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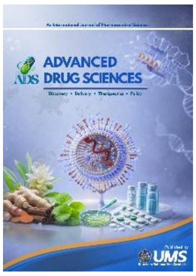

RESEARCH ARTICLE

Comparative Assessment of Antimicrobial Resistance in Poultry-Associated *Escherichia coli* between Commercial and Backyard Poultry Systems in Chattogram, Bangladesh

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ABSTRACT

Background: Poultry-associated *Escherichia coli* is a critical reservoir of multidrug resistance (MDR), posing escalating zoonotic risks at the human-animal interface. Comparative risk assessment of MDR *E. coli* between commercial farm and backyard systems remains understudied in Chattogram, Bangladesh. **Objective:** This study comparatively assessed the bacterial load, antimicrobial resistance profiles, and MDR burden of *E. coli* in poultry litter samples from both production systems. **Method:** Bacterial load was evaluated, followed by biochemical tests to identify probable bacterial species, and *E. coli* identification through culture and polymerase chain reaction. Additionally, the antimicrobial sensitivity pattern of *E. coli* to antimicrobial agents was determined using the disk diffusion method. Descriptive statistical analysis was performed to assess the association of knowledge and practice of antimicrobial agents across sources. **Results:** Farm and backyard samples yielded mean bacterial loads of 141.78 and 134.74 Colony-Forming Units per milliliter, respectively. Both systems harbored overlapping yet compositionally distinct MDR bacterial communities, with *Acinetobacter* exclusively recovered from backyard samples. Furthermore, universal ampicillin and tetracycline resistance (100%) was observed in farm isolates, with predominant ciprofloxacin resistance, while backyard isolates retained broader gentamicin susceptibility. Descriptive analysis revealed that antibiotic full-dose completion was observed in 92.6% of farms, whereas it was observed in 19.1% of backyard systems. Some respondents from both systems reported concurrent illnesses in humans and chickens following the use of human medicines in poultry. **Conclusion:** These findings necessitate production-system-specific stewardship strategies and integrated One Health surveillance frameworks to mitigate MDR *E. coli* transmission in high-density poultry production regions.

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1. INTRODUCTION

Antibiotic resistance is substantially driven by the inappropriate use of antibiotics in food animals [1]. Generally, farmers administer sub-therapeutic doses of antibiotics to broiler flocks without proper knowledge. They also do not wait for the recommended withdrawal period required for the elimination of antibiotics from the chicken's body. Instead, chickens are sold to consumers before complete antibiotic clearance, which may contribute to the spread of antimicrobial resistance (AMR) among human consumers [2]. Not only poultry chickens but also other sources, such as fish, cattle, goat, sheep, etc., food-producing animals, play a crucial role in amplifying resistance factors that eventually converge within populations of human pathogens [3, 4].

Bangladesh's poultry industry is diversified, with both intensive commercial farms and traditional family flocks. About one-third of animal-source protein comes from poultry, which is essential for rural livelihoods, income, and food security. The national chicken population is ~320 million, with about 90,000 registered commercial farms; ~150,000 commercial broiler and layer farms have been reported. With dynamic interactions in marketplaces and live-bird chains, the production and distribution network is structured around four primary sub-systems: backyard deshi, commercial Sonali, commercial broiler, and commercial layer. Many small, inexperienced commercial producers run "fragile" businesses with inadequate biosecurity and a strong reliance on unofficial finance and dealer networks. Viruses are transmitted from various settings, such as live bird markets (LBMs), commercial farms, and backyard settings in both urban and peri-urban settings [5-7].

Currently, broiler chickens act as significant amplifiers of antibiotic resistance determinants to humans, primarily through resistant *Escherichia coli* harbored in the food chain [8-10]. The prevalence of antibiotic resistance is increasing worldwide, with South Asian countries bearing a disproportionately high burden of resistant strains [11]. Additionally, poultry production ranks among the most intensive consumers of veterinary antimicrobials worldwide, with low and middle-income countries bearing a disproportionate burden of unregulated usage, largely due to inadequate biosecurity infrastructure [12].

Furthermore, multidrug-resistant (MDR) *E. coli* has been extensively recorded in a variety of South Asian chicken production systems, particularly in Bangladesh and India [13, 14]. MDR *E. coli* is frequently resistant to three or more antibiotic classes. Additionally, *E. coli* that was isolated from both backyard and commercial broiler chickens has been found as a potential source of crucial zoonotic transmission across various poultry production systems [15].

In Bangladesh, the combination of smallholder backyard systems and commercial broiler operations near dense human populations produces particularly harsh epidemiological conditions for the amplification and cross-system transmission of antibiotic resistance [11, 12]. However, the differences in resistance profiles between backyard and commercial systems remain inconsistent and poorly described. Although regional studies have documented the general prevalence of antibiotic resistance in Bangladeshi poultry, no specific, comparative risk assessment of the AMR profile of *E. coli* between commercial farm and backyard systems in the Chattogram region, where poultry density and human-animal interfaces are among the highest in the nation [16, 17]. Furthermore, the environmental dimension of resistance transmission via contamination of litter, soil, and the coop floor has remained underexplored in system-level comparative analyses.

Therefore, this study aimed to compare the prevalence, AMR profiles, and MDR burden of *E. coli* isolated from commercial farm and backyard poultry production systems in Hathazari, Chattogram, integrating microbiological, biochemical, molecular, and epidemiological approaches to provide a comprehensive One Health risk assessment of MDR *E. coli* transmission dynamics across divergent poultry production systems.

2. MATERIALS AND METHODS

2.1. Ethical approval

The study received approval from the ethical committee of Chattogram Veterinary and Animal Sciences University (Memo no: CVASU/Dir (R&E) EC/2026/17/26, Date:28/01/2026). We obtained informed consent after discussing the informed consent form with each participant.

2.2. Study design, location, and duration:

We conducted a cross-sectional study at commercial and backyard poultry farms in Hathazari Upazila, Chattogram district, Bangladesh, from February 2026 to March 2026.

