















Drivers of antimicrobial resistance in layer poultry farming: Evidence from high prevalence of multidrug-resistant *Escherichia coli* and enterococci in Zambia

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Abstract

Background and Aim: Inappropriate use of antimicrobials exacerbates antimicrobial resistance (AMR) in the poultry sector. Information on factors driving AMR in the layer poultry sector is scarce in Zambia. This study examined the drivers of AMR in the layer poultry sector in the Lusaka and Copperbelt Provinces of Zambia.

Materials and Methods: This cross-sectional study employed a structured questionnaire in 77 layer poultry farms in the provinces of Lusaka and Copperbelt, Zambia, from September 2020 to April 2021. Data analysis was conducted using Stata version 16.1. Antimicrobial resistance was defined as the presence of multidrug resistance (MDR) isolates. Multivariable regression analysis was used to identify drivers of AMR.

Results: In total, 365 samples were collected, from which 339 (92.9%) *Escherichia coli* and 308 (84.4%) *Enterococcus* spp. were isolated. Multidrug resistance was identified in 39% of the *E. coli* and 86% of the *Enterococcus* spp. The overall prevalence of AMR in layer poultry farms was 51.7% (95% confidence interval [CI]: 40.3%–63.5%). Large-scale farmers (Adjusted odds ratio [AOR] = 0.20, 95% CI: 0.04%–0.99%) than small-scale and farmers who were aware of AMR than those who were unaware (AOR = 0.26, 95% CI: 0.08%–0.86%) were less likely to experience AMR problems.

Conclusion: This study found a high prevalence of AMR in layer poultry farming linked to the type of farm management practices and lack of AMR awareness. Evidence of high MDR in our study is of public health concern and requires urgent attention. Educational interventions must increase AMR awareness, especially among small- and medium-scale poultry farmers.

Keywords: antimicrobial resistance, drivers, *Escherichia coli*, poultry, risk factors, Zambia.

Introduction

Antimicrobial resistance (AMR) is a public health problem that has been linked to the inappropriate use of antibiotics [1–4] and increased morbidity

and mortality worldwide [5, 6]. Antimicrobial resistance has increased medical costs and negatively impacted global economy [7, 8]. Furthermore, the emergence of multidrug resistance (MDR) has made the treatment of infections challenging [9–11]. If MDR is not addressed, it is estimated to cause many deaths and lead to the next pandemic [12–17].

The development of AMR in poultry is complex and has been linked to many factors [18, 19]. In addition to bacterial resistance [20–22], overuse and misuse of antibiotics have worsened the AMR problem [23–27]. Most farmers use antibiotics to

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promote growth, prevent infections, and treat microbial infections in livestock and plants [28–33]. This may cause microorganisms to develop resistance to different classes of antimicrobials [29, 34]. The inappropriate use of antibiotics in poultry has contributed to increased AMR of *Escherichia coli* and enterococci [35–38]. These are commensal bacteria in the gastrointestinal tract of animals, but may become pathogenic once they develop AMR or acquire virulence factors [39–43].

Most antibiotics for humans and animals are prescribed empirically, without confirming the causative pathogen and its antimicrobial susceptibility [44–46]. In addition, most antibiotics are prescribed in lower doses and for a shorter duration, leading to persistent subpatent disease [45, 47, 48]. Conversely, some prescribers tend to prescribe antibiotics for a longer period, increasing the risk of AMR [49, 50]. This has been worsened by a lack of laboratory diagnostic facilities for pathogen detection and susceptibility testing, leading to wrong drug prescriptions [51]. Most of these practices have been exacerbated by easy access to antibiotics, usually without prescription [52, 53].

