurity Practices in Selected Poultry Farms (n = 75) in Maiduguri, Borno State, Nigeria**

| Measure                   | Category           | Frequency (n) | Percentage (%) |
|---------------------------|--------------------|---------------|----------------|
| Quarantine of new birds   | Yes                | 14            | 18.7           |
|                           | No                 | 61            | 81.3           |
| Disinfection of equipment | Regular            | 12            | 16.0           |
|                           | Occasional         | 28            | 37.3           |
|                           | Never              | 35            | 46.7           |
| Visitor control           | Restricted         | 17            | 22.7           |
|                           | Not restricted     | 58            | 77.3           |
| Foot baths                | Yes                | 13            | 17.3           |
|                           | No                 | 62            | 82.7           |
| Protective clothing       | Always             | 19            | 25.3           |
|                           | Sometimes          | 26            | 34.7           |
|                           | Never              | 30            | 40.0           |
| Disposal of dead birds    | Proper             | 21            | 28.0           |
|                           | Improper           | 54            | 72.0           |
| Cleaning of drinkers      | Daily              | 18            | 24.0           |
|                           | Weekly             | 29            | 38.7           |
|                           | Occasionally/Never | 28            | 37.3           |
| Cleaning of feeders       | Daily              | 15            | 20.0           |
|                           | Weekly             | 27            | 36.0           |
|                           | Occasionally/Never | 33            | 44.0           |
| Vaccination               | Regular            | 52            | 69.3           |
|                           | Irregular          | 18            | 24.0           |
|                           | Never              | 5             | 6.7            |
| Rodent/pest control       | Yes                | 24            | 32.0           |
|                           | No                 | 51            | 68.0           |
| Record keeping            | Yes                | 11            | 14.7           |
|                           | No                 | 64            | 85.3           |

**Table 9: Awareness of Antimicrobial Resistance and One Health Concepts among Poultry Farmers (n = 75) in Maiduguri, Borno State, Nigeria**

| Variable                                             | Category       | Frequency (n) | Percentage (%) |
|------------------------------------------------------|----------------|---------------|----------------|
| Heard of AMR                                         | Yes            | 19            | 25.3           |
|                                                      | No             | 56            | 74.7           |
| Knowledge of causes of AMR                           | Good           | 6             | 8.0            |
|                                                      | Fair           | 13            | 17.3           |
|                                                      | Poor           | 56            | 74.7           |
| AMR as public health issue                           | Yes            | 15            | 20.0           |
|                                                      | No             | 60            | 80.0           |
| Training on AMR                                      | Yes            | 5             | 6.7            |
|                                                      | No             | 70            | 93.3           |
| Knowledge of prudent antibiotic use                  | Good           | 9             | 12.0           |
|                                                      | Fair           | 18            | 24.0           |
|                                                      | Poor           | 48            | 64.0           |
| Heard of One Health                                  | Yes            | 11            | 14.7           |
|                                                      | No             | 64            | 85.3           |
| AMR transmission poultry-to-human                    | Yes            | 14            | 18.7           |
|                                                      | No             | 61            | 81.3           |
| Source of AMR information                            | Radio/TV       | 8             | 10.7           |
|                                                      | Health workers | 5             | 6.7            |
|                                                      | Veterinarians  | 4             | 5.3            |
|                                                      | Fellow farmers | 2             | 2.7            |
|                                                      | None           | 56            | 74.7           |
| Belief antibiotics can be stopped when birds improve | Yes            | 52            | 69.3           |
|                                                      | No             | 23            | 30.7           |
| Belief antibiotics can be shared among farmers       | Yes            | 41            | 54.7           |
|                                                      | No             | 34            | 45.3           |
| Awareness of antimicrobial residues in eggs          | Yes            | 12            | 16.0           |
|                                                      | No             | 63            | 84.0           |