2.3. Sample and Data Collection

Following ethical approval, we collected litter from the 27 commercial poultry farms and soil and/or coop floor samples from 27 households with backyard poultry. The study was conducted within a geographically restricted area, Hathazari, and the sample size was balanced by the availability of eligible farms to enable comparative analysis between the two production systems, despite limited statistical power due to the small sample size. After collection, samples were preserved in cool boxes at 4°C and transported immediately to the microbiology laboratory. The sampling protocol was implemented by trained personnel in accordance with standardized biosafety procedures. We collected farm-level data and knowledge information from the owners or the farmers through a well-structured questionnaire that captured 17 key variables, particularly poultry system type, chicken breeds, flock demographics, vaccination protocols, antibiotic usage patterns, human-animal health interactions, and biosecurity awareness levels, with responses coded using predetermined categorical scales to ensure data consistency and analytical rigor **(Supplementary Appendix A1)**.

2.4. Laboratory evaluation

2.4.1. Quantitative Bacterial Load Assessment

To calculate the bacterial load in the environmental samples, we evaluated each sample for bacterial colony count by the spread plate technique. Serial dilutions of the homogenized samples were prepared in normal saline to a final dilution of 10^{-10}. The 10^{-7} dilution was then taken from the series of diluted samples for plating. We used nutrient agar media or differential media such as MacConkey agar medium and Eosin Methylene Blue (EMB) agar to grow enteric bacteria [18, 19]. A specific volume of the diluted sample was spread onto nutrient agar plates using the spread plate technique. The plates were then incubated at 37°C for 24 hours. The colonies were then counted, and the bacterial load was determined in colony-forming units per milliliter (CFU/mL) using the standard formula. The nutrient agar media followed by Ebomah et al (2018) [20].

2.4.2. Bacterial Isolation and Identification

Distinct, isolated colonies from each nutrient agar plate were used for bacterial isolation and identification. Each colony was transferred and cultured again on a new nutrient agar medium to isolate a pure bacterial strain. These pure cultures were streaked on plates, and isolated single colonies were used for biochemical tests to determine their identities. Based on biochemical test results, probable *E. coli* isolates were selected and grown on selective and differential media, including MacConkey and EMB agar, for definitive identification. Colonies with distinct colony morphology on MacConkey agar and green metallic sheen on EMB agar were regarded as *E. coli* isolates.

2.5. Biochemical Characterization of the presence of probable multiple bacterial species

Certain biochemical assays were systematically performed to achieve definitive species-level identification of isolated bacterial strains and to confirm the presence of *E. coli* through phenotypic characterization. The IMViC tests, comprising indole production and citrate utilization assays, were employed for *E. coli* identification due to their high specificity and reliability in differentiating closely related Enterobacteriaceae species based on distinct metabolic profiles [21].

Similarly, other enzyme tests, namely, oxidase activity (identifying cytochrome oxidase enzyme in the electron transport chain), and triple sugar iron (TSI) fermentation patterns (assessing glucose, lactose, and sucrose fermentation with hydrogen sulfide production), were performed to provide metabolic profiling and enhance identification accuracy **(Supplementary Figure S1)**.

Each biochemical test was performed according to established protocols using commercially available reagents and media, with isolated colonies inoculated into appropriate test systems and incubated under specified conditions (~37°C for 18-24 hours). After spread plating of all 27 samples from each group, well-isolated colonies were subcultured to obtain pure single colonies. Morphologically similar colonies were excluded to minimize duplication.

2.6. Molecular Characterization

The *E. coli* isolates from both production systems were subjected to polymerase chain reaction (PCR) based molecular characterization targeting a species-specific genetic marker. The uidA gene, which encodes β-D-glucuronidase, is highly conserved across most *E. coli* strains and is frequently used as a molecular target for detection. However, uidA homologs and β-glucuronidase activity have been reported in some non-*E. coli* Enterobacteriaceae. Therefore, given the potential cross-reactivity of the uidA gene with closely related Enterobacteriaceae, this study adopted a combined approach integrating biochemical profiling and molecular characterization to enhance the accuracy of *E. coli* identification.

For deoxyribonucleic acid (DNA) isolation, the pure colonies were suspended in 200 µL of sterile distilled water in a 1.5 mL eppendorf tube and vortexed. The cells were lysed for at least 15 seconds. Genomic DNA was then extracted using the Jena Bioscience kit [22]. Species-specific confirmation of the isolate *E. coli* was done using conventional PCR targeting the uidA gene with universal primers, followed by Ramirez-Martinez et al. (2015) [23] and Bej et al. (1991)[24]. The primers were forward: AAAACGGCAAGAAAAAGCAG; Reverse: ACGCGTGGTTACAGTCTTGCG. The product size was 147bp.

PCR was performed in a 10 µL reaction volume containing 5 µL GoTaq® PCR Master Mix, 0.5 µL each of forward and reverse primers, 1 µL of genomic DNA template, and 3 µL of nuclease-free water. Amplification was performed in a T100™ thermocycler (Bio-Rad, USA) with an initial denaturation at 94 °C for 10 min, followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 58 °C for 45 s, and extension at 72 °C for 1 min, with a final extension at 72 °C for 10 min. PCR products were then stored at 4–8 °C until further analysis.

The PCR products were analyzed by agarose gel electrophoresis and visualized under UV illumination to confirm the presence of the expected amplicon size (~147 bp for the uidA gene), by comparing with the marker gene.

2.7. Antibiogram Testing and Multidrug Resistance Determination

Antibiogram testing was performed to determine the resistance profiles of the isolates against ampicillin, tetracycline, ciprofloxacin, and gentamicin. The analysis was conducted using the standard Kirby-Bauer disk diffusion method on Mueller-Hinton agar (MHA) in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines [25]. The study selected ampicillin, tetracycline, ciprofloxacin, and gentamicin because these antibiotics represent commonly used antimicrobial classes in poultry production and veterinary practice in Bangladesh. These antibiotics were also chosen to reflect local antimicrobial usage patterns and field relevance of study area. The selected panel included representatives from four distinct antimicrobial classes: β-lactams (ampicillin), tetracyclines (tetracycline), fluoroquinolones (ciprofloxacin), and aminoglycosides (gentamicin). Bacterial suspensions were standardized and uniformly inoculated onto MHA plates, followed by the placement of antibiotic disks including ampicillin, tetracycline, ciprofloxacin, and gentamicin. The plates were incubated overnight, and after incubation, zones of inhibition were measured and interpreted according to CLSI breakpoints to categorize isolates as susceptible, intermediate, or resistant. MDR was defined as resistance to at least 3 classes of antibiotics.

