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Research Article

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Antimicrobial Resistance in *Escherichia coli* from Market Birds in Surabaya: Potential Public Health Implications of Multidrug Resistance

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ABSTRACT

Background: Birds possess a wide flight range and high mobility, enabling them to disseminate microorganisms across diverse ecosystems. The bird trade in traditional markets further facilitates bacterial transmission through close contact among birds, humans, and the environment, posing potential public health risks.

Aims: This study aimed to determine the prevalence of *Escherichia coli*, as well as its antimicrobial resistance (AMR) and multidrug resistance (MDR) profiles, among birds traded at the Kupang Bird Market in Surabaya.

Methods: A cross-sectional study was conducted in 2025 using 40 cloacal swab samples collected from pigeons (*Columba livia*), zebra doves (*Geopelia striata*), Javanese turtle doves (*Streptopelia bitorquata*), and sooty-headed bulbuls (*Pycnonotus aurigaster*). Isolation and identification of *E. coli* were performed using MacConkey agar, Gram staining, and biochemical tests. Antimicrobial susceptibility against ampicillin, tetracycline, trimethoprim–sulfamethoxazole, aztreonam, chloramphenicol, and streptomycin was evaluated using the Kirby–Bauer disk diffusion method following CLSI guidelines.

Results: *Escherichia coli* was isolated from 82.5% (33/40) of the samples. The highest resistance rate was observed against tetracycline (33.3%), followed by ampicillin and streptomycin (6.0%), and trimethoprim–sulfamethoxazole (3.0%), whereas all isolates were susceptible to aztreonam and chloramphenicol. The predominant pattern was resistance to tetracycline alone. Additionally, one isolate (3.0%) was classified as MDR, exhibiting resistance to ampicillin, tetracycline, and trimethoprim–sulfamethoxazole.

Conclusion: This study demonstrates that birds traded at traditional markets harbor antimicrobial-resistant *E. coli*, representing a potential public health concern at the animal–human–environment interface. Continuous AMR surveillance in live bird markets is essential to support early detection and strengthen the implementation of the One Health approach.

INTRODUCTION

Birds are a group of animals characterized by high mobility and the ability to travel over vast distances. These characteristics enable them to play a significant role in the dispersal of microorganism, including both commensal and pathogenic organisms, across geographic regions and animal populations. Beyond wild birds, captive and traded birds also pose epidemiological risks because their frequent movement along trade routes facilitates the transmission of microorganisms between locations (Mbuthia & Hoza, 2025). Futhermore, birds can serve as potential reservoirs of antimicrobial-resistant bacteria, linking the wildlife, domestic animal,

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human, and environmental sectors within the One Health framework (Cooper-beknazarova et al., 2026).

Urban bird markets, including those in Surabaya, serve as trading hubs that bring together various bird species from breeders, captive breeding facilities, and different geographic regions into a single location. These conditions promote close interactions among birds, traders, buyers, and the surrounding environment, thereby increasing the likelihood of microorganism transmission. Factors such as high bird population density, the close proximity of cages, constant bird movement, and the accumulation of droppings within the trading area can contaminate cages, feed, drinking water, and environmental surfaces. In additional to increasing the risk of spreading infectious agents, these conditions also facilitate the dissemination of antimicrobial-resistant bacteria through direct contact and environmental contamination. Consequently, bird markets serve as an interface between animals, humans, and the environment, contributing to the transmission and dissemination of microorganism within the One Health framework (Adebowale et al., 2022a; Hasan et al., 2025).

Escherichia coli is a commensal bacterium that naturally inhabits the avian digestive tract. However, it is also recognized as an indicator bacterium for antimicrobial resistance surveillance because it reflects fecal contamination and serves as a reservoir for various antimicrobial resistance genes (Ramos et al., 2020). Its ability to acquire and transfer resistance determinants through plasmids and other mobile genetic elements enables *Escherichia coli* to readily develop antimicrobial resistance, including multidrug-resistant (MDR), thereby playing a significant role in the dissemination of antimicrobial resistance among animals, humans, and the environment (Koutsianos et al., 2020; Kuntaman et al., 2025; Newman et al., 2021).

