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COMPARATIVE ASSESSMENT OF *ESCHERICHIA COLI* CONTAMINATION AND ANTIMICROBIAL RESISTANCE ACROSS BROILER, NATIVE CHICKEN, AND DUCK PRODUCTION UNITS

Kusumandari Indah Prahesti [1,2], Ratmawati Malaka [2,*], Ridwan Amiruddin [3], Muflihanah Muflihanah[4], Farida Nur Yuliati [2], Sudirman Baco [2], Wempie Pakiding [2], Yuni Syafa Wati[5], Sri Wahyuni[5] and Ayu Puspita Sari[5]

[1]Agricultural Science Doctoral Study Program, Postgraduate School, Hasanuddin University, Makassar, 90245, Indonesia
[2]Department of Animal Production, Faculty of Animal Science, Hasanuddin University, Makassar, 90245, Indonesia
[3]Department of Epidemiology, Faculty of Public Health, Hasanuddin University, Makassar, 90245, Indonesia
[4]Disease Investigation Center, Maros, South Sulawesi, Indonesia
[5]Animal Husbandry Study Program, Faculty of Animal Science, Hasanuddin University

Corresponding author: malaka_ag39@yahoo.co.id

ABSTRACT

Variations in poultry housing systems and biosecurity levels can influence *Escherichia coli* persistence and antimicrobial resistance (AMR) dynamics at the farm level. This study compared *E. coli* contamination across cloacal swabs, drinking water, and litter samples among broiler, native chicken, and duck units. Furthermore, it mapped antibiotic resistance profiles, the prevalence of multidrug resistance (MDR), and their correlations with specific poultry hosts. A total of 127 samples (comprising cloacal swabs, drinking water, and litter) were collected across broiler, native chicken, and duck units. *E. coli* was enumerated and tested for susceptibility against eight antibiotics using the disc diffusion method. Data were analyzed using Kruskal–Wallis, Mann–Whitney, and Spearman's correlation tests. The results revealed that *E. coli* concentrations differed significantly between housing units across all sample types ($P<0.01$). Duck units exhibited the highest contamination (median 16 $\log_{10}$), while broilers showed the lowest (median 5.53 $\log_{10}$). The highest resistance was observed against ampicillin (59.3%), ceftriaxone (44.4%), gentamicin (40.7%), and tetracycline (40.7%). Notably, 44.4% of the isolates were MDR, though all remained susceptible to meropenem. Spearman's correlation revealed that broiler isolates were positively correlated with resistance (e.g., ceftazidime, $r=0.77$; ampicillin, $r=0.67$), whereas duck and native chicken isolates were not. Consequently, mitigating MDR risks requires optimizing water/drainage infrastructure in duck units and enforcing stricter antimicrobial stewardship in broiler production.

Keywords: *Escherichia coli*, Poultry housing system, Antimicrobial resistance, Multidrug resistance.

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1. INTRODUCTION

The global poultry sector plays an important role in providing animal protein and maintaining food security worldwide. To meet demand for increased production, the sector implements management and husbandry systems tailored to production capacity and product characteristics. In practice, poultry are kept in various housing systems that offer different levels of biosecurity. The three most common systems are intensive broiler farming in closed houses, semi-intensive rearing of native chickens, and open-water access systems for duck farming (Berg, 2002; Conan et al., 2012; Wilson, 2021). Variations in housing affect bacterial persistence and spread on farms (Zhao et al., 2019; Schreuder et al., 2020), necessitating mapping bacterial transmission patterns and conducting biosecurity evaluations for each system. Recent comparative surveys confirm that biosecurity implementation differs markedly between production systems; Mbatidde et al. (2024) reported that semi-intensive poultry farms applied considerably more structural and operational biosecurity measures, such as footbaths and disinfectant use, than free-range systems, while Amalraj et al. (2024) similarly found that conventional indoor duck, breeder, and turkey operations scored higher on standardized biosecurity indices than free-range layer and broiler farms. These disparities matter because inconsistent biosecurity directly shapes the opportunity for environmental bacteria to persist, multiply, and spread within a flock.

In livestock environments, *E. coli* is the primary indicator bacterium used to assess the sanitation status and fecal contamination levels (Khan & Gupta, 2020; Anjum et al., 2021; Zarić et al., 2023). At the local scale, the risks of *E. coli* dissemination and resistance vary by commodity type and management practices (Parveen et al., 2006; Ibrahim

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et al., 2019; Sonnenschein-Swanson et al., 2025; Hayajneh, 2026). In intensive farming units, such as broilers, high antibiotic use generates a strong selective pressure for the emergence of resistance genes (Turcotte et al., 2020; Mughini-Gras et al., 2022; Bhattarai et al., 2024; Nechitailo et al., 2024). Leclercq et al. (2024) demonstrated that commensal multidrug-resistant *E. coli* strains persist in broiler flocks primarily through recirculation from the rearing environment rather than through vertical transmission alone, while Eraky & Abd El-Ghany (2024) isolated multidrug-resistant avian pathogenic *E. coli* carrying multiple virulence and resistance genes from commercial broiler breeds in Egypt. Comparable patterns have also been documented in Asian production systems, where Zhou et al. (2024) reported a high prevalence of virulence-associated and resistance genes among *E. coli* isolated across the feeding cycle of Arbor Acres broilers in China.