2.8. Statistical Analysis

Descriptive statistics were calculated, and Fisher's exact test was performed to determine the associations between poultry management practices, patterns of antimicrobial usage, and awareness levels across the different production systems [26]. Fisher's exact test was used to compare two groups because it performs better with categorical data and small sample sizes, providing exact p-values without relying on large-sample approximations. The statistical analysis was carried out using the SciPy library (SciPy. Stats module) of Python 3.0, where each variable was cross-tabulated against poultry system categories (backyard and commercial farm) [27]. Significant associations were identified with p-values < 0.05.

3. RESULTS

3.1. Bacterial Viability and Load Comparison

Bacterial load measurements, expressed in CFU/ml, were assessed across 54 samples, with 27 individual samples from both farm and backyard sources. Among the samples from the commercial farm, the bacterial load exhibited considerable inter-sample variability, with a calculated mean of 141.78 CFU/ml, ranging from a minimum of 23 CFU/ml to a maximum of 412 CFU/ml, (Figure 1a). Notable peaks were also recorded at samples 6 (250 CFU/ml), 7 (270 CFU/ml), 15 (240 CFU/ml), 23 (224 CFU/ml), and 27 (410 CFU/ml), while several samples including samples 3 (50 CFU/ml), 10 (60 CFU/ml), and 26 (55 CFU/ml) registered comparatively lower counts.

In backyard samples, bacterial load demonstrated an even wider range, with a mean of 134.74 CFU/ml, spanning from 19 CFU/ml (samples 9 and 15) to a peak of 616 CFU/ml (sample 7), (Figure 1b). Prominent elevations were observed at samples 1 (256 CFU/ml), 6 (428 CFU/ml), 16 (357 CFU/ml), and 22 (310 CFU/ml), interspersed with markedly lower values at samples 8 (55 CFU/ml), 11 (40 CFU/ml), 13 (25 CFU/ml), and 26 (36 CFU/ml). Though the mean bacterial load was marginally higher in farm samples (141.78 CFU/ml) compared to backyard samples (134.74 CFU/ml), the backyard samples exhibited a substantially greater absolute peak load.

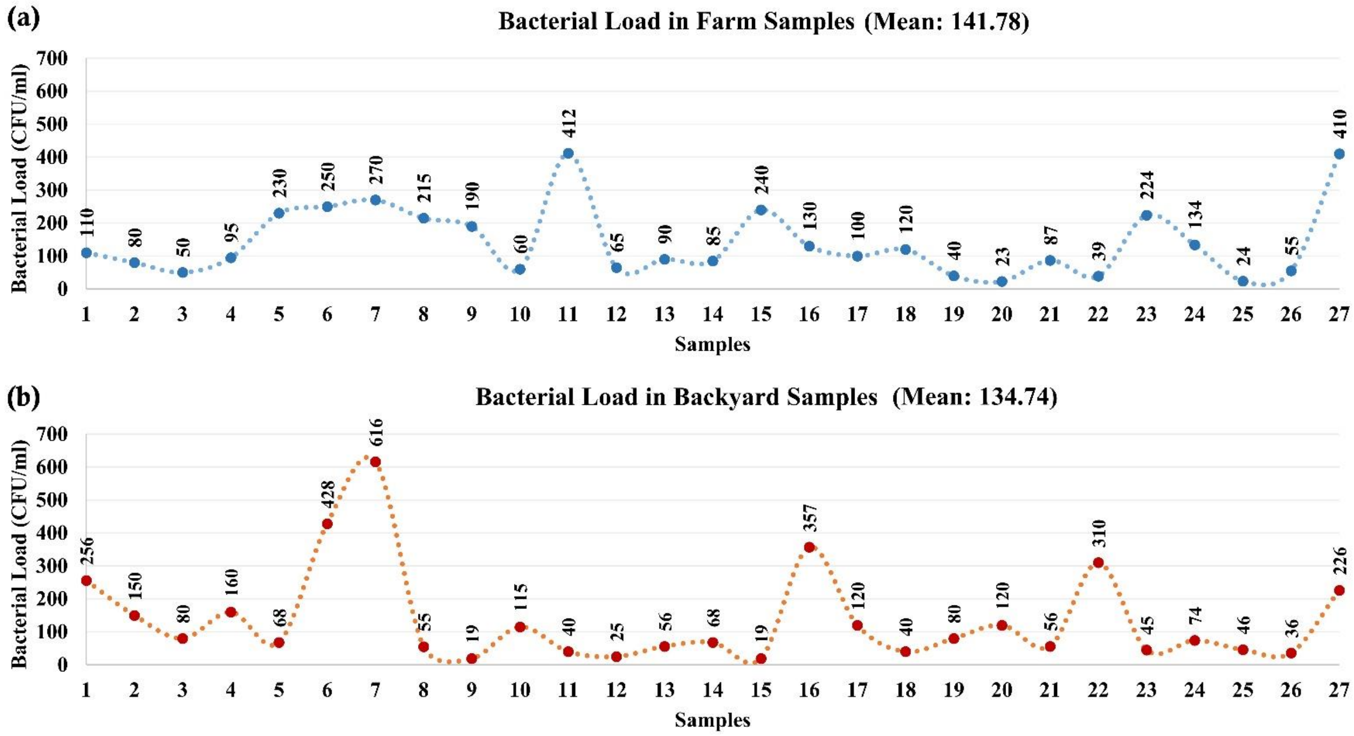

Figure 1. Comparing the bacterial load of farm and backyard samples. Here, Blue color denotes Farm samples, and red color denotes Backyard samples.

3.2. Biochemical Evaluation

We identified several bacterial isolates based on a series of biochemical tests (Table 1). Among the farm isolates, 01 was characterized by positive citrate and motility reactions, alkaline slant and butt in TSI, and a positive oxidase test, with Gram-negative morphology, and was identified as the genus Pseudomonas. The farm isolate 02 demonstrated positive indole and urease reactions with an acid slant and alkaline butt, assuming as Proteus. While isolate 03 exhibited indole and urease positivity, H_2S production, and an acid-acid TSI profile, consistent with Citrobacter. Isolate 04 remained unreactive across citrate, MIU, and oxidase tests, with an acid slant and alkaline butt, and was recommended as Citrobacter. *E. coli* 05 displayed the characteristic combination of positive motility, indole, and urease, an acid-acid TSI profile, and gas production. The four *Klebsiella* isolates (06–09) showed varying profiles: *Klebsiella* 06 was citrate-positive with an acid slant and alkaline butt; *Klebsiella* 07 and 08 were positive for motility, indole, and urease. Finally, *Klebsiella* 09 was motility and urease-positive without indole production. Additionally, Enterobacter 10 was citrate-positive with an acid-acid TSI profile and gas production.

