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Molecular identification of the *bla*$_{\text{TEM}}$ gene in *Escherichia coli* isolated from quail in Surabaya

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ABSTRACT

Antimicrobial resistance (AMR) represents a major global public health concern driven in part by extensive antibiotic use in food-producing animals. Poultry production, including quail farming, plays a significant role in the dissemination of resistant bacteria due to intensive management systems and frequent antimicrobial exposure. *Escherichia coli*, a common intestinal commensal and opportunistic pathogen, is widely used as an indicator organism for monitoring AMR, particularly extended-spectrum β-lactamase (ESBL) production mediated by genes such as *bla*$_{\text{TEM}}$. However, data on ESBL-associated resistance in quails marketed in Indonesia remain limited. This study aimed to detect the presence of the *bla*$_{\text{TEM}}$ gene in *E. coli* isolated from quails sold in traditional markets in Surabaya, Indonesia. A total of 150 cloacal swab samples were collected from five traditional markets between November and December 2024. Isolation and identification of *E. coli* were performed using conventional bacteriological and biochemical methods. Antimicrobial susceptibility testing was conducted using the Kirby–Bauer disk diffusion method in accordance with CLSI guidelines. Polymerase chain reaction (PCR) was used to detect the *bla*$_{\text{TEM}}$ gene among aztreonam-resistant isolates. *E. coli* was isolated from 148 of 150 samples (99%). The highest resistance rates were observed against ciprofloxacin (33.1%), tetracycline (22.2%), and aztreonam (13.5%), while resistance to kanamycin and chloramphenicol remained low. Multidrug-resistant (MDR) *E. coli* was identified in 2.7% of isolates. PCR analysis revealed the presence of the *bla*$_{\text{TEM}}$ gene in five aztreonam-resistant isolates, confirming the circulation of ESBL-associated resistance. These findings indicate that quails marketed in traditional markets may serve as reservoirs of antimicrobial-resistant *E. coli*. Continuous surveillance, prudent antimicrobial use, and improved hygiene practices are essential to mitigate the spread of ESBL-producing bacteria within a One Health framework.

Introduction

Antimicrobial resistance (AMR) has emerged as one of the most critical global public health challenges, threatening the effective prevention and treatment of bacterial infections in both humans and animals (Al-Khalaifah *et al.*, 2025). The widespread and often unregulated use of antibiotics in food-producing animals has been identified as a major driver of the emergence and dissemination of resistant bacteria (Enshaie *et al.*, 2025). In particular, the poultry sector plays a significant role in the epidemiology of AMR due to intensive farming practices, high animal densities, and frequent antimicrobial exposure (Elbehiry and Marzouk, 2025). These conditions facilitate the selection and spread of resistant bacteria along the food production chain and into the environment (Widodo et al., 2023; Rahayu *et al.*, 2025).

Escherichia coli is a commensal bacterium commonly found in the intestinal tract of humans and animals; however, it is also recognized as an important opportunistic pathogen (Foster-Nyarko and Pallen, 2022). Because of its ability to readily acquire and disseminate resistance genes through horizontal gene transfer, *E. coli* is widely used as an indicator organism for monitoring antimicrobial resistance (Aabed *et al.*, 2021). Of particular concern is the emergence of *E. coli* strains producing extended-spectrum β-lactamases (ESBLs), enzymes capable of hydrolyzing a wide range of β-lactam antibiotics, including penicillins and third-generation cephalosporins (Husna *et al.*, 2023). ESBL-producing *E. coli* pose a serious threat to both veterinary and human medicine, as they limit therapeutic options and increase the risk of treatment failure (Ansharieta et al., 2021; Woerde *et al.*, 2023).

Among the various ESBL-encoding genes, the *bla*$_{\text{TEM}}$ gene remains one of the most frequently reported worldwide (Effendi *et al.*, 2022). Initially described in clinical isolates, *bla*$_{\text{TEM}}$ has since been widely detected in bacteria originating from food animals, retail meat, and environmental sources (Telli *et al.*, 2025). The dissemination of *bla*$_{\text{TEM}}$-harboring *E. coli* in food-producing animals is of particular concern, as these bacteria can be transmitted to humans through direct contact, contaminated food products, or environmental pathways (Ahadini *et al.*, 2025). Molecular detection of resistance genes such as *bla*$_{\text{TEM}}$ is therefore essential to better understand the epidemiology of ESBL-producing bacteria and to support effective AMR surveillance strategies (Mustika *et al.*, 2024).