Multidrug resistance (MDR) is a condition in which bacteria become resistant to multiple classes of antibiotics (Wibisono et al., 2021). The increasing prevalence of multidrug-resistant *Escherichia coli* is a global public health concern because it reduces the effectiveness of antibiotic therapy and increases the risk of disseminating resistant bacteria across the human-animal-environment interface. In Indonesia, research on antimicrobial resistance in *Escherichia coli* have primarily focused on food producing poultry, whereas information on MDR *Escherichia coli* isolated from birds traded in bird markets remains very limited. As gathering points for various bird species originating from different geographic regions, bird markets may facilitate the circulation of antimicrobial resistant bacteria and promote their dissemination. Therefore, this study aimed to identify the presence of *Escherichia coli*, determine antimicrobial resistance profiles, and evaluate the prevalence of multidrug resistance (MDR) among *Escherichia coli* isolates obtained from cloacal swabs of birds traded at the Kupang Bird Market in Surabaya, thereby providing baseline data to support One Health based antimicrobial resistance surveillance.

MATERIALS AND METHODS

Study design

This observational study employed a cross-sectional design and was conducted in November 2025 at the Kupang Bird Market in Surabaya, East Java, Indonesia. Bacterial isolation, identification, and antimicrobial susceptibility testing were performed at the Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Wijaya Kusuma University Surabaya.

Determination of Sample Size and Sampling

This study employed a purposive sampling method targeting bird species commonly traded at the Kupang Bird Market in Surabaya. A total of 40 cloacal swab samples were collected, comprising 10 samples each from rock pigeons (*Columba livia*), zebra doves (*Geopelia striata*), Javanese turtle doves (*Streptopelia bitorquata*), and sooty-headed bulbuls (*Pycnonotus aurigaster*). An equal number of samples was collected from each species to ensure balanced representation of the four target species for *Escherichia coli* isolation and identification, as well as antimicrobial resistance profiling. The sample size was selected to provide an initial exploratory assessment of the occurrence of *E. coli* and antimicrobial resistance among the selected bird species traded at the study site, rather than to provide a population-level estimate for all birds traded in Surabaya. The samples were obtained from birds sold by different vendors at the Kupang Bird Market.

The inclusion criteria comprised birds that appeared clinically healthy, belonged to the target species, and could be safely sampled via cloacal swabbing. Birds showing signs of severe illness or those from which cloacal swabs could not be safely collected were excluded from the study. Cloacal samples were collected aseptically using sterile cotton swabs inserted into the cloaca and immediately placed in tubes containing Buffered Peptone Water (BPW) as a transport medium. All samples were transported in a cooler maintained at approximately 4°C to the Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Wijaya Kusuma University Surabaya, where bacterial isolation was performed (Candra et al., 2022; Wibisono et al., 2021).

Isolation and Identification of *Escherichia coli*

Cloacal swab samples in Buffered Peptone Water (BPW) were streaked onto MacConkey agar (MCA) plates and incubated aerobically at 37°C for 18–24 hours. Colonies showing typical lactose fermenting characteristics (pink colonies) were selected for further identification by Gram staining and biochemical tests, including Triple Sugar Iron Agar (TSIA), Sulfide Indole Motility (SIM), Simmons Citrate Agar (SCA), Methyl Red (MR), and Voges–Proskauer (VP) tests, following standard microbiological procedures (Kendek et al., 2024; Wibisono et al., 2022).

Antimicrobial Susceptibility Testing and Detection of Multidrug Resistance

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method on Mueller –Hinton Agar (MHA) in accordance with the Clinical and Laboratory Standards Institute guidelines (CLSI, 2024). Bacterial suspensions were adjusted to a 0.5 McFarland turbidity standard before being inoculated onto MHA plates and tested against ampicillin (10 µg; penicillin), tetracycline (30 µg; tetracycline), trimethoprim–sulfamethoxazole (25 µg; folate pathway inhibitor), aztreonam (30 µg; monobactam), chloramphenicol (30 µg; phenicol), and streptomycin (10 µg; aminoglycoside). Following incubation at 37°C for 18–24 h, the diameters of the inhibition zones were measured and interpreted as susceptible, intermediate, or resistant according to CLSI criteria. Isolates exhibiting resistance to at least one antimicrobial agent in three or more antimicrobial classes were classified as multidrug-resistant (MDR). The frequency and resistance patterns of MDR isolates were subsequently analyzed (CLSI, 2024).