Among the backyard isolates, *E. coli* 01 demonstrated positive motility, indole production, gas production, and an acid-acid TSI profile, though Gram stain data were unavailable. Proteus 02 showed citrate and motility positivity with an acid slant and alkaline, while *Pseudomonas* 03 was positive for citrate, motility, and oxidase with an alkaline-alkaline TSI profile. *Acinetobacter* 04 was citrate-positive with a purely alkaline TSI profile and negative oxidase, and *Klebsiella* 05 and 07 were both citrate-positive with acid slant and alkaline butt profiles. *Enterobacter/Citrobacter* 06 was distinguished by citrate and urease positivity, an acid-acid TSI profile, and gas production. Klebsiella 08 was urease-positive with an alkaline-alkaline TSI, *Klebsiella* 09 showed a variable indole reaction with an acid-acid TSI profile, and Proteus 10 demonstrated positive motility with an alkaline slant and acid butt **(Supplementary Figure S2)**.

Table 1. Biochemical characterization of the presumptive 10 best bacterial colonies.

Isolate ID	Citrate (+/–)	MIU – Motility (+/–)	MIU – Indole (+/–)	MIU – Urease (+/–)	TSI – Slant (K/A)	TSI – Butt (K/A)	TSI – Gas (+/–)	TSI – H_2S (+/–)	Oxidase +/–)	Gram (+/–)
					Farm					
Pseudomonas 01	+	+	-	-	K	K	-	-	+	-
Proteus 02	-	-	+	+	A	K	-	-	-	-
Citrobacter 03	-	-	+	+	A	A	-	+	-	-
Citrobacter 04	-	-	-	-	A	K	-	-	-	-
E. coli 05	-	+	+	+	A	A	+	-	-	-
Klebsiella 06	+	-	-	-	A	K	-	-	-	-
Klebsiella 07	-	+	+	+	K	K	-	-	-	-
Klebsiella 08	-	+	+	+	A	K	-	-	-	-
Klebsiella 09	-	+	-	+	A	K	-	-	-	-
Enterobacter 10	+	-	-	-	A	A	+	-	-	-
					Backyard					
E. coli 01	-	+	+	-	A	A	+	-	-	N/A
Proteus 02	+	+	-	-	A	K	-	-	-	-
Pseudomonas 03	+	+	-	-	K	K	-	-	+	-
Acinetobacter 04	+	-	-	-	K	K	-	-	-	-
Klebsiella 05	+	-	-	-	A	K	-	-	-	-
Enterobacter / *Citrobacter* 06	+	-	-	+	A	A	+	-	-	-
Klebsiella 07	+	-	-	-	A	K	-	-	-	-
Klebsiella 08	-	-	-	+	K	K	-	-		-
Klebsiella 09	-	-	V	-	A	A	-	-		-
Proteus 10	-	+	-	-	K	A	-	-		-

+ = Positive, - = Negative, A = Acid, K = Alkaline, V = Variable.

Overall, the presumptive bacterial genera identified from farm samples comprised *Pseudomonas spp.*, *Proteus spp.*, *Citrobacter*, *Klebsiella spp.*, *Enterobacter*, and *E. coli*, whereas backyard samples harbored *E. coli*, *Pseudomonas spp.*, *Enterobacter/Citrobacter*, *Klebsiella spp.*, *Acinetobacter spp.*, and *Proteus spp.*, suggesting a broadly overlapping yet compositionally distinct bacterial community structure between the two poultry production environments.

3.3. Molecular Profiling

Molecular profiling confirms the tentative biochemical identification of isolates, as phenotypic characterization alone is insufficient to unequivocally differentiate closely related species. PCR amplification targeting the uidA gene, which encodes the enzyme β-Dglucuronidase and serves as a highly specific molecular marker for *E. coli*.

Representative isolates from farm and backyard poultry sources were subjected to molecular analysis, with PCR-amplified products subsequently visualized by agarose gel electrophoresis (Figure 2). The presence of discrete amplification bands (~147 bp) further confirmed the molecular identity of the respective isolates as *E. coli*.

3.4. Antimicrobial Susceptibility Patterns

To avoid duplicate phenotypes and ensure adequate purity for reliable antimicrobial susceptibility testing, two isolates from the backyard group were excluded after purification and quality assessment. Consequently, to maintain a balanced comparison between the study groups, antibiogram analysis was performed on 25 representative isolates from each group. Zone of inhibition measurements were performed for all 50 isolates from both farm and backyard sources against a panel of commercially used antibiotics and interpreted in accordance with CLSI breakpoint criteria **(Supplementary Figure S3)** to determine the antimicrobial susceptibility patterns of poultry-associated bacterial isolates. Among farm isolates, ampicillin (10 µg) and tetracycline (30 µg) yielded no measurable zones of inhibition across all 25 isolates from farm samples. Among backyard isolates, ampicillin (10 µg) produced zones of 0 mm for the majority of isolates, with isolated exceptions at isolates 3 (16 mm), 4 (12 mm), 5 (10 mm), 11 (6 mm), 18 (12 mm), 19 (6 mm), and 21 (4 mm), most of which remained at or below the resistance breakpoint (≤13), except isolate 3 (16 mm) fell in the range of intermediate sensitive range (1416 mm) (Figure 3a). Tetracycline (30 µg) showed predominantly zero inhibition zones, with sparse activity recorded at isolates 3 (18 mm), 11 (8 mm), and 18 (6 mm), and a value of 4 mm at isolate 22, all falling below or at the resistance threshold except for isolate 3, which approached the intermediate range (15-18 mm) (Figure 3b).