Quail (*Coturnix coturnix*) production has increased substantially in Indonesia due to high consumer demand, rapid growth rates, and relatively low production costs (Oke *et al.*, 2025). Quails are commonly marketed through traditional markets, where biosecurity measures are often limited and sanitary conditions may be suboptimal (Jansson *et al.*, 2025). These environments may facilitate the persistence and spread of antimicrobial-resistant bacteria, including ESBL-producing *E. coli* (Martinez-Laorden *et al.*, 2023). Despite the growing importance of quail production, data on antimicrobial resistance and ESBL-associated genes in quail-derived bacterial isolates remain limited, particularly in Indonesia (Hedman *et al.*, 2020).

Surabaya, one of the largest metropolitan cities in Indonesia, hosts numerous traditional markets that serve as major distribution points for poultry products (Sumiati *et al.*, 2025). The close interaction between live

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animals, vendors, and consumers in these markets increases the potential risk of zoonotic transmission of resistant bacteria (Hasan *et al*., 2025). Surveillance of ESBL-producing *E. coli* in quails marketed in Surabaya is therefore crucial from both veterinary and public health perspectives, especially within the One Health framework that recognizes the interconnectedness of human, animal, and environmental health (Rahayu *et al*., 2025).

This study aimed to molecularly identify the presence of the bla_{TEM} gene in *Escherichia coli* isolated from quail cloacal swabs collected from traditional markets in Surabaya. By combining conventional bacteriological methods with polymerase chain reaction (PCR) analysis, this research provides valuable insights into the occurrence of bla_{TEM}-harboring *E. coli* in quail populations. The findings are expected to contribute to the existing knowledge on antimicrobial resistance in food-producing animals and to support the development of appropriate control and prevention strategies to mitigate the spread of ESBL-producing bacteria.

Materials and methods

Ethical approval

Ethical clearance for this research was granted by the Animal Ethics Committee, Faculty of Veterinary Medicine, Universitas Wijaya Kusuma Surabaya, Indonesia (Approval No. 170-KKE-2025).

Study design

The study was carried out between November and December 2024 using a total of 150 cloacal swab samples collected from quails obtained from five traditional markets in Surabaya, Indonesia, namely Turi, Bratang, Cemara Pabean, Kupang, and Benowo. Sample collection was performed using a random sampling approach. This research employed an exploratory and observational laboratory-based design and was conducted at the Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Universitas Wijaya Kusuma Surabaya.

The culture media utilized included Eosin Methylene Blue Agar (EMBA; Oxoid CM0069), Triple Sugar Iron Agar (TSIA; Himedia M021), Simmons Citrate Agar (SCA; Himedia M099), Sulfide Indole Motility (SIM; Himedia M181), Methyl Red (MR; Himedia GM070), Voges–Proskauer (VP; Himedia GM070), and Mueller–Hinton Agar (MHA; Himedia M173).

Isolation and identification of *Escherichia coli*

Cloacal swab samples were initially enriched in Buffered Peptone Water (BPW) prior to inoculation onto EMBA plates. EMBA is a selective and differential medium containing peptone, lactose, sucrose, eosin, and methylene blue. The presence of methylene blue inhibits Gram-positive bacteria, facilitating selective growth of Gram-negative enteric organisms. In addition, eosin and methylene blue function as pH indicators, while lactose and sucrose act as fermentable carbohydrates to differentiate coliform bacteria.

Colonies exhibiting a characteristic green metallic sheen on EMBA were presumptively identified as *E. coli*. These isolates were subsequently subjected to Gram staining and a series of biochemical assays, including TSIA, SCA, SIM, and MR–VP tests, to confirm their identity.

Antimicrobial susceptibility testing

Antibiotic susceptibility was assessed using the Kirby–Bauer disk diffusion method in combination with the well diffusion technique. The diameters of inhibition zones were measured and interpreted in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2020).

The antibiotics evaluated included aztreonam, ciprofloxacin, tetracycline, erythromycin, and streptomycin. Based on CLSI criteria, isolates were classified as susceptible, intermediate, or resistant (Widodo *et al*., 2022). Isolates demonstrating resistance to three or more antimicrobial classes were defined as multidrug-resistant (MDR) (Khairullah *et al*., 2023).