Data Analysis

Data were analyzed descriptively and are presented as frequencies (n) and percentages (%) to describe the prevalence of *Escherichia coli*, antimicrobial resistance profiles, and the occurrence of multidrug resistance (MDR).

RESULTS AND DISCUSSION

Isolation and Identification of *Escherichia coli*

Isolation and identification revealed that 33 of 40 (82.5%) cloacal swab samples collected from birds were positive for *Escherichia coli*, whereas 7 (17.5%) samples were negative (Table 1). Among the bird species examined, the highest prevalence of *Escherichia coli* was observed in *Perkutut* (Zebra Dove), *Puter Pelung* (Javanese Turtle Dove), and *Kutilang* (Sooty-headed Bulbul), each with a prevalence 90% (9/10). In contrast, rock pigeons showed the lowest prevalence at 60% (6/10). The overall prevalence of *Escherichia coli* (82.5%) observed in cloacal swabs from birds traded at the Kupang Bird Market in Surabaya indicates that this bacterium is commonly present in the intestinal microbiota of traded birds. As a commensal bacterium, *Escherichia coli* may contribute to fecal contamination of the market environment through fecal shedding, thereby representing a potential source of bacterial dissemination. The prevalence observed in the present study is comparable to that reported by Rizal et al. (2024), who detected *Escherichia coli* in 76.5% of cloacal swab samples collected from chickens sold in traditional markets in Indonesia, suggesting that *Escherichia coli* is widely distributed among birds marketed in traditional trading systems.

The variation in *Escherichia coli* prevalence among bird species, ranging from 60% in rock pigeons to 90% in zebra doves, Javanese turtle doves, and sooty-headed bulbuls, suggests that colonization by this bacterium may be influenced by biological characteristics and husbandry related factors, such as the birds' origin, stocking density, and exposure to fecally contaminated environments. Although these specific factors were not evaluated in the present study, the consistently high prevalence of *Escherichia coli* across all bird species indicates that this bacterium is widely among birds traded at the market. These findings warrant attention because bird markets serve as convergence points where multiple bird species from different geographic regions are housed in close proximity, increasing the

Table 1. Results of *Escherichia coli* isolation and identification from bird cloacal swabs

Bird	Sample	*Escherichia coli*
Columba livia (Merpati)	10	60% (6/10)
Geopelia striata (Perkutut)	10	90% (9/10)
Streptopelia bitorquata (Puter-pelung)	10	90% (9/10)
Pycnonotus aurigaster (Kutilang)	10	90% (9/10)
Total	40	82,5% (33/40)

potential for bacterial transmission and environmental contamination through fecal shedding. Within the One Health framework, such markets may facilitate the circulation of bacteria among animals, humans, and the environment. Although *Escherichia coli* is a normal component of the avian intestinal microbiota, it is widely recognized as an indicator organism for fecal contamination and antimicrobial resistance surveillance. Therefore, the high prevalence of *Escherichia coli* observed in traded birds highlights the importance of continuous surveillance to support the early detection of antimicrobial resistant bacteria and strengthen veterinary public health and One Health surveillance programs (Ahmed & Gulhan, 2024; Cooper-beknazarova et al., 2026).

Colonies of *Escherichia coli* isolated on MacConkey Agar (MCA) (Figure 1) appeared pink, round, discrete, and dry, indicating lactose fermentation. Gram staining revealed short, pink to red, rod-shaped Gram-negative bacteria. Biochemical identification showed reactions characteristics of *Escherichia coli***:** Triple Sugar Iron Agar (TSIA) showed an acid slant and acid butt (A/A) with gas production but no hydrogen sulfide (H_2S) production; Simmons Citrate Agar (SCA) was negative; Sulfide Indole Motility (SIM) medium showed positive indole production and motility but negative H_2S production; Methyl Red (MR) was positive; and Voges–Proskauer (VP) was negative.

The colony characteristics observed on MacConkey agar, together with the Gram staining and biochemical test results, were consistent with the phenotypic characteristics of *Escherichia coli* reported in previous studies. The pink colonies on MacConkey agar indicated lactose fermentation, whereas the Gram staining and biochemical profiles (TSIA, SCA, SIM, MR, and VP) confirmed the identity of the isolates as a *Escherichia coli*, a Gram-negative member of the family *Enterobacteriaceae*. These findings demonstrate that conventional culture based methods remain reliable for the isolation and preliminary identification of *Escherichia coli* from avian cloacal swab samples before antimicrobial susceptibility testing (Jomehzadeh et al., 2022).