Reports have shown that a lack of awareness and knowledge of antimicrobial use (AMU) among veterinary drug dispensers has contributed to the development of antimicrobial-resistant infections in poultry [54, 55]. Veterinary drug vendors or agrovet dispensers often possess inadequate knowledge regarding poultry AMU and AMR, resulting in insufficient information provided to the farmers about antibiotic indications, dose, frequency, and duration [56]. In addition, veterinary drug vendors do not provide adequate information to farmers on the importance of biosecurity, withdrawal period, use of prescriptions, and veterinary expert consultations [53]. This may lead to the irrational use of antimicrobials in poultry and increase the risk of AMR. Consistently, the risks of AMR emergence and spread are high when poultry farmers have poor awareness and knowledge about AMU and AMR [57–59], leading to the misuse of antibiotics [60–63]. Furthermore, farmers with poor knowledge of AMR tend to be unaware of the withdrawal period of antibiotics, risking AMR development in poultry products and increasing the possibility of transmitting AMR pathogens to humans [64, 65].

The lack of standard treatment guidelines in the poultry sector has contributed to the development of AMR in some countries [66, 67]. Under these circumstances, there is no standardized treatment for diseases, and laboratory results do not inform the prescription of antimicrobials [66]. In humans, standard treatment guidelines are critical for promoting consistency, diagnosing correctly, and treating disease, thereby improving patient quality of care [68, 69]. Similarly, the development and implementation of, as well as adherence to, standard treatment guidelines in animal health are critical in promoting the rational use of antibiotics and reducing AMR [70–72].

Poor regulation of access to antibiotics is one of the major drivers of AMR [24, 73]. Unregulated antibiotic access causes individuals to have access to plenty, usually cheap, antibiotics without prescriptions, which promotes irrational use [24, 74]. Poor regulation of antibiotic use in poultry has negatively impacted human health [75]. In addition, poor disease and AMR surveillance systems have contributed to the emergence and spread of AMR [73, 76]. A lack of microbiological diagnostics, which are critical for monitoring the trends in AMR, has further worsened the problem [76, 77].

In Zambia, the excessive use of antibiotics in the poultry sector has contributed to the emergence of AMR [65, 78, 79]. Most Zambian poultry farmers have poor knowledge, negative attitudes, and poor practices toward AMU and AMR [65, 80]. The AMR situation in Zambia has been worsened by many factors [79]. Some of these factors include accessing antibiotics without prescription [65, 81], poor awareness of AMR [65, 80], inappropriate use of antibiotics [65, 78, 80, 81], and inappropriate prescribing of antibiotics [82–85].

Therefore, this study aimed to examine the drivers contributing to AMR development in layer poultry farming in the provinces of Lusaka and Copperbelt, Zambia.

Materials and Methods

Ethical approval and Informed consents

This study was approved by the ERES Converge ethics committee with a protocol ID # Ref No. 2019-Dec-004. We also obtained approval from the Zambia National Health Research Authority. All participants were informed about the purpose of the study, and they provided verbal and written consent to participate in the study. This study was conducted according to the guidelines of the Declaration of Helsinki.

Study period and location

A cross-sectional study was conducted in layer poultry farms from September 2020 to April 2021 in the Copperbelt and Lusaka Provinces of Zambia. These two study sites produce the majority of Zambia's poultry and poultry products [86] and have the highest number of households and populations [87, 88], which may translate into an increased need for poultry products. All poultry farmers involved in rearing layer hens in the production age who provided informed consent were included in the study. In addition, all layer chickens were to be healthy to minimize sampling of sick chickens that could have been on antimicrobial treatment. Chickens that were still in the withdrawal period after antimicrobial therapy were also excluded from this study.

Sample size estimation

Sample size was estimated using Ausvet Epitools (<https://epitools.ausvet.com.au/>). The estimation was performed at 5% desired precision, 95% confidence

level, and 50% estimated proportion, as described in a similar study [89]. Based on registers from the District Veterinary Offices and the Poultry Association of Zambia, the number of active layer poultry farms was estimated at 96 (56 in Lusaka and 40 in the Copperbelt). In total, 77 farms (45 from Lusaka and 32 from the Copperbelt) were included in this study based on consent from the farm owners.