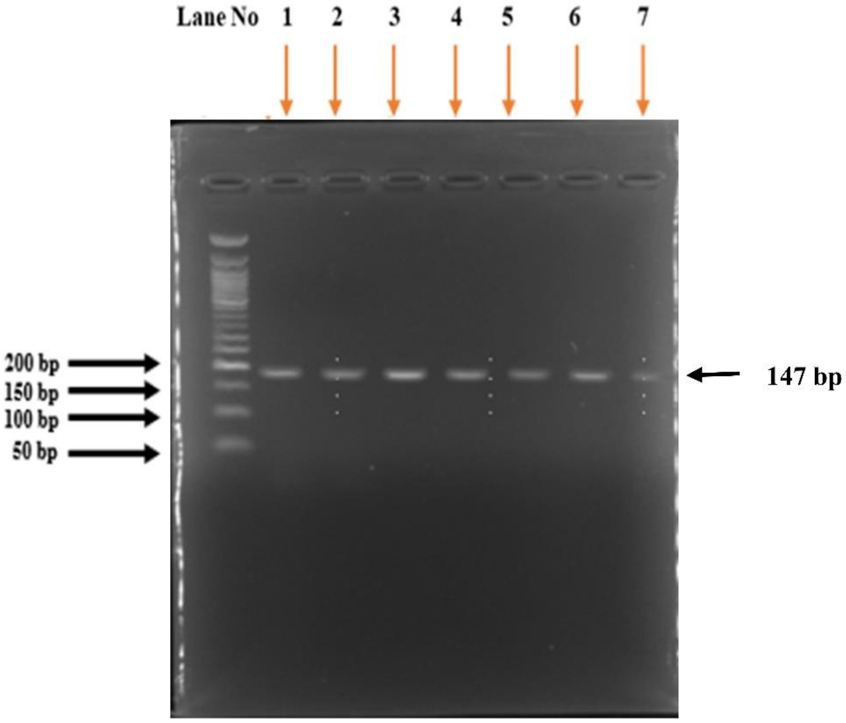

Figure 2. uidA gene-specific PCR amplification bands were visualized in an agarose gel. Here, a 50 bp DNA ladder has been used, and lanes 1–7 show the expected amplicon size of 147 bp.

In the case of farm isolates, gentamicin (10 µg) inhibition zones of two isolates reached a maximum of 22 mm (isolate 10) and 20 mm (isolate 15), which fell within the sensitive threshold (≥15) (Figure 3c). On the other hand, gentamicin (10 µg) demonstrated a markedly broader and more consistent activity profile among backyard isolates relative to farm isolates, with zones ranging from 0 mm (isolate 17) to a peak of 28 mm (isolate 9), and several isolates recording values at or above the sensitive threshold (≥15), including isolates 1 (22 mm), 4 (24 mm), 5 (24 mm), 6 (20 mm) (Figure 3c).

Ciprofloxacin (5 µg) produced zones ranging from 0 mm across the majority of isolates to a peak of 12 mm (isolate 14) and 10 mm (isolates 10 and 15), with 8 mm (isolates 2 and 22) and 10 mm (isolates 5 and 25), all of which fell below the sensitive threshold (≥21), suggesting widespread ciprofloxacin resistance among farm isolates (Figure 3d). On the contrary, Ciprofloxacin (5 µg; sensitive: ≥21 mm) showed variable inhibition among backyard isolates, with zones reaching 26 mm (isolate 3) and 24 mm (isolate 4), along with values of 22 mm (isolate 13), 20 mm (isolate 14), and 18 mm (isolates 1 and 17). This suggests that a subset of backyard isolates retained susceptibility to ciprofloxacin, whereas most of the others recorded within the resistant threshold (≤15) (Figure 3d).