In free-range systems for native chickens and ducks, the risk of *E. coli* exposure is heavily influenced by direct interactions with the environment (Delpont et al., 2026). Specifically, in duck houses, the presence of open-water access and accumulation of bedding material (litter) are suspected to provide highly favorable conditions for bacterial contamination and multiplication, even though management practices generally do not use antibiotics as intensively as in broiler units (Zhao et al., 2019; Sharmila et al., 2023). Nevertheless, waterfowl are not exempt from the multidrug-resistance burden; Kerek et al. (2025) reported an estimated multidrug-resistance prevalence of nearly 80% among clinical *E. coli* isolates from ducks in Hungary, with resistance to enrofloxacin, neomycin, amoxicillin, and florfenicol identified as the strongest predictors of the multidrug-resistant phenotype. For native, free-range chickens, resistant *E. coli* and related enteric bacteria have similarly been recovered despite comparatively lower reported antibiotic use, suggesting that environmental exposure pathways, scavenging behavior, and contact with contaminated soil and water contribute independently to resistance carriage (Worku et al., 2025).

The poultry sector has been globally identified as a major reservoir for the spread of *E. coli* resistance genes through the food chain, requiring strict surveillance across the entire production chain (Hossain et al., 2020; Sevilla-Navarro et al., 2022; Medrano et al., 2025). Monitoring this bacterium is critical, as the World Health Organization (WHO) classifies Enterobacterales (*Escherichia coli*) as a "Critical" priority pathogen in the 2024 Bacterial Priority Pathogens List, reflecting the growing threat posed by antimicrobial resistance (AMR) and multidrug-resistant bacterial infections (WHO, 2024; EFSA, 2025). This classification was reaffirmed in the 2024 update of the WHO Bacterial Priority Pathogens List, in which third-generation cephalosporin- and carbapenem-resistant Enterobacterales, including *E. coli*, were again ranked in the critical priority quartile alongside *Klebsiella pneumoniae* and *Acinetobacter* spp. (WHO, 2024). At the continental level, Munk et al. (2024) mapped the livestock resistome across Europe and confirmed that the composition and abundance of resistance genes in food-producing animals, including poultry, remain closely linked to antimicrobial usage patterns and farm management practices, reinforcing the need for sector- and system-specific surveillance rather than a single generalized approach. The increased risk of resistance is driven by the extensive use of antimicrobials for prophylactic purposes and as growth promoters in the livestock and poultry industries (Efendi et al., 2022; Thompson et al., 2023; Ijaz et al., 2024; Usman et al., 2025). At the molecular level, Xue et al. (2024) further showed that avian pathogenic *E. coli* isolates frequently carry conserved virulence gene clusters alongside resistance determinants, indicating that virulence and resistance traits may co-disseminate within poultry populations rather than evolving independently.

Despite this expanding body of literature on poultry-associated AMR, studies that simultaneously quantify *E. coli* contamination levels across multiple sample types, including cloacal swabs, drinking water, and litter, and correlate these burdens with phenotypic resistance profiles across different housing types within a single, controlled farm area remain limited. Most existing surveys focus on a single production system or a single sample matrix, which restricts direct comparison of contamination and resistance dynamics between intensive, semi-intensive, and open-water poultry housing operating under comparable environmental and geographic conditions (Sevilla-Navarro et al., 2022; Thongratsakul et al., 2023; Kerek et al., 2024). A controlled, multi-system comparison within one teaching or research farm setting therefore offers a valuable opportunity to isolate the effect of housing type from confounding regional and management variability, and to generate evidence that can directly inform system-specific biosecurity and antimicrobial stewardship recommendations

This study aimed to compare E. coli contamination levels in cloacal swabs, drinking water, and litter samples from broiler, native chicken, and duck units within a teaching farm area. Furthermore, this study determined the antibiotic resistance profiles and MDR prevalence and analyzed the correlation between these resistance profiles and poultry type.

2. MATERIALS AND METHODS

2.1. Study Area

This study was conducted at the poultry facilities of the Faculty of Animal Science, Hasanuddin University, Makassar, South Sulawesi Province, Indonesia. The Faculty of Animal Science comprises three poultry units: broilers, native chickens, and ducks. The broiler unit is a commercial-scale production facility with intensive livestock industry standards; the native chicken unit serves as the experimental unit; and the duck unit serves as the pedagogical unit for

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teaching and student practice. Fig. 1 shows the locations of the poultry units.