Antimicrobial Resistance Profile of *Escherichia coli*

Antimicrobial susceptibility testing of 33 *Escherichia coli* isolates showed the highest resistance to tetracycline at 33.3% (11/33), followed by ampicillin and streptomycin at 6.0% (2/33) and trimethoprim-sulfamethoxazole at 3.0% (1/33). No resistance to aztreonam and chloramphenicol was detected. Most isolates remained susceptible to ampicillin (93.9%), trimethoprim-sulfamethoxazole (97.0%), and aztreonam (100%) (Table 2). The predominance of tetracycline resistance suggests that this antimicrobial continues to exert selective pressure on commensal *Escherichia coli* populations circulating in traded birds. In contrast, resistance to ampicillin and trimethoprim-sulfamethoxazole remained relatively low, while all isolates were susceptible to aztreonam. These findings are consistent with previous studies in wild and captive bird, which identified tetracycline as one of the antimicrobial agents with the highest resistance rates in *Escherichia coli*, likely reflecting its extensive and long-term use in animal production systems (Afayibo et al., 2022; Ahmed & Gulhan, 2024; Wibisono et al., 2020).

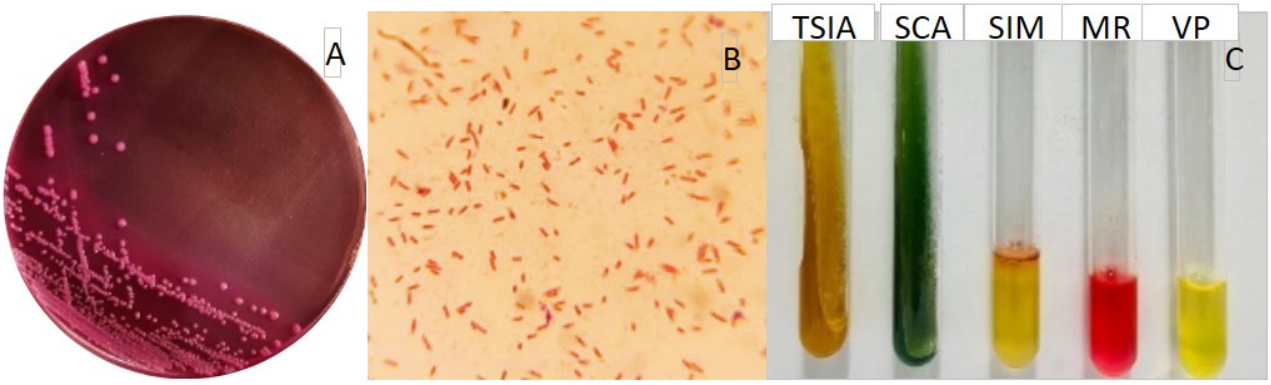


Figure 1. Isolation and identification of *Escherichia coli*: (A) MacConkey Agar (MCA); (B) Gram staining; (C) biochemical tests using Triple Sugar Iron Agar (TSIA), Simmons Citrate Agar (SCA), Sulfide Indole Motility (SIM), Methyl Red (MR), and Voges–Proskauer (VP) media.

Across the bird species examined, tetracycline resistance was detected in isolates from all four species, whereas ampicillin resistance was identified only in isolates from zebra doves and sooty-headed bulbuls. Resistance to trimethoprim-sulfamethoxazole was detected in a single isolate from a sooty-headed bulbul (Table 2). The relatively low prevalence of resistance to ampicillin and trimethoprim-sulfamethoxazole, together with the absence of aztreonam-resistant isolates, suggests that exposure to these antimicrobial agents remains uncommon among *Escherichia coli* isolates recovered from traded birds. However, the presence of tetracycline-resistant isolates warrants attention because *Escherichia coli* is recognized as an indicator bacterium capable of acquiring and disseminating antimicrobial resistance genes through plasmids and other mobile genetic elements. Bird markets, where birds from different geographic regions are housed in close proximity, may facilitate the circulation of antimicrobial resistant bacteria and resistance determinants among animals, humans, and the environment. Therefore, continuous antimicrobial resistance surveillance in traded birds is essential to support the implementation of the One Health approach (Ahmed & Gulhan, 2024).