**Table 10: Association between Biosecurity Practices and Occurrence of *Klebsiella* Species in Poultry Farms (n = 75) in Maiduguri, Borno State, Nigeria**

| Practice                | Category           | No. of Farms | Farms Positive n (%) | $\chi^2$ | p-value |
|-------------------------|--------------------|--------------|----------------------|----------|---------|
| Equipment disinfection  | Regular/Occasional | 40           | 3 (7.5)              | –        | 0.1737  |
|                         | Never              | 35           | 7 (20.0)             |          |         |
| Foot baths              | Yes                | 13           | 1 (7.7)              | –        | 1.0000  |
|                         | No                 | 62           | 9 (14.5)             |          |         |
| Quarantine of new birds | Yes                | 14           | 1 (7.1)              | –        | 0.6767  |
|                         | No                 | 61           | 9 (14.8)             |          |         |
| Visitor control         | Restricted         | 17           | 1 (5.9)              | –        | 0.4389  |
|                         | Not restricted     | 58           | 9 (15.5)             |          |         |
| Record keeping          | Yes                | 11           | 0 (0.0)              | –        | 0.3409  |
|                         | No                 | 64           | 10 (15.6)            |          |         |
| Protective clothing     | Always/Sometimes   | 45           | 4 (8.9)              | –        | 0.1853  |
|                         | Never              | 30           | 6 (20.0)             |          |         |

**Footnote:** Percentages are row percentages within each biosecurity category

Fisher's exact test was used where expected cell frequencies were <5.

**Table 11: Association between Antimicrobial Use Practices and Multidrug Resistance in *Klebsiella* Isolates (n = 27) in Maiduguri, Borno State, Nigeria**

| Practice                  | Category                         | No. of Isolates | MDR Isolates n (%) | $\chi^2$ | p-value |
|---------------------------|----------------------------------|-----------------|--------------------|----------|---------|
| Source of prescription    | Veterinarian                     | 6               | 1 (16.7)           | 2.422    | 0.2979  |
|                           | Self-prescription/Fellow farmers | 17              | 9 (52.9)           |          |         |
|                           | Extension agent                  | 4               | 2 (50.0)           |          |         |
| Reason for antibiotic use | Treatment                        | 10              | 2 (20.0)           | –        | 0.1071  |
|                           | Prevention/Growth promotion      | 17              | 10 (58.8)          |          |         |
| Adherence to dosage       | Always                           | 8               | 1 (12.5)           | –        | 0.0433* |
|                           | Sometimes/Never                  | 19              | 11 (57.9)          |          |         |
| Withdrawal period         | Observed                         | 6               | 1 (16.7)           | –        | 0.1819  |
|                           | Not observed                     | 21              | 11 (52.4)          |          |         |
| Veterinary consultation   | Yes                              | 8               | 1 (12.5)           | –        | 0.0433* |
|                           | No                               | 19              | 11 (57.9)          |          |         |

**Footnote:** Antimicrobial use practices are farm-level variables, while MDR is an isolate-level variable. The analysis does not account for clustering of isolates within farms.

Fisher's exact test was used where expected cell frequencies were <5.

\*Statistically significant at  $p < 0.05$ ; MDR = multidrug-resistant

**Table 12: Association between Educational Level and Antimicrobial Use/Biosecurity Practices Among Poultry Farmers (n = 75) in Maiduguri, Borno State, Nigeria**

| Practice                | Educational Level | Good Practice n (%) | Poor Practice n (%) | $\chi^2$ | p-value |
|-------------------------|-------------------|---------------------|---------------------|----------|---------|
| Veterinary consultation | Tertiary          | 8 (38.1)            | 13 (61.9)           | 5.324    | 0.0698  |
|                         | Secondary         | 5 (18.5)            | 22 (81.5)           |          |         |
|                         | Primary/None      | 3 (11.1)            | 24 (88.9)           |          |         |
| Adherence to dosage     | Tertiary          | 12 (57.1)           | 9 (42.9)            | 14.58    | 0.0007* |
|                         | Secondary         | 7 (25.9)            | 20 (74.1)           |          |         |
|                         | Primary/None      | 2 (7.4)             | 25 (92.6)           |          |         |
| Withdrawal period       | Tertiary          | 9 (42.9)            | 12 (57.1)           | 12.34    | 0.0021* |
|                         | Secondary         | 4 (14.8)            | 23 (85.2)           |          |         |
|                         | Primary/None      | 1 (3.7)             | 26 (96.3)           |          |         |
| Record keeping          | Tertiary          | 7 (33.3)            | 14 (66.7)           | 13.27    | 0.0013* |
|                         | Secondary         | 2 (7.4)             | 25 (92.6)           |          |         |
|                         | Primary/None      | 0 (0.0)             | 27 (100.0)          |          |         |
| Protective clothing     | Tertiary          | 11 (52.4)           | 10 (47.6)           | 12.85    | 0.0016* |
|                         | Secondary         | 6 (22.2)            | 21 (77.8)           |          |         |
|                         | Primary/None      | 2 (7.4)             | 25 (92.6)           |          |         |
| Equipment disinfection  | Tertiary          | 9 (42.9)            | 12 (57.1)           | 16.89    | 0.0002* |
|                         | Secondary         | 3 (11.1)            | 24 (88.9)           |          |         |
|                         | Primary/None      | 0 (0.0)             | 27 (100.0)          |          |         |

**Footnote:** Percentages are row percentages within each educational level. Total number of farmers: Tertiary = 21, Secondary = 27, Primary/None = 27.