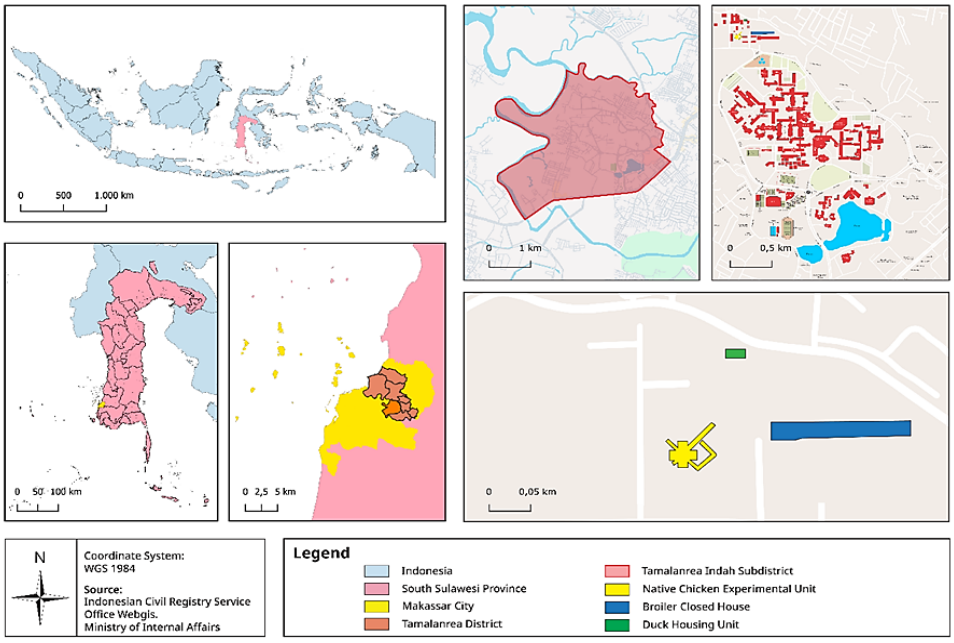

Fig. 1: Map of sampling locations at the Hasanuddin University poultry facility.

2.2. Sample Collection

The total number of samples was 127, comprising 45 broilers, 36 native chickens, and 46 ducks, collected from drinking water, litter, and cloacal swabs. Samples from the broiler house were randomly collected at 4 weeks of age, representing the inlet, middle, and outlet sections (Badrah et al., 2021). Native samples were taken from the finisher-phase chicken house, namely a 6×6 m^2 communal barn containing eight breeding pens of 1.25×2.5 m^2 each with a 1:6 male-to-female ratio. Duck samples were collected from a communal barn containing ducks older than 1 year. The duck communal barn contains breeding pens of 3×4 m^2 with a 3:22 male-to-female ratio. For the native chicken and duck units, breeding pens were selected using a simple random sampling technique, and all birds present within each selected pen at the time of sampling were included; drinking water and litter samples were likewise collected from randomly selected points within each pen, following standard sampling principles for cross-sectional livestock studies (Thrusfield, 2018).

Drinking water samples were collected from nipples connected to water pipes supplying water from a drinking water storage tank, whereas for ducks, samples were collected from drinking water containers. Cloacal swab samples were collected using sterile cotton swab sticks moistened with normal saline. The stick was inserted into the cloaca and placed in a sterile tube. Litter samples were aseptically collected from several spots in each poultry house. All samples were placed in sterile containers and transported to the laboratory in an ice box.

2.3 *Escherichia coli* Enumeration

E. coli enumeration was performed using the pour plate method with serial 10-fold dilutions, following the guidelines from the Bacteriological Analytical Manual (Feng et al., 2020). Aliquots from the serially diluted samples were plated on Eosin Methylene Blue (EMB) agar and incubated at 37°C for 24 hours (Fig. 2a). Units of measurement were $\log_{10}$ CFU/g for litter samples and $\log_{10}$ CFU/mL for drinking water and cloacal swab samples. Subsequently, typical colonies exhibiting a metallic green sheen on the EMB agar were selected as presumptive *E. coli*. These isolates were further subjected to confirmation tests, including Gram staining, indole test, Methyl Red Test, Voges-Proskauer Test, and Citrate Test (IMViC) (Fig. 2b). *E. coli* strain American Type Culture Collection (ATCC) 25922 was used as a control (Souza et al., 2025).

2.4. Antibiotic Susceptibility Test (AST)

A total of 27 representative E. coli isolates were purposefully selected and subjected to AST to ensure adequate representation across all poultry types and matrices. AST was performed using the disc diffusion method against eight types of antibiotics: ampicillin (AMP) 10 µg, tetracycline (TE) 30 µg, sulfamethoxazole-trimethoprim (SXT) 1.25/23.75 µg, meropenem (MEM) 10 µg, gentamicin (CN) 10 µg, chloramphenicol (C) 30 µg, amoxicillin-clavulanic

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acid (AMC) 20/10 µg, and ceftriaxone (CRO) 30 µg. *E. coli* cultures with a concentration of 1-2×10^8 CFU/ml (equivalent to McFarland 0.5) were streaked onto Mueller-Hinton agar (MHA), after which antibiotic discs were placed using sterile forceps. The inhibition zones were interpreted according to the Clinical and Laboratory Standards Institute (CLSI, 2021).