Data collection

Layer poultry farmers

We used a previously validated questionnaire to collect data from the farmers [90]. To allow for face and content validation, the questionnaire was reviewed by public health and epidemiological experts from the University of Zambia. This allowed for the prevalidation of the questionnaire for simplicity, relevance, accuracy, clarity, and logic. Next, we conducted a pilot study that included 12 farmers from Monze Township, Southern Province, Zambia, who were excluded from the final analysis of the main study findings. The pilot study helped assess the questionnaire's consistency in collecting data. Cronbach's α for the final questionnaire was 0.78, indicating acceptable internal consistency. The final questionnaire had two sections: Section A included questions on farm epidemiological data, such as type of farmer, gender of the owner, age of chickens, and type of floor; and section B included questions on antibiotic usage, use of prescriptions to access antibiotics, use of antibiotics to prevent infections, use of antibiotics to improve production, consulting veterinarians, administration of antibiotics, knowledge of withdrawal observation period, biosecurity practices at the farm, and awareness of AMR. Face-to-face interviews, which lasted for 20–30 min per participant, were conducted by three researchers. The participants were allowed to ask questions freely regarding poultry infections, antibiotics, and AMR. A brochure with information about AMR and its risk factors was given to the respondents after the interview.

Poultry houses

All chickens that met the inclusion criteria were selected using a simple random method. Three chickens were randomly selected per 25 m² of the poultry house. The cloacal region of chicken was swabbed to collect samples, which were then pre-enriched in 10 mL of buffered peptone water (BPW) (Oxoid, Basingstoke, UK). Samples collected in Lusaka were transported to the Public Health Laboratory at the School of Veterinary Medicine, University of Zambia, within 8 h. Samples collected in the Copperbelt province were placed in BPW immediately after collection, stored at the district veterinary offices at 2°C–8°C, and transported a day later to Lusaka for further processing. In total, 365 cloacal swab samples were collected and processed to isolate and identify *E. coli* and *Enterococcus* spp.

Isolation and identification and confirmation of *E. coli* and enterococci

The isolation and identification of *E. coli* were performed using conventional methods and biochemical tests, as described by Mudenda *et al.* [91]. Presumptive *E. coli* isolates were confirmed using 16S rRNA gene sequencing [92]. Enterococci isolation and identification were performed using standard operating procedures, as described by Mudenda *et al.* [93], and confirmed using 16S rRNA gene sequencing [93].

Antimicrobial susceptibility testing for *E. coli* and enterococci

Antimicrobial susceptibility testing was performed using the disk diffusion test [91, 94]. *Escherichia coli* and enterococci were exposed to a panel of 13 and 9 antibiotics of human and poultry importance in Zambia, respectively. Antibiotics were chosen based on the World Health Organization recommendations to test when conducting surveillance studies [95]. The zones of inhibition were categorized qualitatively as resistant (R), intermediate (I), and susceptible (S), and interpreted using the 2020 Clinical and Laboratory Standard Institute guidelines [96]. Resistance of isolates to ≥ 3 antibiotics from different classes was referred to as MDR [97].

Statistical analysis

The collected data were entered in Microsoft Excel (Microsoft, Redmond, WA, USA), and statistical analyses were performed using Stata version 16.1 (Stata Corp., College Station, Texas, USA). The zones of inhibition were analyzed using WHONET 2020 (<https://whonet.org/software.html>) and reported as R, I, and S. Because the continuous variable (age of chickens) was not normally distributed (confirmed by using QQ-plot), it was summarized using the median and interquartile range (IQR). Categorical variables were reported as frequencies (%). The Wilcoxon-rank sum test, Fisher's exact test, and Pearson's Chi-square test were used where appropriate. The prevalence of AMR was calculated by dividing the number of AMR cases by the total number of respondents assessed and then multiplying by 100. Clopper-Pearson's exact method was used to calculate 95% binomial confidence interval (CI) of the proportion of AMR cases. In this study, a farm was classified as having AMR if at least one isolate of *E. coli* or enterococci recovered from the farm had resistance to at least one antibiotic from three classes (positive AMR status = 1 and negative AMR status = 0). Univariate and multivariate binary logistic regression were used to assess AMR-related factors. Any variable with $p < 0.2$ from the univariate analysis was included to build the multivariate model. To avoid inflating the rate of type I error, the continuous variable (age of chickens) was not categorized in regression analysis. Investigator-led best model selection was used to build the final model. Forward and backward selection methods were used to obtain a parsimonious model. Differences in deviances were used to assess any possible terms to add to the

final model. Assessment of interactions between significant variables in the final model showed no statistical significance. The Hosmer-Lemeshow goodness-of-fit statistic was used to further assess the goodness-of-fit of the final model. Two-sided $p < 0.05$ was considered statistically significant.