\*Statistically significant at  $p < 0.05$

## DISCUSSION

The present study documents a substantial prevalence of *Klebsiella* species (21.6%) in commercial layer chickens and their farm environments in Maiduguri, Borno State, Nigeria. This finding aligns with the growing body of evidence identifying poultry as a significant reservoir for *Klebsiella* species, particularly *K. pneumoniae*, across different poultry farm settings in Nigeria (Ugbo *et al.*, 2024; Irekeola *et al.*, 2025) and other countries in the world (Effah *et al.*, 2020; Al-Aalim *et al.*, 2024). The observed prevalence is consistent with reports from Nigeria, where Ugbo *et al.* (2024) documented a 17.8% isolation rate from poultry environmental swabs, while Irekeola *et al.* (2025), in a systematic review, reported varying prevalence rates across different regions of Nigeria. The findings of the present study also agrees with reports from other African nations including Egypt and Ethiopia, where comparable carriage rates have been reported in commercial and smallholder poultry operations (Kahin *et al.*, 2024; El Baz *et al.*, 2026).

The predominance of *K. pneumoniae* (70.4%) among the recovered isolates corroborates the species' established ecological niche within the poultry gastrointestinal tract and its status as the most clinically significant member of the genus (Franklin-Alming *et al.*, 2021; Kowalczyk *et al.*, 2022; Ugbo *et al.*, 2024). The recovery of *K. oxytoca* (22.2%) and *K. variicola* (7.4%) further highlights the species diversity within poultry-associated *Klebsiella* populations, with *K. variicola* being notably restricted to environmental sources (feed and water) in this study. This source-specific distribution pattern suggests potential ecological specialization among *Klebsiella* species and may reflect differential survival or colonization dynamics within the poultry production environment. The absence of a statistically significant association between sample category and *Klebsiella* occurrence contrasts with some previous studies that have reported higher recovery rates from cloacal swabs compared to environmental samples (El Baz *et al.*, 2026; Karacan Sever *et al.*, 2026). However, the comparatively high prevalence observed in surface swabs (20.0%) and the environmental detection of *Klebsiella* in feed and water samples collectively indicate widespread environmental contamination within the poultry production ecosystem. This environmental dissemination is particularly concerning given the capacity of *Klebsiella* species to persist in farm environments and serve as a

continuous source of exposure for colonized and susceptible birds (Deleza *et al.*, 2025; Korves *et al.*, 2025).

The striking and statistically significant association between deep litter management and *Klebsiella* colonization (42.9% compared to 7.5% in battery cages) represents one of the most salient findings of this investigation. This observation is mechanistically plausible and aligns with the known epidemiology of *Klebsiella* in poultry production systems. The deep litter system, characterized by the accumulation of organic bedding material, provides an enriched substrate for bacterial proliferation and environmental persistence. The constant contact between birds and contaminated litter material facilitates fecal-oral transmission and cloacal colonization, while the elevated ammonia concentrations and moisture levels typical of deep litter environments may compromise respiratory and intestinal mucosal barriers, enhancing susceptibility to colonization (Deleza *et al.*, 2025; El Baz *et al.*, 2025).

The substantially lower prevalence in battery cage systems (7.5%) likely reflects the physical separation of birds from their excreta, reduced environmental contamination, and the more controlled microclimate inherent to cage housing. This finding is consistent with the broader literature indicating that management intensification does not uniformly increase infectious disease risk; rather, the specific housing system and its associated hygiene dynamics are critical determinants of pathogen transmission (Taylor and Hurnik, 1996; Choi, 2025). The practical implication is clear: targeted interventions in deep litter operations, including enhanced litter management, regular disinfection, and improved ventilation, are urgently needed to mitigate *Klebsiella* colonization in commercial layer flocks.