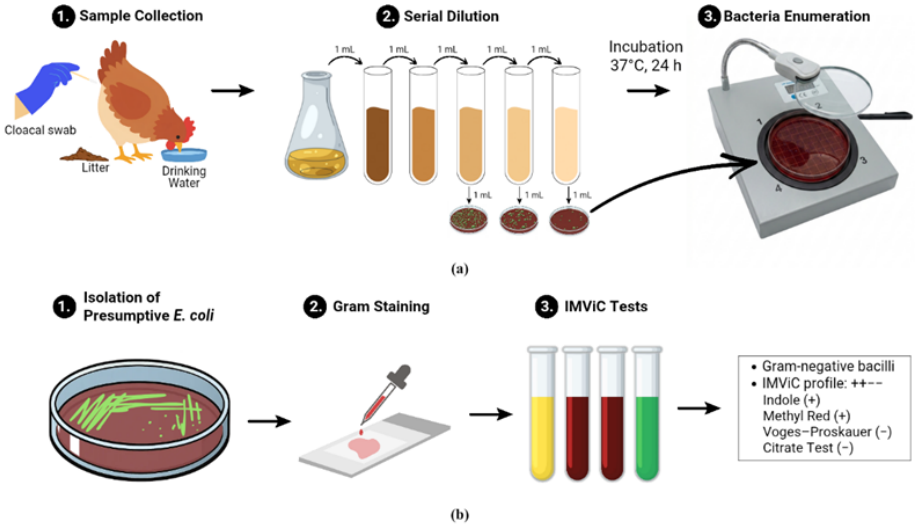

Fig. 2: Schematic illustration of (a) sample collection and bacteria enumeration method, (b) *E. coli* colony confirmation tests.

2.5. Statistical Analysis

The *E. coli* concentration data from each poultry unit were tabulated and processed using a Microsoft Excel spreadsheet. Subsequent data analysis was conducted using IBM® SPSS® Statistics 24. The *E. coli* concentrations for each sample type per poultry unit were summarized descriptively and presented as quartile distributions. Non-parametric tests were applied to examine the differences in bacterial concentrations across samples from each poultry type. The Kruskal-Wallis Test was used for comparisons between groups. Variables with a P≤0.05 were subjected to further analysis using the Mann-Whitney U test. Spearman's rank correlation was used to analyze the correlation between antibiotic resistance in isolates and poultry type, using RStudio version 4.5.2.

3. RESULTS

3.1. Housing and Biosecurity Management of Poultry Units

All poultry maintenance units included in this study were teaching farms affiliated with the Faculty of Animal Science, Hasanuddin University, Indonesia. These three units primarily support education and research by providing a comprehensive farm management model for students and researchers, although they differ in operational scale. Table 1 presents the housing management of each poultry unit, including the operational scale, housing system, bedding material, antibiotic administration, and biosecurity procedures.

Table 1: Comparison of Operational Scale, Housing Systems, Biosecurity Practices, and Antibiotic Administration across Poultry Units

Parameters	Poultry Units		
	Broiler chicken	Native chicken	Duck
Operational scale	Industrial	Research, experimental	Teaching, practicum
Housing system	Closed	Opened	Opened
Bedding material	Rice husk	Rice husk	Wood shavings
Drinking water source	Ground water	Ground water	Ground water
Biosecurity level	High	Moderate	Moderate
Drinking water disinfection	Routine, scheduled	Every 2-3 months	Not performed
Footbath at entrance	Available	Available	Available
Litter replacement	At cycle onset; conditionally if moist or caked	Every 2-3 months	>12 months
Vaccination program	Avian influenza, Newcastle disease, Infectious bursal disease	Avian influenza, Newcastle disease, Infectious bursal disease	Not performed
Antibiotic administration	scheduled	Incidental	Not performed

The broiler unit operated in a closed-house system measuring 12×120 m², accommodating up to 20,000 birds. The biosecurity program is meticulously documented through Standard Operating Procedures (SOPs), with visitor access restrictions, ensuring that all workers fully comprehend and adhere to the procedures. Disinfection of barn buildings and equipment is conducted post-harvest and during preparation for chick-in. Mid-cycle disinfection is performed as necessary, particularly during outbreaks. As part of partnership management, the company specifies the

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type, dosage, schedule, and method of disinfectant application. The temperature within the housing was maintained at 26-28°C, and the humidity was 60-80%. Antibiotics were administered based on a predetermined schedule. Feeding was automated, and drinking water was supplied through a closed system utilizing nipple drinkers. Rice husks were used as the bedding material and were routinely turned or replaced every one to two weeks. The frequency may increase depending on barn conditions, weather, and poultry population density, particularly when humidity increases or manure accumulation leads to ammonia odors.

The native unit operates as an open house and serves as a research breeding facility, covering all stages from hatching, day-old chicks, starter, and grower to the productive phase. The biosecurity level is moderate because the commodity is believed to naturally resist diseases. The biosecurity program is not yet comprehensively documented and currently exists as written instructions displayed in the housing area along with verbal instructions. Similar to the broiler units, the native housing utilized a closed drinking water system with nipple drinkers. Drinking water is disinfected with chlorine every two to three months, and antibiotics are administered incidentally in response to disease outbreaks.

The duck unit housed Mojosari and Alabio duck breeds. This unit is limited to educational and practical purposes. The ducks were reared in an open house covering a total area of 200 m^2 (20 m ×10 m), with bedding material made of wood shavings that were rarely replaced, causing them to become compacted over time. Ducks are considered natural poultry and are more disease-resistant; therefore, biosecurity is moderate. The enclosure is cleaned as needed; vaccination is never carried out, and antibiotics are not administered to the animals. A bathing area is provided in the duck enclosure because ducks are waterfowl that depend on access to open water.