Results

Basic characteristics of layer poultry farms

Overall, 77 layer poultry farmers were enrolled in this study, of which 70 (90.9%) were males. Approximately one-half (39 [50.7%]) of the respondents were large-scale farmers, and 39 (50.7%) reported never using prescriptions when accessing antimicrobials. A larger proportion (66 [85.7%]) used antibiotics for poultry, 66 (85.7%) never consulted a veterinarian, 45 (58.4%) used antibiotics for infection prophylaxis, and 40 (52.0%) used antimicrobials to improve production. Nearly all (90.9%) who owned a farm were male, 75 (97.4%) had a concrete type of

floor in the poultry house, and 70 (90.9%) had biosecurity measures in place. Furthermore, 61 (79.2%) reported using farm workers to administer antimicrobials, 48 (62.3%) knew about the withdrawal period, and 41 (53.3%) were aware of AMR. The overall median age of chickens at the time of assessment was 53 weeks (IQR: 38–68). In addition, there was evidence of an association between AMR and the person who administered antibiotics ($p = 0.038$) and awareness of AMR ($p = 0.015$) (Table-1).

Antimicrobial susceptibility tests for *E. coli*

The findings demonstrated that *E. coli* isolates were highly resistant to tetracycline (54.6%), ampicillin (54.0%), and cefotaxime (30.4%) and highly susceptible to meropenem (94.7%), chloramphenicol (85.8%), and ceftazidime (85.3%) (Table-2).

Antimicrobial susceptibility tests for enterococci

Enterococci isolates were highly resistant to tetracycline (80.5%), erythromycin (53.6%), and

Table-1: Basic characteristics of layer farms by the AMR status (n = 77).

| Characteristic | Total population n (%) | Antimicrobial resistance | | p-value |
|---------------------------------------|---------------------------|--------------------------|-----------------|--------------|
| | | No, n = 37 (%) | Yes, n = 40 (%) | |
| Type of farmer | | | | |
| Small-scale | 18 (23.4) | 6 (16.2) | 12 (30.0) | 0.348 |
| Medium-scale | 20 (26.0) | 11 (29.7) | 9 (22.5) | |
| Large-scale | 39 (50.7) | 20 (54.1) | 19 (47.5) | |
| Gender of owner | | | | |
| Male | 70 (90.9) | 36 (97.3) | 34 (85.0) | 0.110 |
| Female | 7 (9.1) | 1 (2.7) | 6 (15.0) | |
| Age of chickens median (IQR) | 53 (38–68) | 54 (48–68) | 51.5 (36–68.5) | 0.482 |
| Type of floor | | | | |
| Concrete | 75 (97.4) | 36 (97.3) | 39 (97.5) | 1.000 |
| Soil | 2 (2.6) | 1 (2.7) | 1 (2.5) | |
| Antibiotic use | | | | |
| Yes | 66 (85.7) | 34 (91.9) | 32 (80.0) | 0.136 |
| No | 11 (14.3) | 3 (8.1) | 8 (20.0) | |
| Use of prescription | | | | |
| Yes | 38 (49.4) | 17 (46.0) | 21 (52.5) | 0.565 |
| No | 39 (50.7) | 20 (54.1) | 19 (47.5) | |
| Prevention of infection | | | | |
| Yes | 45 (58.4) | 25 (67.6) | 20 (50.0) | 0.118 |
| No | 32 (41.6) | 12 (32.4) | 20 (50.0) | |
| Improving production | | | | |
| Yes | 37 (48.1) | 17 (46.0) | 20 (50.0) | 0.722 |
| No | 40 (52.0) | 20 (54.1) | 20 (50.0) | |
| Consulting a veterinarian | | | | |
| Yes | 66 (85.7) | 30 (81.1) | 36 (90.0) | 0.264 |
| No | 11 (14.3) | 7 (18.9) | 4 (10.0) | |
| Antibiotic administration | | | | |
| Farm owner | 16 (20.8) | 4 (10.8) | 12 (30.0) | 0.038 |
| Farm worker | 61 (79.2) | 33 (89.2) | 28 (70.0) | |
| Knowledge of the observation period | | | | |
| Yes | 48 (62.3) | 23 (62.2) | 25 (62.5) | 0.976 |
| No | 29 (37.7) | 14 (37.8) | 15 (37.5) | |
| Biosecurity practices | | | | |
| Yes | 70 (90.9) | 32 (86.5) | 38 (95.0) | 0.251 |
| No | 7 (9.1) | 5 (13.5) | 2 (5.0) | |
| Aware of AMR | | | | |
| Yes | 36 (46.8) | 12 (32.4) | 24 (60.0) | 0.015 |
| No | 41 (53.3) | 25 (67.6) | 16 (40.0) | |
| Overall prevalence of AMR, % (95% CI) | | 51.9 (40.3–63.5) | | |