The progressive increase in *Klebsiella* prevalence with advancing age—from 13.6% in growing pullets to 36.0% in adult layers—though not statistically significant, is biologically meaningful and consistent with the epidemiology of opportunistic bacterial colonization. This age-related trend likely reflects cumulative exposure over time, with older birds having greater opportunity to acquire *Klebsiella* from environmental sources and from horizontal transmission within the flock. Additionally, the physiological demands of egg production and the associated metabolic and immunological changes in adult layers may contribute to increased susceptibility to colonization (Theocharidi *et al.*,

2022; Drakul *et al.*, 2025). The progressive nature of this trend, if confirmed in larger longitudinal studies, would have significant implications for flock management, suggesting that older birds may represent a particularly important target for surveillance and intervention strategies.

The antimicrobial resistance profile of the *Klebsiella* isolates reveals a concerning pattern of resistance across multiple critically important antimicrobial classes. The high resistance rates observed against ampicillin (70.4%) and tetracycline (66.7%) are consistent with the extensive and often indiscriminate use of these agents in Nigerian poultry production, as documented in the present study's farmer survey and corroborated by previous investigations in the region (Gebremeskel *et al.*, 2023; Ugbo *et al.*, 2024; El Baz *et al.*, 2025; Utami *et al.*, 2025). The elevated resistance to trimethoprim-sulfamethoxazole (59.3%) further reflects the widespread availability and use of this combination in poultry practice.

Of particular concern is the resistance observed against third-generation cephalosporins, with ceftriaxone, cefotaxime, and ceftazidime each exhibiting resistance rates of 40.7% to 48.1%. These antimicrobials are categorized as critically important for human medicine, and the presence of resistant *Klebsiella* in poultry represents a significant public health threat (Mitman *et al.*, 2022; Huynh *et al.*, 2025; Kashosi *et al.*, 2026). The detection of carbapenem (meropenem) resistance in 18.5% of isolates is especially alarming, as carbapenems constitute last-resort therapeutic options for severe infections caused by multidrug-resistant Gram-negative bacteria (Rida *et al.*, 2018; WHO, 2024; Salem *et al.*, 2026). These findings align with the systematic review by Elshamy and Aboshanab (2020), which documented high resistance to penicillins and third- and fourth-generation cephalosporins in poultry-associated *Klebsiella* isolates globally, with resistance to carbapenems and polymyxins being particularly pronounced in African and Asian settings. The relatively lower resistance rates observed against amikacin (18.5%) and meropenem (18.5%) suggest that these agents may retain some efficacy against poultry-associated *Klebsiella* in this setting, although the presence of any carbapenem resistance is clinically significant. Gentamicin and chloramphenicol exhibited intermediate resistance profiles, with resistance rates of 18.5% and 29.6%, respectively.

The finding that 44.4% of *Klebsiella* isolates were multidrug-resistant (resistant to  $\geq 3$  antimicrobial classes) is consistent with the global trend of

increasing MDR prevalence in poultry-associated *Klebsiella*. A study from China reported that 87.88% of ESBL-producing *K. pneumoniae* strains from broiler chickens were MDR (Li *et al.*, 2022), while investigations in Indonesia and Egypt have documented MDR rates ranging from 54% to 100% (Darniati *et al.*, 2025; Enany *et al.*, 2025). The detection of one extensively drug-resistant (XDR) isolate (3.7%) is of particular concern, as XDR phenotypes severely limit therapeutic options and represent a sentinel indicator of escalating resistance in the poultry production environment (Enany *et al.*, 2025).

The mean MAR index of  $0.38 \pm 0.22$ , with 44.4% of isolates exhibiting MAR indices  $\geq 0.21$  (categorized as high risk), provides quantitative evidence of extensive antimicrobial exposure in the source environments. According to established interpretation criteria, MAR indices exceeding 0.2 indicate that isolates originate from environments with high antimicrobial selection pressure (Krumperman, 1983; Muthu *et al.*, 2022; Isaiah *et al.*, 2025). The absence of any isolate with a MAR index of 0.00 (indicating no antimicrobial resistance) and the absence of pansusceptible isolates further substantiate the pervasive influence of antimicrobial use on the resistance ecology of poultry-associated *Klebsiella* in Maiduguri. These findings are consistent with the whole-genome sequencing study from Bangladesh, which documented high MAR indices in poultry-derived *K. pneumoniae* and identified genomic determinants of resistance that pose threats to both poultry and human health (Munim *et al.*, 2024).