3.2. *E. coli* Concentration

Table 2 summarizes the sample source, type, size, and *E. coli* concentration in the observed poultry units. *E. coli* concentrations vary among different types of poultry. The duck unit exhibited the highest contamination load, with a stable median value of 16 $\log_{10}$ units across all sample types, indicating a uniformly and highly contaminated environment. In contrast, the broiler group showed the lowest bacterial concentration, particularly in drinking water samples (median, 5.53 $\log_{10}$ CFU/mL). *E. coli* concentrations ranged from 9.07 to 16.64 $\log_{10}$ CFU/g in litter samples, 4.83 to 16.48 $\log_{10}$ CFU/mL in drinking water, and 8.13 to 16.69 $\log_{10}$ CFU/mL in cloacal swab samples.

Table 2: Distribution of *E. coli* concentration in observed poultry units

Sample Type	Sample Size	*E. coli* Concentration*				
		Minimum	Q1	Median	Q3	Maximum
Litter						
Broiler	15	9.07	9.54	9.85	10.27	10.65
Native	12	12.63	13.22	13.42	14.08	14.29
Duck	8	16.30	16.39	16.42	16.52	16.64
Drinking water						
Broiler	15	4.83	5.27	5.53	5.77	6.40
Native	12	8.96	9.33	9.65	9.94	10.23
Duck	8	16.05	16.18	16.26	16.42	16.48
Cloacal swab						
Broiler	15	8.13	9.10	9.50	9.73	10.32
Native	12	8.58	9.22	9.74	10.20	10.49
Duck	30	15.96	16.11	16.21	16.30	16.69

*Measurement unit: $\log_{10}$ CFU/g for litter samples and $\log_{10}$ CFU/mL for drinking water and cloacal swab samples. Q1: first quartile (25th percentile); Q3: third quartile (75th percentile).

Confirmation of *E. coli* isolates showed a metallic green sheen on EMB agar and Gram-negative, rod-shaped bacteria (Fig. 3). The IMViC test profiles were ++--, showing positive indole and Methyl Red, but negative Voges-Proskauer and Citrate. The positive indole test was indicated by the formation of a bright red ring after addition of Kovács reagent, positive methyl red test was indicated by the broth turning distinctly red upon the addition of the methyl red indicator; the negative Voges-Proskauer test indicated by the lack of color change after the addition of Barritt's A and B reagents, negative citrate tests were observed as *E. coli* cannot utilize citrate as carbon source (Widodo et al., 2023).

The Kruskal-Wallis test results (Table 3) showed a highly significant difference ($P<0.01$) in *E. coli* concentration across poultry types in all sample types. The concentration of *E. coli* in native and duck was significantly higher than that in broilers in all sample types. In litter samples, *E. coli* concentrations were 4- to 7-$\log_{10}$ units higher in native and duck than in broilers (geometric means 13.52±0.56 and 16.45±0.13 vs. 9.88±0.53 $\log_{10}$ CFU/g, respectively; $P<0.01$). For drinking water samples, the *E. coli* concentrations were significantly 4-log higher in native and 11-$\log_{10}$ higher in duck compared to broiler (geometric means 9.64±0.42 and 16.28±0.17 vs. 5.52 ± 0.44 $\log_{10}$ CFU/mL, respectively; $P<0.01$).

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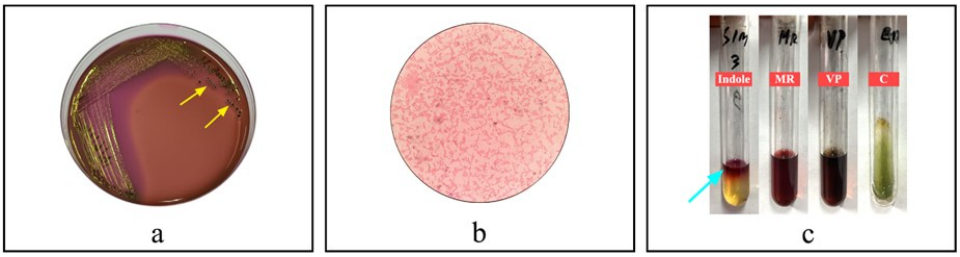

Fig. 3: *E. coli* isolate confirmation. Metallic green sheen on EMB Agar (yellow arrow) (a), Gram-negative bacilli (b), IMViC profile: ++--. MR, Methyl Red; VP, Voges-Proskauer; C, Citrate.

Table 3: Geometric means of *E. coli* concentration from samples in poultry units

Sample type	Broiler	Native	Duck	P Value
Drinking Water (log_{10} CFU/mL)	5.52±0.44[a]	9.64±0.42[b]	16.28±0.17[c]	0.00
Litter (log_{10} CFU/g)	9.88±0.53[a]	13.52±0.56[b]	16.45±0.13[c]	0.00
Cloacal swab (log_{10} CFU/mL)	9.33±0.65[a]	9.68±0.64[b]	16.22±0.16[c]	0.00

Values (mean + SE) bearing different superscripts on the same row indicate significant differences (P<0.01).

3.3. Antibiotic Susceptibility Test (AST)

The AST results showed the highest resistance rate in the ampicillin group (59.3%), followed by ceftriaxone (44.4%), gentamicin (40.7%), tetracycline (40.7%), sulfamethoxazole-trimethoprim (25.9%), amoxicillin-clavulanic acid (7.4%), and chloramphenicol (7.4%). All isolates were susceptible to meropenem (Fig. 4). A total of 12 out of 27 (44.4%) isolates examined showed multidrug resistance to three to five antibiotics. Fig. 5 shows the AST results of *E. coli* isolates from each poultry type.