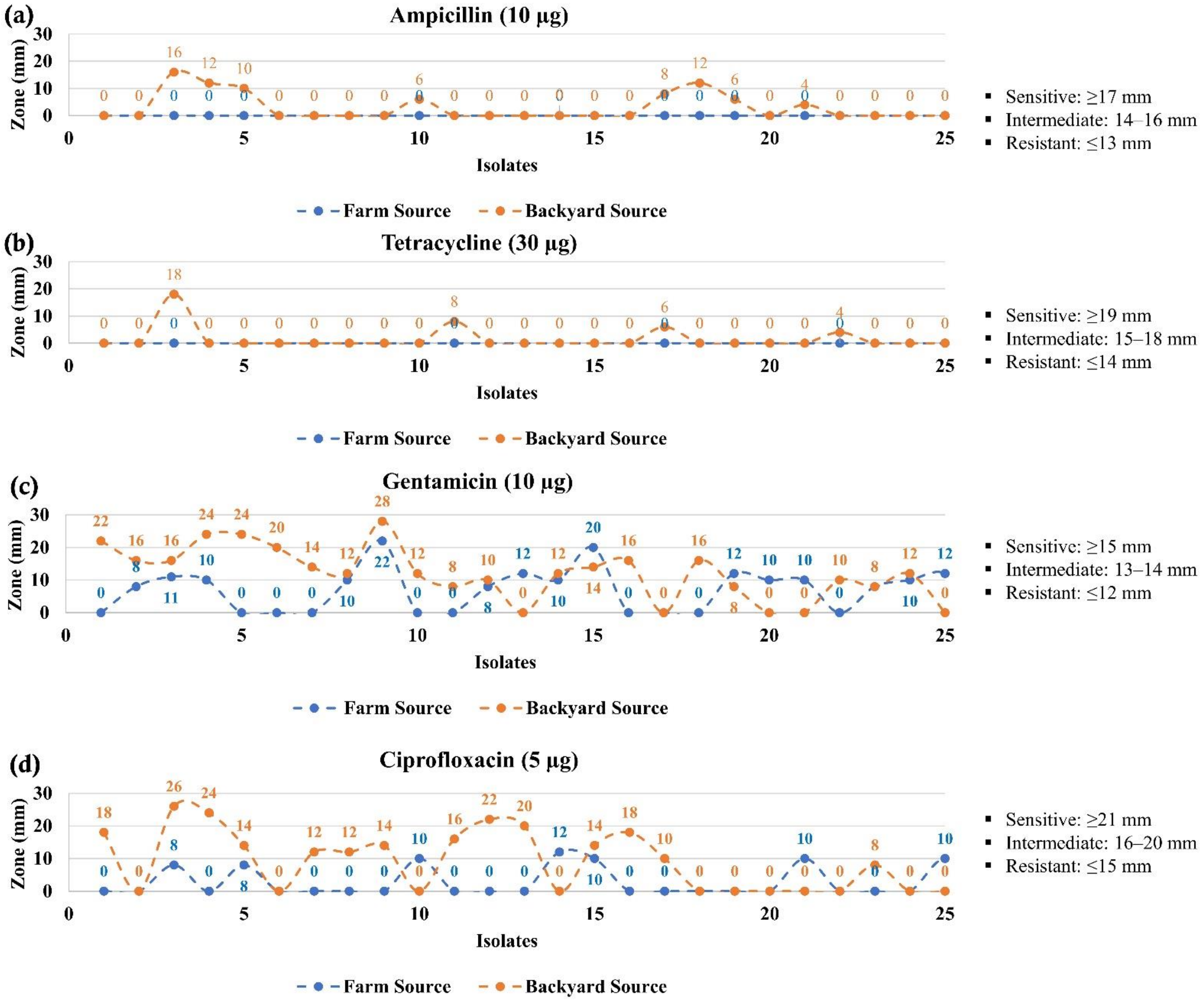

Figure 3. Zone of inhibition determination based on the general CLSI Guideline of commercially used antibiotics in chicken against bacterial isolates from the farm and backyard sources. (a), (b) Ampicillin and tetracycline against bacterial isolates from backyard sources, respectively, and (c) and (d) gentamycin and ciprofloxacin against bacterial isolates from both farm (blue) and backyard (orange) sources, respectively.

3.5. Knowledge of and practice of antimicrobial agents among farmers

In the statistical analysis, there was some association between the use of antimicrobial agents and knowledge and practice of antimicrobial agents among the farmers. Among responders, a statistically significant association was observed between completion of the antibiotic course and the source of antibiotic recommendation among farm chicken respondents (p = 0.038) (Figure 4a). Respondents who used antibiotics based on veterinary recommendations showed the highest rate of full-dose completion, where 92.6% completed the antibiotic course properly. In contrast, respondents who followed a seller recommendation or made a self-decision demonstrated markedly lower completion rates, with only 3.7% in each group completing the full dose.

Similarly, a statistically significant association was also observed between completion of the antibiotic course and awareness of zoonotic transmission among farm chicken respondents (p = 0.037) (Figure 4b). Respondents who completed the full antibiotic dose demonstrated a markedly higher level of awareness regarding zoonotic disease transmission, with 96.3% aware that diseases can spread from chickens or other animals to humans.

Among farm chicken respondents, *E. coli* awareness varied by the source of antibiotic recommendations

(Figure 4c). Respondents who received antibiotic recommendations from veterinarians demonstrated comparatively higher awareness of *E. coli*, including knowledge that it exists in chickens and can spread diseases and antibiotic resistance to humans. In contrast, respondents relying on seller recommendations or self-decisions showed relatively lower awareness levels, with a statistically significant value (p=0.007).

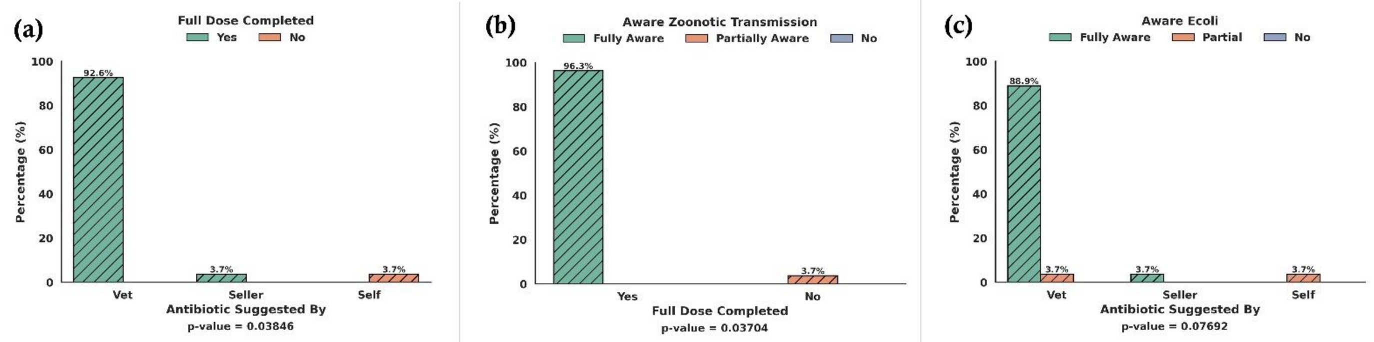

Figure 4. Statistical association (Fisher's exact test) between antibiotics and related phenomena for farm chickens. Here, (a) denotes the association between full dose completion of antibiotics and antibiotic suggestions; (b) denotes the association between full dose completion of antibiotics and aware zoonotic transmission; and (c) denotes the association between full dose completion of antibiotics and aware *E. coli.*

Given the common rural practice of administering human medicines to poultry, respondents were also asked whether chickens and humans became sick simultaneously or within a close time period, and whether human medicines were used in chickens. Analysis of the farm chicken data revealed a strongly significant association between simultaneous sickness in chickens and humans and the use of human medicines in chickens. Respondents who reported the use of human medicines in chickens were more likely to report that chickens and humans became sick at the same time. suggesting a possible link between inappropriate medication practices, disease transmission, and public health awareness.

On the other hand, among backyard chicken respondents, incomplete antibiotic dose administration was most common among those who used antibiotics at their own discretion without following any veterinary recommendation (Figure 5a). Approximately 42.3% of respondents who administered antibiotics on their own did not complete the full course.