Fischer's exact/Pearson Chi-square/Wilcoxon Rank sum test, IQR=Interquartile range, 95% CI=95% confidence interval, AMR=Antimicrobial resistance, bold values represent statistical significance at $p < 0.05$

Table-2: Antimicrobial resistance patterns of *Escherichia coli* isolates.

| Antibiotic name | n (%) R | n (%) I | n (%) S | % R 95%CI |
|-------------------------------|------------|-----------|------------|-----------|
| Amoxicillin/Clavulanic acid | 25 (7.4) | 32 (9.4) | 282 (83.2) | 13.1–21.3 |
| Ampicillin | 183 (54.0) | 40 (11.8) | 116 (34.2) | 48.5–59.4 |
| Cefotaxime | 103 (30.4) | 39 (11.5) | 197 (58.1) | 25.6–35.6 |
| Ceftazidime | 21 (6.2) | 29 (8.6) | 289 (85.3) | 4.0–9.5 |
| Cefepime | 21 (6.2) | 61 (18.0) | 257 (75.8) | 4.0–9.5 |
| Chloramphenicol | 30 (8.8) | 18 (5.3) | 291 (85.8) | 6.1–12.5 |
| Ciprofloxacin | 86 (25.4) | 80 (23.6) | 173 (51.0) | 20.9–30.4 |
| Gentamicin | 29 (8.6) | 69 (20.4) | 241 (71.1) | 5.9–12.2 |
| Meropenem | 3 (0.9) | 15 (4.4) | 321 (94.7) | 0.2–2.8 |
| Nitrofurantoin | 41 (12.1) | 72 (21.2) | 226 (66.7) | 8.9–16.2 |
| Tetracycline | 184 (54.3) | 52 (15.3) | 103 (30.4) | 49.1–59.9 |
| Trimethoprim/Sulfamethoxazole | 90 (26.5) | 12 (3.5) | 237 (69.9) | 22.0–31.6 |
| Nalidixic acid | 82 (24.2) | 58 (17.1) | 199 (58.7) | 19.8–29.2 |

R=Resistant, I=Intermediate, S=Susceptible, 95% CI=95% Confidence interval, n=number of isolates

Table-3: Antimicrobial resistance patterns of *Enterococcus* spp. (n = 308).