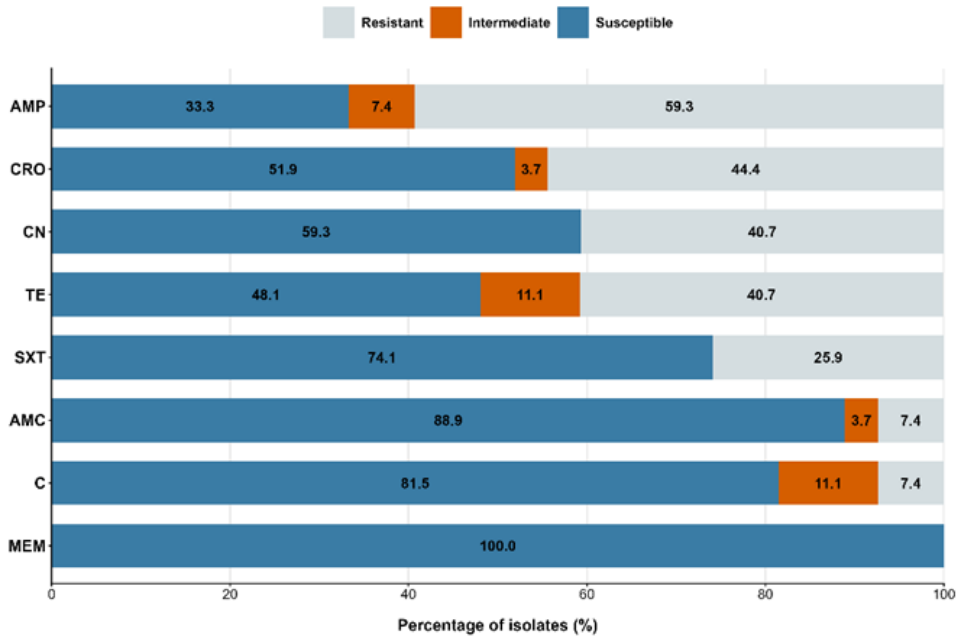

Fig. 4: Antibiotic susceptibility profiles of E. coli isolates. MEM, meropenem; C, chloramphenicol; AMC, amoxicillin–clavulanic acid; SXT, sulfamethoxazole–trimethoprim; TE, tetracycline; CN, gentamicin; CRO, ceftriaxone; AMP, ampicillin.

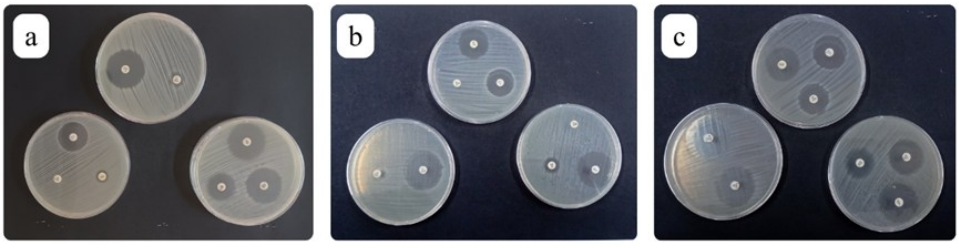

Fig. 5: The antibiotic susceptibility test results of *E. coli* isolates from each poultry type. Broiler (a), duck (b), and native chicken (c).

Visualization of Spearman's rank showed that, in broilers, almost all antibiotics displayed positive correlations, with the strongest correlations observed for CN (gentamicin) at 0.77 and AMP (ampicillin) at 0.67, while AMC showed a negative correlation of -0.29. In ducks, the strongest negative correlations were for CN (r = -0.33) and C (r = -0.31), and only AMC showed a positive correlation (r = 0.28). Native chickens exhibited even stronger negative

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correlations than ducks, particularly for CN (r=-0.50) and AMP (r=-0.45) (Fig. 6). These results indicate that the isolates from native chickens were the most sensitive to the antibiotics tested.