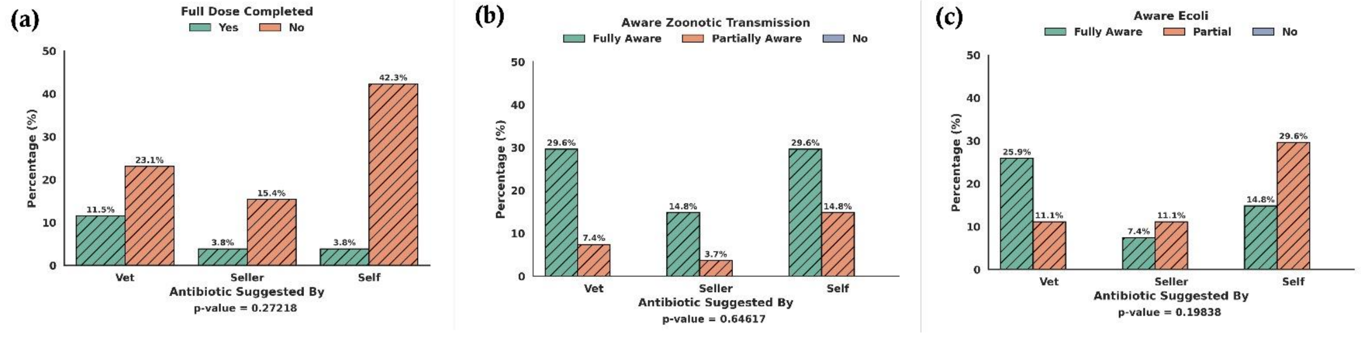

Figure 5. Statistical association (Fisher's exact test) between antibiotics and related phenomena for chickens from backyard sources. Here, (a) denotes the association between full dose completion of antibiotics and antibiotic suggestions; (b) denotes the association between full dose completion of antibiotics and aware zoonotic transmission; and (c) denotes the association between full dose completion of antibiotics and aware *E. coli.*

Furthermore, awareness of zoonotic disease transmission and of *E. coli* was comparatively lower among respondents who did not seek veterinary consultation and instead relied on seller recommendations or self-decisions regarding antibiotic use (Figure 5b, 5c). In contrast, respondents receiving veterinary advice demonstrated relatively greater awareness of the transmission of diseases from chickens and other animals to humans. suggesting the positive role of professional guidance in improving public health awareness and responsible antibiotic use practices.

In comparison with farm systems, backyard poultry systems showed a higher incidence of administering human medicines to chickens. Respondents from backyard settings also more frequently reported the perception that chickens and humans become ill at the same time or in close temporal proximity, accounting for approximately 22.2% of responses. In addition, the proportion of "do not know" responses was comparatively higher among backyard respondents than among farm respondents.

4. DISCUSSION

AMR in chicken production systems is a serious public health concern, especially in low- and middle-income nations where the use of antibiotics is still mostly unregulated [12, 28, 29]. The indiscriminate use of antibiotics in poultry settings has accelerated the emergence and dissemination of MDR bacterial strains, which are capable of zoonotic transmission [30, 31]. The indiscriminate antimicrobials use in poultry can pose serious public health and food safety threat to Bangladesh including animal health and environmental ecosystem as well [32].

Moreover, antimicrobial resistance in poultry-associated *E. coli* constitutes a pressing One Health concern, particularly at the human-animal-environment interface characteristic of high-density poultry production regions [14, 33]. Intensive commercial farming and residential backyard systems coexist in close spatial proximity to densely populated areas in Bangladesh's Chattogram region, providing a habitat that is particularly high risk for interspecies and environmental AMR transmission [34, 35]. Comparative risk evaluations between backyard poultry production and intensive farm systems are still understudied, despite growing worldwide concern. This leaves a substantial knowledge gap regarding the relative contributions of each system to the AMR burden, especially in the case of regional research [8-10, 14, 30]. While regional studies have documented the general prevalence of antibiotic resistance in Bangladeshi poultry, a specific and methodologically rigorous comparative risk assessment of *E. coli* resistance profiles between commercial farm and backyard production systems in this region has remained absent from the literature. Furthermore, the environmental dimension of resistance dissemination through contamination of litter, soil, and the coop floor has been largely unintegrated into comparative assessments. Addressing this gap, the present study compared the prevalence, antimicrobial resistance profiles, and multidrug resistance burden of *E. coli* isolated from commercial farm and backyard poultry production systems in Hathazari, Chattogram, employing integrated microbiological, biochemical, molecular, and epidemiological approaches to construct a comprehensive One Health risk assessment of MDR *E. coli* transmission dynamics across divergent production environments.

Subsequently, bacteriological characterization revealed a broadly overlapping yet compositionally distinct microbial community structure between the two environments. *Pseudomonas*, *Proteus*, *Klebsiella*, *Enterobacter*, *Citrobacter*, and *E. coli* were recovered from both systems, while *Acinetobacter* was identified exclusively among backyard isolates, a genus of growing clinical concern given its intrinsic resistance mechanisms and capacity for horizontal gene transfer with *E. coli* and other Enterobacteriaceae. Mean bacterial loads in backyard samples demonstrated substantially greater peak loads and wider amplitude fluctuation. This suggests the absence of standardized hygiene and waste management protocols characteristic of informal production settings. Comparatively the lower bacterial load was observed in commercial poultry systems. This might be the consequence of frequent use of antibiotics and improved management practices, which exert selective pressure on gut microbiota and reduce overall microbial abundance. In contrast, backyard systems, with limited antimicrobial intervention, tend to harbor higher bacterial loads due to unrestricted environmental exposure [36]. In addition, molecular confirmation of *E. coli* identity via PCR amplification of the uidA gene provided more specificity to the biochemical profiles, ensuring that susceptibility and resistance data were most likely attributed to *E. coli* isolates rather than phenotypically ambiguous Enterobacteriaceae.

Furthermore, according to antimicrobial susceptibility profile, all farm and backyard isolates showed universal resistance to ampicillin and tetracycline, which is consistent with earlier research [37, 38]. This pattern is consistent with the prolonged, widespread, and largely unregulated use of these first-line agents in Bangladeshi poultry production and reflects their well-documented ineffectiveness as therapeutic options for *E. coli* infections in this context. The universal resistance to ampicillin and tetracycline among farm-associated *E. coli* mirrors findings reported from Bangladesh, India, and Pakistan, where these antibiotics rank among the most frequently administered agents in commercial poultry operations, usually without a veterinarian's prescription or dose oversight [39-41].

However, ciprofloxacin resistance was predominant among farm isolates, with inhibition zones for most isolates falling below the resistance breakpoint. This indicates that the fluoroquinolone-resistant *E. coli* has become entrenched in commercial farm environments. It is a crucial finding of the study as a serious public health concern. Besides, in backyard isolates, gentamicin demonstrated broader and more consistent inhibitory activity than farm isolates. This indicates that differential selective pressure, rather than uniform resistance saturation, characterizes the backyard AMR landscape. These differential susceptibility profiles between the two production systems have important implications for risk stratification in regional AMR surveillance and for the prioritization of antibiotic classes in stewardship interventions.