The survey of poultry farmers revealed a pattern of antimicrobial use that is both extensive and frequently inappropriate, providing critical context for the resistance findings. The reported antibiotic use prevalence of 90.7% is consistent with previous studies from Nigeria and other low- and middle-income countries, where antimicrobials are often used as substitutes for adequate biosecurity and husbandry practices (Chah *et al.*, 2022). The predominance of self-prescription (47.1%) and prescription by fellow farmers (27.9%) over veterinary consultation (16.2%) represents a fundamental breach of antimicrobial stewardship principles and creates conditions conducive to the selection and dissemination of resistant bacteria.

The statistically significant association between non-adherence to dosage and MDR, and between lack of veterinary consultation and MDR, provides empirical evidence linking specific antimicrobial use behaviors to resistance outcomes. These findings are

mechanistically coherent: subtherapeutic or inconsistent dosing fails to achieve bactericidal concentrations, thereby promoting the selection of resistant subpopulations, while the absence of veterinary oversight increases the likelihood of inappropriate drug selection, dosing, and duration (Alhaji *et al.*, 2018). The high proportion of farmers reporting use of antibiotics for disease prevention (38.2%) and growth promotion (26.5%) is particularly concerning, as these practices are recognized drivers of antimicrobial resistance and are increasingly discouraged or banned in many jurisdictions (WHO, 2017; Matheou *et al.*, 2025; Raihan *et al.*, 2026).

The finding that 79.4% of farmers did not observe withdrawal periods has direct implications for food safety, as antimicrobial residues may persist in eggs and meat, potentially contributing to the selection of resistant bacteria in consumers and the broader food chain (Adegbeye *et al.* 2024; Bacanlı, 2024; Mashauri *et al.*, 2025). The low rate of record keeping (13.2%) further undermines the capacity for antimicrobial use surveillance and stewardship interventions.

The pervasive biosecurity deficiencies documented in this study—including inadequate equipment disinfection (46.7% never disinfect), absence of foot baths (82.7%), unrestricted visitor access (77.3%), and improper disposal of dead birds (72.0%)—create an environmental milieu conducive to the introduction, persistence, and dissemination of *Klebsiella* and other pathogens. While the associations between individual biosecurity practices and *Klebsiella* occurrence did not achieve statistical significance in this study, the consistent directional trends (e.g., higher positivity rates in farms that never disinfected equipment compared to those that did: 20.0% compared to 7.5%; higher positivity in farms without record keeping: 15.6% compared to 0.0%) suggest that the lack of statistical significance may reflect limited statistical power rather than a true absence of effect. However, these associations may be confounded by other farm-level factors, such as management system, flock size, and antimicrobial use practices, which were not controlled for in this analysis. Multivariable modeling with a larger sample size is needed to disentangle these effects.

The biosecurity deficiencies observed are consistent with findings from other low-resource settings, where economic constraints, limited technical knowledge, and weak regulatory enforcement contribute to suboptimal farm management practices (Ayebare *et al.*, 2025). The association between educational level and biosecurity practices—with tertiary-educated farmers demonstrating significantly better adherence

to dosage, withdrawal period observance, record keeping, protective clothing use, and equipment disinfection—highlights the critical role of education and training in improving antimicrobial stewardship and biosecurity compliance. However, education may be a proxy for socioeconomic status and access to resources, and the observed associations may be confounded by these factors. Future studies should include measures of socioeconomic status and farm resources to disentangle these effects. These findings suggest that targeted educational interventions, particularly for farmers with limited formal education, could yield substantial improvements in on-farm practices.

The profound knowledge deficits regarding antimicrobial resistance and One Health concepts among the surveyed farmers—with only 25.3% having heard of AMR, 14.7% aware of One Health, and 74.7% lacking any source of AMR information—represent a fundamental barrier to effective antimicrobial stewardship. These findings are consistent with surveys from other developing countries, which have documented similarly low levels of AMR awareness among poultry farmers (Nonga *et al.*, 2010; Chah *et al.*, 2022; Halilu *et al.*, 2025). The finding that 69.3% of farmers believed antibiotics could be discontinued when birds showed clinical improvement, and 54.7% believed antibiotics could be shared among farmers, reflects misconceptions that directly contribute to subtherapeutic dosing and the selection of resistant bacteria.

The low awareness of antimicrobial residues in eggs (16.0%) is particularly concerning given the direct implications for food safety and consumer health. These findings are consistent with surveys from other developing countries, which have documented similarly low levels of AMR awareness among poultry farmers (Nonga *et al.*, 2010; Halilu *et al.*, 2025). The association between educational level and various stewardship practices observed in this study reinforces the need for educational interventions that address not only the technical aspects of antimicrobial use but also the fundamental concepts of resistance selection and the interconnectedness of human, animal, and environmental health within the One Health framework.