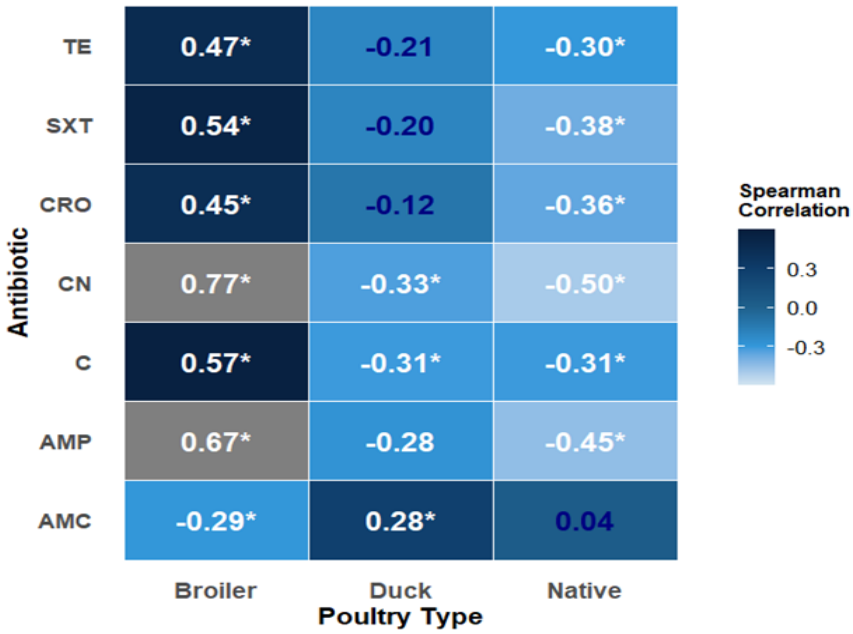

Fig. 6: Heatmap visualization of Spearman's rank correlation between the antibiotic resistance and the source of poultry type (there were no isolates resistant to the antibiotic meropenem, MEM). Asterisks (*) denote |r| ≥ Q1 (0.277); AMC, amoxicillin-clavulanic acid; AMP, ampicillin; C, chloramphenicol; CN, gentamicin; CRO, ceftriaxone; SXT, sulfamethoxazole-trimethoprim; TE, tetracycline.

4. DISCUSSION

The concentration of *E. coli* in the duck unit showed a high and homogeneous distribution across all sample types. Percentile data indicated a narrow range, with median values of 16.42 in litter samples, 16.26 in drinking water, and 16.21 in cloacal swabs, with Q1 values within the same range. The minimal variance between percentiles indicates saturation of fecal contamination. Unlike the broiler and native chicken units, the duck unit showed a plateau effect at the maximum detection limit, suggesting that the entire farming area, including the litter, drinking water, and environment, was consistently contaminated with *E. coli*. Duck behavior, including water-related activities such as bathing and soaking, facilitates widespread bacterial transmission (Babington & Campbell, 2022; Fauszt et al., 2026). The soaking areas became sites where feces and bacteria accumulated. Consequently, constant cross-contamination occurs between feces, water, and the environment through the ducks' wet plumage, leading to uniformly high bacterial loads across all sampled matrices within the unit. This pattern is consistent with more recent longitudinal evidence from duck production systems; Kerek et al. (2025) reported that clinical *E. coli* isolates from Hungarian duck flocks were almost uniformly multidrug-resistant, which the authors linked to the continuous fecal-water cycling inherent to duck husbandry rather than to farm-level antimicrobial dosing alone. Because ducks preen and soak repeatedly throughout the day, bacteria shed in feces are continuously resuspended and redistributed across the pen, preventing the natural decline in bacterial counts normally observed as litter dries under dry-housed rearing (Fauszt et al., 2026).

Broiler and native chicken units that adopted the nipple drinker system showed better control of bacterial contamination in drinking water than duck units. The nipple system acts as a closed channel that minimizes water exposure to environmental pollutants (Fauszt et al., 2026). Closed delivery systems such as nipple drinkers substantially reduce opportunities for fecal-oral recontamination compared with open water sources, since water only briefly contacts the bird's beak and is not left standing in pools where fecal material and biofilm can accumulate (Münster & Kemper, 2024). In contrast, the use of open drinking water containers in duck units, combined with the presence of a bathing pool facility, allows for continuous cross-contamination (Schober et al., 2025); ducks can transfer bacteria from their beaks and wet bodies into open drinking containers after engaging in activities in the bathing area (Mustedanagic et al., 2023), consistent with survey data showing that open, publicly sourced poultry drinking water is more frequently colonized by enteric bacteria than water delivered through enclosed lines. This is the main determining factor in the almost identical concentration of E. coli in duck drinking water and litter, a condition not observed in broiler and native chicken units. A recent study recommended implementing overhead showers. The system successfully promoted wet preening behavior and promote wet preening behavior and behaviorally preferred over conventional nipple drinkers (Archer & House, 2026). Taken together, these observations suggest that the drinking-water delivery system, rather than species-specific hygiene behavior alone, is a major determinant of environmental *E. coli* burden in poultry housing, and that water-line design should be treated as a

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priority biosecurity control point in duck production.

In this study, *E. coli* counts in litter samples were higher than in other sample types for all poultry species. A previous study in Indonesia found that the number of *E. coli* in broiler litter ranged from 2.5×10^7 to 5.4×10^7 CFU/g (Badrah et al., 2021). Blaak et al. (2014) found that the average concentration of *E. coli* in poultry feces ranged from 2.5×10^7 to 4.4×10^{10} in broilers and ranged from 7.8×10^9 to 9×10^{10} in laying hens. Furthermore, Xu et al. (2022) found that the concentration of *E. coli* in fecal samples from pastured poultry farms ranged from 2.70 to 9.57 $\log_{10}$ CFU/g. Another study reported that the *E. coli* concentration in settled dust in poultry farms was 1.6×10^5 (Skóra et al., 2016).