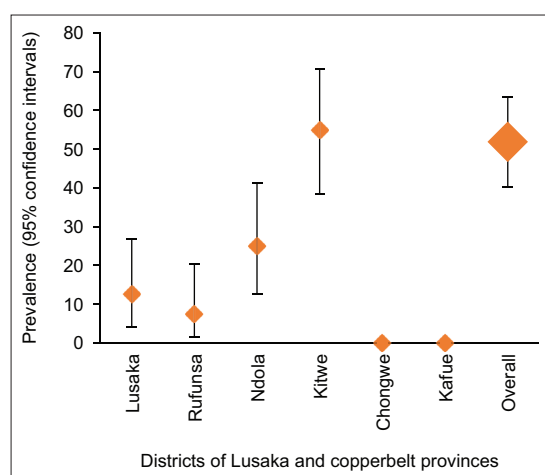
| Antibiotic name | n (%) R | n (%) I | n (%) S | % R 95% CI |
|---------------------------|------------|------------|------------|------------|
| Ampicillin | 113 (36.7) | - | 195 (63.3) | 31.3–42.4 |
| Chloramphenicol | 12 (3.9) | 77 (25) | 219 (71.1) | 2.1–6.9 |
| Ciprofloxacin | 34 (11.0) | 126 (40.9) | 148 (48.1) | 7.9–15.2 |
| Erythromycin | 165 (53.6) | 107 (34.7) | 36 (11.7) | 47.8–59.2 |
| Linezolid | 93 (30.2) | 51 (16.6) | 164 (53.2) | 25.2–35.7 |
| Nitrofurantoin | 20 (6.5) | 49 (15.9) | 239 (77.6) | 4.1–10.0 |
| Quinupristin-dalfopristin | 164 (53.2) | 68 (22.1) | 76 (24.7) | 47.5–58.9 |
| Tetracycline | 248 (80.5) | 22 (7.1) | 38 (12.3) | 75.6–84.7 |
| Vancomycin | 101 (32.8) | 71 (23.1) | 136 (44.2) | 27.6–38.4 |

R=Resistant, I=Intermediate, S=Susceptible, 95% CI=95% Confidence interval, n=number of isolates

Table-4: Prevalence, AMR, and MDR of *E. coli* (n = 339) and *Enterococcus* species (n = 308).

| Characteristics | <i>Escherichia coli</i> n (%) | <i>Enterococcus</i> species n (%) |
|-----------------|-------------------------------|-----------------------------------|
| Prevalence | 339 (92.9) | 308 (84.4) |
| AMR | 327 (96.5) | 306 (99.4) |
| MDR | 219 (64.6) | 265 (86.0) |

AMR=Antimicrobial resistance, MDR=Multidrug resistance

**Figure-1:** Prevalence of antimicrobial resistance problem at farm level among surveyed districts of Lusaka and Copperbelt provinces.

quinupristin-dalfopristin (53.2%), but highly susceptible to nitrofurantoin (77.6%) and chloramphenicol (71.1%) (Table-3).

The prevalence of AMR in *E. coli* isolates was 96.5% (95% CI: 93.73%–98.07%), of which 64.6% (95% CI: 59.22%–69.64%) were MDR. The prevalence of AMR in *Enterococcus* spp. was 99.4%, of which 86.0% (95% CI: 81.7%–89.5%) were MDR (Table-4).

The prevalence of AMR at the farm level is shown in Figure-1. The overall prevalence of AMR was 51.9% (95% CI: 40.3%–63.5%), with the highest being in Kitwe district at 55% (95% CI: 38.5%–70.7%), followed by Ndola district at 25% (95% CI: 12.7%–41.2%), and Lusaka at 12.5% (95% CI: 4.2%–26.8%). Conversely, the lowest prevalence of AMR was in Rufunsa district at 7.5% (95% CI: 1.6%–20.4%).

Results from multivariable logistic regression analysis are shown in Table-5. Factors associated with AMR included the type of farmer and awareness of AMR. Compared to small-scale farmers, large-scale farmers were less likely to have AMR problems (Adjusted odds ratio [AOR] = 0.20, 95% CI: 0.04%–0.99%, $p = 0.049$). In addition, farmers who were aware of AMR were less likely to have AMR problems on their farms than those who were unaware (AOR = 0.26, 95% CI: 0.08%–0.86%, $p = 0.027$).