Antimicrobial susceptibility testing using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (MHA) demonstrated variations in the diameters of inhibition zones surrounding the antibiotic disks (Figure 2). Most antibiotic disks produced clear inhibition zones, whereas one disk produced either a very small or no inhibition zone, indicating resistance of the *Escherichia coli* isolate according to the Clinical and Laboratory Standards Institute (CLSI) criteria. The diameter of the inhibition zone reflects the susceptibility of *Escherichia coli* to the tested

Table 2. Antimicrobial resistance profile of *Escherichia coli* isolates

Sample	*Escherichia coli*	Antibiotic (%)					
		AMP	TE	SXT	ATM	C	S
Columba livia (Merpati)	6/10 (60%)	0/6 (0%)	1/6 (16,7%)	0/6 (0%)	0/6 (0%)	0/6 (0%)	0/6 (0%)
Geopelia striata (Perkutut)	9/10 (90%)	1/9 (11,1%)	5/9 (55,6%)	0/9 (0%)	0/9 (0%)	0/9 (0%)	1/9 (11,1%)
Streptopelia bitorquata (Puter-pelung)	9/10 (90%)	0/9 (0%)	2/9 (22,2%)	0/9 (0%)	0/9 (0%)	0/9 (0%)	0/9 (0%)
Pycnonotus aurigaster (Kutilang)	9/10 (90%)	1/9 (11,1%)	3/9 (33,3%)	1/9 (11,1%)	0/9 (0%)	0/9 (0%)	1/9 (11,1%)
Total	33/40 (82,5%)	2/33 (6%)	11/33 (33,3%)	1/33 (3%)	0/33 (0%)	0/33 (0%)	2/33 (6%)

Note: AMP: Ampicillin, TE: Tetracycline, SXT: Sulfamethoxazole-trimethoprim, ATM: Aztreonam, C: Chloramphenicol, S: Streptomycin, R: Resistant; I: Intermediate; S: Susceptible.

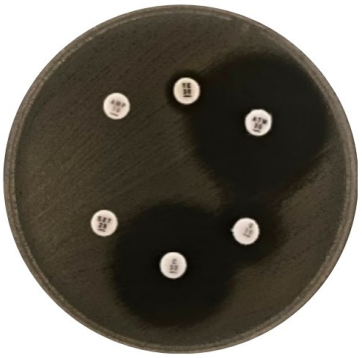


Figure 2. Antimicrobial susceptibility test of *Escherichia coli* isolate obtained from birds traded at Kupang Bird Market, Surabaya, using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar (MHA). AMP = ampicillin (10 µg); TE = tetracycline (30 µg); SXT = trimethoprim–sulfamethoxazole (25 µg); ATM = aztreonam (30 µg); C = chloramphenicol (30 µg); S = streptomycin (10 µg).

antimicrobial agents, with larger zones indicating greater susceptibility and smaller or absent zones indicating resistance. The Kirby-Bauer disk diffusion method performed on MHA is a standardized and widely accepted technique for antimicrobial susceptibility testing and is routinely used to determine bacterial resistance profiles in accordance with Clinical and Laboratory Standards Institute guidelines (CLSI, 2024; Normaliska et al., 2019; Widhi & Saputra, 2021).

Occurrence of Multidrug Resistance (MDR) in *Escherichia coli*

Among the 33 *Escherichia coli* isolates, one isolate (BK3; 3.0%) was classified as multidrug-resistant (MDR), exhibiting resistance to ampicillin, tetracycline, and trimethoprim-sulfamethoxazole while remaining susceptible to aztreonam. Twelve additional isolates showed resistance to a single antimicrobial class and were therefore classified as non-MDR. The most common resistance pattern was resistance to tetracycline alone, followed by resistance to ampicillin alone (Table 3). The predominance of single drug resistance indicates that most resistant isolates had not yet acquired resistance to multiple antimicrobial classes. However, the detection of an MDR isolate is of concern because *Escherichia coli* can act as a reservoir of antimrobial resistance genes that may be disseminated through horizontal gene transfer. Bird markets, where birds from different sources are housed in close proximity, may facilitate the circulation of antimicrobial resistant bacteria and resistance determinants among animals, humans, and the environment. Therefore, continuous antimicrobial resistance surveillance in traded birds is essential to support the implementation of the One Health approach and enable the early detection of emerging resistance (Adebowale et al., 2022b; Athanasakopoulou et al., 2021).