Backyard poultry systems often lack adequate knowledge and practices related to hygienic handling of raw chicken and proper waste management, which may contribute to environmental contamination and human exposure. This finding indicates that the absence of proper veterinary guidance may contribute to irresponsible antibiotic use practices and incomplete dose administration in backyard poultry management. Though commercial farms generally maintain relatively better biosecurity and handling practices, both systems contribute to the dissemination of antimicrobial-resistant bacteria through raw poultry products and improper disposal of untreated waste. The consequence is transmission of AMR and MDR among humans via the food chain and the surrounding environment [42].

The present study revealed several significant associations between antibiotic use practices and AMR-related phenomena that carry direct implications for *E. coli*-mediated resistance transmission in both production systems. In farm settings, a statistically meaningful association was identified between antibiotic use and zoonotic awareness, with full dose completion universally observed when antibiotic administration was guided by a veterinarian or seller. In contrast, self-directed antibiotic use consistently resulted in incomplete dosing, a practice well established as a principal driver of subtherapeutic antibiotic exposure and subsequent selection of resistance in commensal *E. coli* populations. Of clinical significance, among respondents who reported using human medicine in chickens, they simultaneously reported concurrent illness in both chickens and humans, raising serious concern regarding bidirectional *E. coli* transmission pathways operating at the farm-household interface. In backyard settings, the epidemiological risk signal was comparably pronounced. All antibiotic-using respondents reported human illness following chicken illness, suggesting a direct epidemiological link between poultry antibiotic use and adverse human health outcomes in household production settings.

Knowledge-level determinants of risk perception may operate differently across production systems, as evidenced by the strong and statistically significant correlations among *E. coli* awareness, biosecurity awareness, and zoonotic transmission awareness observed only in farm settings and absent among backyard respondents. Consequently, this distinction has obvious consequences for the design and targeting of antibiotic stewardship initiatives. These findings indicate that respondents seeking veterinary advice were comparatively more careful and responsible in administering antibiotics, whereas incomplete dosing practices were more common among those relying on sellers or self-medicating. This finding suggests that respondents with better knowledge of zoonotic transmission were more likely to follow proper antibiotic administration practices and complete the prescribed antibiotic course.

However, the current literature shows mixed findings regarding differences in awareness. Muleme et al. (2023) found that subsistence (rural) farmers in Uganda were more likely to correctly describe antibiotics than commercial farmers [43]. On the contrary, Mudenda et al. (2022) portrayed the opposite in Zambia: commercial farmers were more aware of AMR than medium-scale farmers [44]. The evidence indicates AMR risk in rural settings is driven by a multi-level awareness system.

Despite providing the first integrated microbiological, biochemical, molecular, and epidemiological comparative assessment of MDR *E. coli* across commercial farm and backyard poultry production systems in the Chattogram region, the use of self-reported data for antibiotic use practices and illness co-occurrence introduces recall and social desirability biases, while small sub-group sample sizes in certain strata may have constrained the statistical power of associative inferences. Moreover, the absence of whole-genome sequencing precluded characterization of specific resistance genes, mobile genetic elements, and plasmid-mediated mechanisms, hereby limiting the assessment of horizontal gene transfer associated with the observed AMR burden. Another important point is that the cross-sectional design precludes causal inference, and the geographic restriction to Hathazari, Chattogram, which limits broader generalizability.

5. LIMITATIONS OF THE STUDY

While this study provides a detailed comparison of multidrug-resistant *E. coli* across poultry systems, there are some limitations and future directions to note. The first limitation of the study is the small sample size, which could undermine the power of the findings. Furthermore, this investigation was unable to establish causality; moreover, because it was conducted in Hathazari, Chattogram district, it could not provide comprehensive insight into the state of affairs in other areas. At the molecular level, the lack of whole-genome sequencing precluded the identification of resistance mechanisms, including mobile genetic elements and specific resistance genes. The restricted antibiotic panel used for susceptibility testing, although based on commonly used antimicrobial classes in the local poultry setting, may have limited a more comprehensive characterization of antimicrobial resistance, and inclusion of additional agents, such as third-generation antibiotics, could have strengthened the resistance profiling. Although the cross-sectional design and geographic restriction limit causal inference and broader generalizability, these constraints also define a clear trajectory for future research, particularly longitudinal, multi-site investigations incorporating whole-genome sequencing and environmental sampling of litter, soil, and drainage water. These are needed to fully understand the dynamics of antimicrobial resistance and to address the environmental transmission gap identified in this study.

6. CONCLUSION

The findings collectively demonstrate that commercial farm and backyard systems harbor clinically significant MDR bacteria, including *E. coli* populations, yet differ meaningfully in their resistance landscapes, behavioral risk determinants, and knowledge-level predictors of AMR. Both systems are significant contributors to AMR due to prolonged and improper antibiotic use, as evidenced by resistance to drugs such as ampicillin and tetracycline. The resistance profile also varies between farm types: commercial farms show ciprofloxacin resistance, while backyard farms have higher bacterial loads, including *Acinetobacter*. The study highlights a direct link between poor antibiotic use and concurrent infections in humans and chickens, suggesting possible pathogen movement between hosts. These findings underscore the need to address antibiotic resistance in each farming system individually. Hence, the urgent need for production system-specific antibiotic stewardship strategies, strengthened veterinary oversight, genomic epidemiology, and One Health AMR surveillance frameworks that integrate human, animal, and environmental resistance data at the regional level.

Supplementary Materials

The supplementary materials supporting the findings of this study are available with the online version of this article as **Supplementary Appendix A1** and **Supplementary Figures S1–S3**.

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Institutional Review Board Statement

The study received approval from the ethical committee of Chattogram Veterinary and Animal Sciences University (Memo no: CVASU/Dir (R&E) EC/2026/17/26, Date:28/01/2026). We obtained informed consent after discussing the informed consent form with each participant.

Informed Consent Statement

Informed consent was obtained from all participants involved in the study.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Artificial Intelligence (AI) Declaration

The authors declare that all scientific ideas presented in this manuscript, including conceptualization, content, data analysis, and data interpretation, are their own; no generative AI tools were used. During the preparation of this work, the authors received limited assistance from DeepseekR to improve language, clarity, and readability. After using this tool/service, the authors reviewed and edited the content as needed and took full responsibility for the published article.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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