Discussion

This study identified the drivers of AMR in layer poultry farms in Lusaka and Copperbelt provinces using *E. coli* and enterococci as indicator organisms. The study found that the prevalence of AMR in layer

Table-5: Factors associated with AMR in layer poultry farms.

| Characteristic | COR (95% CI) | p-value | AOR (95% CI) | p-value |
|-------------------------------------|-------------------|--------------|-------------------|--------------|
| Type of farmer | | | | |
| Small-scale | Ref | | Ref | |
| Medium-scale | 0.41 (0.11–1.53) | 0.184 | 0.31 (0.06–1.59) | 0.161 |
| Large-scale | 0.48 (0.15–1.52) | 0.210 | 0.20 (0.04–0.99) | 0.049 |
| Gender of owner | | | | |
| Male | Ref | | | |
| Female | 6.35 (0.73–55.54) | 0.095 | | |
| Age of chickens | 0.99 (0.98–1.02) | 0.703 | | |
| Type of floor | | | | |
| Concrete | Ref | | | |
| Soil | 0.92 (0.06–15.31) | 0.955 | | |
| Antibiotic use | | | | |
| No | Ref | | Ref | 0.537 |
| Yes | 2.83 (0.69–11.63) | 0.148 | 1.88 (0.25–14.05) | |
| Use of prescription | | | | |
| No | Ref | | | |
| Yes | 0.77 (0.31–1.88) | 0.566 | | |
| Prevention of infection | | | | |
| No | Ref | | | |
| Yes | 2.08 (0.83–5.26) | 0.120 | | |
| Improving production | | | | |
| Yes | Ref | | | |
| No | 0.85 (0.35–2.08) | 0.722 | | |
| Consulting a veterinarian | | | | |
| No | Ref | | | |
| Yes | 0.48 (0.13–1.78) | 0.271 | | |
| Antibiotic administration | | | | |
| Farm owner | Ref | | Ref | 0.411 |
| Farm worker | 0.28 (0.08–0.98) | 0.046 | 0.48 (0.09–2.74) | |
| Knowledge of the observation period | | | | |
| No | Ref | | | |
| Yes | 0.99 (0.39–2.48) | 0.976 | | |
| Biosecurity practices | | | | |
| No | Ref | | Ref | 0.223 |
| Yes | 0.34 (0.06–1.85) | 0.211 | 0.27 (0.03–2.21) | |
| Aware of AMR | | | | |
| No | Ref | | Ref | 0.027 |
| Yes | 0.32 (0.13–0.81) | 0.017 | 0.26 (0.08–0.86) | |

COR=Crude odds ratio, AOR=Adjusted odds ratio, 95% CI=95% confidence interval, AMR=Antimicrobial resistance, bold values represent statistical significance at $p < 0.05$, variables not appearing in the final multivariable model (blank spaces) were dropped from the final model

poultry farms was 51.9%. The prevalence of AMR for the isolated organism was 92.6% for *E. coli* and 84.4% for *Enterococcus* spp. The prevalence of MDR for *E. coli* was 39% and that of *Enterococcus* spp. was 86%. Large-scale poultry farmers were less likely to have AMR in their poultry than small-scale farmers, because commercial farmers employed professionals trained in managing an enterprise. In addition, farmers who were aware of AMR were less likely to have AMR in their poultries than those who were unaware.

In our study, the prevalence of MDR was high, translating into a serious AMR problem in layer poultry farms. This was evidenced by the high AMR and MDR rates of *E. coli* and enterococci. Consistently, other studies have reported high AMR rates of *E. coli* and enterococci [91, 93, 98–100]. The findings from our study and other studies may be partly due to the inappropriate use of antibiotics in the poultry sector [65]. In addition, these findings could be due to the natural phenomenon of AMR, where *E. coli* and enterococci develop natural resistance to

antibiotics [101]. Therefore, the observed AMR levels in this study in both *E. coli* and enterococci were too high to be solely accounted for by the evolution of natural resistance.