Based on the antimicrobial susceptibility test results, one of the 33 *Escherichia coli* isolates (3.0%) was classified as multidrug resistant (MDR). The MDR isolate originated from a sooty-headed bulbul, corresponding to an MDR prevalence of 11.1% (1/9) among *Escherichia coli* isolates recovered from this species (Table 3). The isolate (BK3) exhibited resistance to ampicillin (β-lactams), tetracycline (tetracycline class), and trimethoprim-sulfamethoxazole (antifolate), thereby fulfilling the MDR criterion of resistance to at least one antimicrobial agent in three different antimicrobial classes. Although MDR was detected in only a single isolate, its presence demonstrates that birds traded in traditional bird markets may harbor *Escherichia coli* strains resistant to multiple antimicrobial classes. As a commensal

Table 3. Antibiotic resistance patterns

Sample code	Antibiotic						Resistance Pattern	MDR
	AMP	TE	SXT	ATM	C	S		
BM 7	S	R	S	S	S	S	TE	Non MDR
BP 2	S	R	S	S	S	S	TE	Non MDR
BP 3	S	S	S	S	S	R	S	Non MDR
BP 4	S	R	S	S	S	S	TE	Non MDR
BP 6	S	R	S	S	S	S	TE	Non MDR
BP 7	S	R	S	S	S	S	TE	Non MDR
BP 9	R	S	S	S	S	S	AMP	Non MDR
BP 10	S	R	S	S	S	S	TE	Non MDR
BPP 3	S	R	S	S	S	S	TE	Non MDR
BPP 9	S	R	S	S	S	S	TE	Non MDR
BK 3	R	R	R	S	S	S	AMP/TE/SXT	MDR
BK 7	S	R	S	S	S	S	TE	Non MDR
BK 8	S	S	S	S	S	R	S	Non MDR
BK 9	S	R	S	S	S	S	TE	Non MDR

Note: AMP: Ampicillin, TE: Tetracycline, SXT: Sulfamethoxazole-trimethoprim, ATM: Aztreonam, C: Chloramphenicol, S: Streptomycin, MDR: Multidrug Resistance, R: Resistant; I: Intermediate; S: Susceptible. BM: Burung Merpati – rock pigeons, BP: Burung Perkutut - Zebra Dove, BPP: Burung Puter Pelung - Javanese Turtle Dove, BK: Burung Kutilang - Sooty-headed Bulbul.

bacterium, *Escherichia coli* can act as a reservoir of antimicrobial resistance genes that may be disseminated to other bacteria through plasmids and other mobile genetic elements, highlighting the potential of traded birds in the spread of antimicrobial resistance within the One Health interface (Mandal et al., 2022; Michaelis & Grohmann, 2023; Putri et al., 2023).

This study has several limitations, particularly the relatively small sample size of 40 birds and the use of a purposive sampling approach, which may limit the representativeness and generalizability of the findings to the broader population of birds traded in Surabaya. Therefore, the observed prevalence of *E. coli* and antimicrobial resistance should be interpreted as an initial assessment of the selected bird species at the study site rather than as a population-level estimate. Further studies involving larger sample sizes, probability-based sampling, and multiple bird markets are warranted to confirm and extend these findings.

CONCLUSION

This study demonstrated a high prevalence of *Escherichia coli* among birds traded at the Kupang Bird Market, Surabaya, with tetracycline resistance being the predominant antimicrobial resistance pattern and the detection of multidrug-resistant (MDR) isolates. These findings indicate that traded birds may carry antimicrobial-resistant *E. coli* and highlight the importance of continuous One Health-based antimicrobial resistance surveillance in bird markets.

AUTHORS CONTRIBUTION

F.J.W. Conceptualization, study design, data curation, formal analysis, data interpretation, and writing of the original draft. F.M.W. Data curation, formal analysis, review and editing of the manuscript. B.B.M.N. Sample collection, laboratory investigation, antimicrobial susceptibility testing, and data acquisition. All authors have read, critically reviewed, and approved the final version of the manuscript.

"The authors declare that there is no conflict of interest with the parties involved in this research".

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