The recovery of antimicrobial-resistant and MDR *Klebsiella* from commercial layer chickens and their environment in Maiduguri has significant zoonotic and public health implications. *Klebsiella pneumoniae* is recognized as a leading cause of healthcare-associated infections, including

pneumonia, bloodstream infections, and urinary tract infections, and the emergence of MDR and carbapenem-resistant strains has been designated by the World Health Organization as a critical priority for research and development of new antibiotics (WHO, 2024; El Baz *et al.*, 2026). The presence of such strains in poultry production systems creates potential pathways for zoonotic transmission through direct contact with colonized birds, consumption of contaminated poultry products, and environmental dissemination of resistant bacteria and resistance genes (Silva-Bea *et al.*, 2024; Munim *et al.*, 2024; Li *et al.*, 2025b).

The detection of *K. variicola* in feed and water samples is notable, as this species has been increasingly recognized as an emerging human pathogen with clinical presentations overlapping those of *K. pneumoniae* (Rodríguez-Medina *et al.*, 2019; Ohno *et al.*, 2025; Yin *et al.*, 2025). The environmental recovery of this species suggests that feed and water may serve as previously underappreciated reservoirs for clinically relevant *Klebsiella* species in poultry operations. The exclusive recovery of *K. variicola* from feed and water samples, with no isolation from cloacal or surface swabs, suggests that these environmental sources may serve as specific reservoirs for this species. This finding warrants further investigation into the survival and transmission dynamics of *K. variicola* in poultry production systems.

Several limitations of this study warrant consideration. First, the cross-sectional design precludes assessment of temporal relationships between antimicrobial use practices and the emergence of resistance. Second, the relatively small sample size ( $n = 125$ ) and the small number of isolates ( $n = 27$ ) limit the precision of prevalence estimates and the statistical power to detect associations. Larger studies are needed to confirm these findings. Third, antimicrobial susceptibility testing was performed using disk diffusion, which does not provide minimum inhibitory concentration data and cannot detect all resistance mechanisms. Fourth, the absence of molecular characterization of resistance determinants and virulence factors limits inferences regarding the genetic basis of resistance and the pathogenic potential of the isolates. Fifth, the farmer survey relied on self-reported practices, which may be subject to social desirability bias. Finally, the study was conducted in a single geographical region, and the findings may not be generalizable to other settings with different management practices, antimicrobial use patterns, and environmental

conditions. The study was conducted in a conflict-affected region, which may have unique challenges related to veterinary service delivery, antimicrobial supply chains, and farm management practices.

## CONCLUSION

This study provides comprehensive evidence that commercial layer chickens and their farm environments in Maiduguri, Borno State, constitute significant reservoirs of antimicrobial-resistant *Klebsiella* species. The overall prevalence was 21.6%, with cloacal swabs yielding the highest recovery, followed by surface swabs, water, and feed. Speciation revealed the predominance of *K. pneumoniae*, followed by *K. oxytoca* and *K. variicola*, with the latter recovered exclusively from feed and water. Deep litter management was a significant risk factor for colonization compared to battery cages. Critically, 44.4% of isolates were multidrug-resistant, with high resistance to ampicillin, tetracycline, and trimethoprim-sulfamethoxazole, while one isolate exhibited extensively drug-resistant phenotype. The mean multiple antibiotic resistance index was  $0.38 \pm 0.22$ , with 44.4% of isolates exceeding the 0.21 high-risk threshold. Non-adherence to dosage and absence of veterinary consultation were significantly associated with multidrug resistance, while tertiary education was significantly associated with better biosecurity and stewardship practices. These findings provide evidence that commercial layer farms in Maiduguri are potential sources of occupational and public health risks, highlighting the urgent need for integrated One Health surveillance, antimicrobial stewardship, and enhanced biosecurity interventions. Based on the findings of the present study, it is therefore recommended that targeted interventions should prioritize deep litter farms, where colonization risk was highest. Deep litter farms should implement daily litter turning, regular litter replacement (every 2–3 weeks), and use of litter amendments (e.g., lime, zeolites) to reduce moisture and ammonia levels. Ventilation systems should be upgraded to ensure adequate air exchange, with targeted ammonia levels below 25 ppm. Veterinary oversight should be strengthened through mandatory prescription-only dispensing of antimicrobials, with regular farm visits.

## Competing Interests

The authors declare that they have no competing interests.

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