This study found that large-scale layer poultry farmers were less likely to encounter AMR problems in their flocks. These findings are similar to those reported in China, where small- and medium-scale poultry farmers were more likely to misuse antibiotics than commercial farmers [102]. A study in Bangladesh reported that small-scale farmers were more likely to use antibiotics without consulting veterinary professionals, thereby increasing the risk of AMR in their farms [103]. Consequently, all small-scale poultry farmers were reported to have accessed antibiotics from their feed and chick sellers and used them to promote the growth of their chickens [103]. This practice was inappropriate because the poultry farmers missed out on expert information from pharmacy professionals and animal health personnel. Small-scale poultry farmers tend to misuse and overuse antibiotics due to

a lack of antibiotic regulation, resources, and access to professional veterinary services [104]. Large-scale farmers are involved in rearing a larger number of layer chickens than small- and medium-scale farmers; hence, they usually vaccinate their layers to prevent infections [90]. Large-scale farmers are more likely to adhere to biosecurity guidelines than small- and medium-scale farmers, because they have to secure their high capital investment [90]. Adherence to biosecurity practices reduces the use of antibiotics in animal health [32, 105–107], and subsequently, reduces the risk of AMR development. In addition, large-scale farmers may employ knowledgeable employees who can follow the rules of biosecurity and vaccination. This could explain why medium- and large-scale farms have lower AMR rates than small- and medium-scale farms.

Our findings showed that layer poultry farmers who were aware of AMR were less likely to have AMR problems on their farms than those who were unaware. These findings agree with those of other studies that reported low awareness of AMR among poultry farmers [53, 59–61, 64]. A study in Bangladesh reported that most small-scale farmers had poor knowledge of AMR and farm management practices, resulting in increased risk of infection and overuse of antibiotics [108]. A study in Nigeria found that most poultry farmers who were unaware of AMR were inappropriately using antibiotics for growth promotion and disease prevention [109]. Similarly, a study in Nepal reported that most poultry farmers who were unaware of AMR were not compliant with the withdrawal period of antibiotics, used antimicrobials as growth promoters, and continuously used critically important antimicrobials, increasing the risk of AMR development [59]. Thus, policy enforcement is needed to monitor the use of antimicrobials in the poultry sector and improve outreach and educational activities in poultry farms [59]. In addition, AMR awareness must be promoted among farmers through sustainable mass media programs [110].

Our study revealed that most farmers used antibiotics that were accessed without prescriptions. Some farmers used antibiotics for prophylaxis and growth promotion. These findings are similar to those reported in other studies [53, 103, 109, 111, 112]. These practices by poultry farmers are potential contributing factors to the development of AMR. Therefore, there is a need for heightened antimicrobial stewardship and AMR surveillance programs in the poultry sector to increase awareness and promote the rational use of antibiotics [113–119]. Moreover, there is a need to promote behavioral change among poultry farmers regarding inappropriate AMU [119–122].

Our study highlights the potential drivers of AMR in poultry in Zambia. However, the results might have been better with a qualitative study because we collected data using a cross-sectional study. Consequently, we could not use focus group

discussions due to the emphasis on adhering to the COVID-19 prevention measures. Despite these limitations, our findings concerning AMR and intervention measures in poultry are important for health authorities and decision-makers.

Conclusion

This study found a high prevalence of AMR in layer poultry farming, especially among small-scale layer poultry farmers who were mostly unaware of AMR. The drivers of AMR identified in this study demonstrate the need to provide educational interventions to poultry farmers for disease prevention practices. Finally, there is a need to increase awareness of AMR and the contributing factors, especially among small- and medium-scale farmers. This can be done during outreach services in poultry farms and through activities, such as educational workshops and conferences, with the farmers.

Authors' Contributions

StM and JBM: Conceptualized the study. StM, KY, MAH, GM, and GS: Collected the data and conducted laboratory work. StM, MoM, and JBM: Performed data analysis. StM, FNB, KY, MuM, SyM, MoM, MAH, VD, SKM, GS, GM, MeM, BMH, and JBM interpreted the data. StM, KY, MuM, SyM, MoM, VD, SKM, FNB, GS, MeM, BMH, and JBM: Wrote the original draft preparation. StM, MuM, SyM, GM, MoM, KY, MAH, VD, SKM, FNB, GS, MeM, BMH, and JBM: Reviewed and edited the manuscript. StM, FNB, KY, MuM, SyM, MoM, MAH, VD, SKM, GM, MeM, BMH, and JBM revised the manuscript for important intellectual content. MuM, SyM, BMH, and JBM: Supervised the project. All authors have read, reviewed, and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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