In our study, the highest resistance was observed for ampicillin (59.3%). High ampicillin resistance in our study is similar to previous studies in Indonesia (Hardiati et al., 2021; Indrawati et al., 2021; Faridah et al., 2023). High antibiotic resistance levels were also observed in E. coli isolated from poultry-related samples in Indonesia, which concurs with our E. coli observations: tetracycline 57.1%, ampicillin 33.3%, ceftriaxone 23.8%, gentamicin 23.8%, and chloramphenicol 9.5% (Ariyanti et al., 2025). In Indonesia, a study found that *E. coli* resistant to ampicillin (ranging from 68.43 to 100%), streptomycin (68.42 to 95%), erythromycin (73.68 to 88.24%), tetracycline (15 to 70 %), and sulfamethoxazole-trimethoprim (52.64 to 94.12%) were detected in cloacal swab samples of laying hens and broilers; overall MDR levels were 63.16 to 88.23% in laying hens and 90 to 100% in broilers (Faridah et al., 2023). Antibiotic resistance of *E. coli* isolated from poultry has also been reported in other countries (Blaak et al., 2014; Zou et al., 2021; Khong et al., 2023). Furthermore, high MDR *E. coli* isolated from poultry has also been reported in Türkiye (82.83%) (Sever et al., 2022), Bangladesh (76%) (Mandal et al., 2022), Nepal (91.6%) (Bhattarai et al., 2024), and Nigeria (92.9%) (Aworh et al., 2020). More regional surveys reinforce the high ampicillin resistance observed in the present study. Veloo et al. (2025) reported ampicillin resistance in 76.1% of *E. coli* isolates from Malaysian poultry farm environments and found that resistance rates were significantly higher in broiler farm environments (91.3%) than in indigenous farm environments (64.6%), a pattern consistent with more intensive antimicrobial use in commercial broiler systems. Likewise, Rizal et al. (2024) reported a higher prevalence of multidrug-resistant and extended-spectrum β-lactamase-producing *E. coli* in broiler chickens than in local chickens sold at the same market in West Java, Indonesia. In an Indonesian traditional-market setting, Ferasyi et al. (2025) similarly found *E. coli* resistance to ampicillin reaching 100% across broiler, human, and environmental samples, reflecting the widespread and largely unregulated use of this antibiotic class in Southeast Asian poultry value chains.

Spearman's rank correlation between poultry type and antibiotic resistance showed that broilers were dominated by strong positive correlations, whereas ducks and native chickens were dominated by negative correlations. *E. coli* isolates from broilers tended to be resistant to seven of the antibiotics tested, except for AMC, which showed a negative correlation (r=-0.29), indicating that broiler isolates remained sensitive to amoxicillin-clavulanate. This suggests selection pressure from more intensive antibiotic use in broiler units than in other poultry. Isolates from native chickens and ducks showed negative values for most antibiotics, indicating that isolates from ducks and native chickens are relatively less likely to exhibit resistance than isolates from broiler units. This pattern of strong positive correlation among resistance traits in broilers, contrasted with weak or negative correlations in ducks and native chickens, mirrors findings from other production-system comparisons in the region. Using a similar correlation and co-existence network approach, Roy et al. (2025) found that *E. coli* isolates from Bangladeshi broiler flocks displayed extensive co-resistance linkages among unrelated antibiotic classes, which the authors attributed to shared selective pressure from concurrent and prophylactic antimicrobial use rather than resistance to a single drug class in isolation. Veloo et al. (2025) reported a comparable divergence between production systems, finding that the multiple antibiotic resistance index of *E. coli* recovered from broiler farm environments was roughly double that recovered from indigenous farm environments in Malaysia, indicating that intensively managed systems generate more tightly linked resistance profiles than extensive systems. Similarly, Mbatidde et al. (2024) linked the more frequent and structured antimicrobial use reported on semi-intensive Ugandan poultry farms to a broader, more coordinated resistance phenotype than that observed on free-range farms, where antimicrobial use was sporadic, and resistance traits appeared more randomly distributed.

Together, these findings support the interpretation that the strong positive correlations among resistance traits in broiler isolates in the present study reflect a shared, intensive selection pressure exerted by routine antimicrobial use in closed broiler housing, whereas the weaker and largely negative correlations in native chicken and duck isolates are consistent with the more heterogeneous, lower-intensity antimicrobial exposure typical of semi-intensive and open-range poultry systems. The negative correlation observed for amoxicillin-clavulanate resistance among broiler isolates further suggests that this antibiotic combination, which is less commonly used in Indonesian broiler production than first-line agents such as ampicillin and tetracycline, has not yet been subjected to comparable selective pressure (Bhattarai et al., 2024).

5. CONCLUSION

This study demonstrates that the variability in *E. coli* load and antibiotic resistance profiles is primarily determined by the biosecurity procedures and environmental management of each poultry unit. The duck unit with

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an open-water access system exhibited the highest and most homogeneous E. coli contamination across all sample matrices compared with the broiler and native chicken units. Interestingly, despite the duck unit harboring the highest bacterial load, the predominantly negative Spearman's correlation values indicate that the increase in bacterial population is not linearly related to resistance to the eight antibiotics tested. This suggests that environmental factors, specifically water and litter, are more dominant as pathogen-multiplying agents than the selective pressure exerted by antibiotics. Therefore, structural modifications to the drainage system of duck houses are urgently required to reduce the rate of *E. coli* proliferation, and strengthening antimicrobial stewardship must be prioritized in the broiler unit to prevent the emergence of multidrug-resistant (MDR) strains. Furthermore, future studies should expand the geographical scope to track resistance dynamics while developing environmentally adaptive biocontrol strategies using natural disinfectants to suppress pathogens without increasing the risk of MDR.

Declarations

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