Polymerase Chain Reaction (PCR)

PCR analysis was conducted to detect the presence of resistance-related genes in *E. coli* isolates. PCR is a molecular technique that amplifies specific DNA sequences using the thermostable Taq DNA polymerase through repeated cycles of denaturation, annealing, and extension. The enzyme's thermal stability, derived from Thermus aquaticus, allows efficient DNA amplification under high-temperature conditions, making PCR suitable for bacterial gene detection and molecular screening.

Two sets of specific primers were employed. The primer pair consisting of Forward (5′-ATGAGTATTCAACATTTCCG-3′) and Reverse (5′-CTGACAGTTACCAATGCTTA-3′) amplified an 867 bp target fragment with an annealing temperature of 57°C. PCR products were analyzed by agarose gel electrophoresis to confirm the presence of the bla_{TEM} gene (Ahadini *et al*., 2025).

Results

A total of 150 cloacal swab samples obtained from quails in five traditional markets in Surabaya were analyzed to determine the presence of *E. coli*. Of these, *E. coli* was successfully isolated from 148 samples, corresponding to a prevalence of 99%. Complete positivity (30/30) was observed in samples from the Turi, Bratang, and Cemara Pabean markets, whereas slightly lower detection rates were recorded in Kupang and Benowo, each with 97% positivity (29/30) and one negative sample (Table 1). On Eosin Methylene Blue Agar (EMBA), presumptive *E. coli* colonies displayed a characteristic metallic green sheen, indicative of lactose fermentation (Figure 1). Gram staining confirmed that the isolates were Gram-negative bacilli, as evidenced by their pink rod-shaped appearance (Figure 2).

Table 1. *E. coli* isolation and identification table.

Traditional market	Number of samples	*E. coli* (%)	
		Positive	Negative
Turi	30	100% (30/30)	0% (0/30)
Bratang	30	100% (30/30)	0% (0/30)
Cemara Pabean	30	100% (30/30)	0% (0/30)
Kupang	30	97% (29/30)	3% (1/30)
Benowo	30	97% (29/30)	3% (1/30)
Total	150	99% (148/150)	1% (2/150)

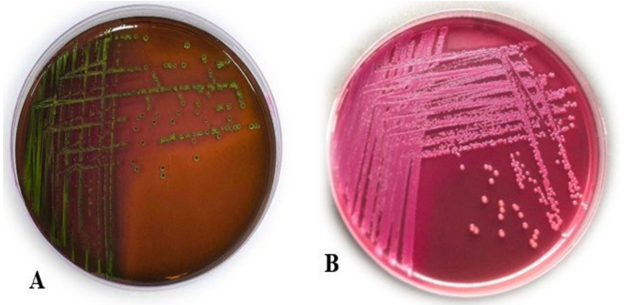

Figure 1. (A) *E. coli* bacterial colony on Eosin Methylene Blue Agar (EMBA) media; (B) *E. coli* bacterial colony on Mac Conkey Agar (MCA) media.

Biochemical characterization of the cloacal swab isolates demonstrated typical *E. coli* profiles. On Triple Sugar Iron Agar (TSIA), all isolates produced a color shift from red to yellow, reflecting the fermentation of glucose, lactose, and sucrose, with gas production observed and no hydrogen sulfide (H_2S) formation. The Simmons Citrate Agar (SCA) test yielded negative results, while the Sulfide Indole Motility (SIM) assay was positive for indole production, as indicated by the formation of a red ring following the addition of Kovach's reagent. The Methyl Red (MR) test was positive, whereas the Voges–Proskauer (VP) test remained negative (Figure 3). Collectively, these biochemical findings confirmed the identity of the isolates as *E. coli*.

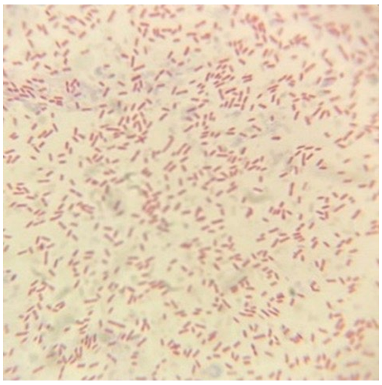

Figure 2. Microscopic examination of *E. coli* with Gram staining.

Figure 3. Biochemical test on *E. coli* bacterial isolates.

Antimicrobial susceptibility testing was performed on all 148 *E. coli* isolates against five commonly used antibiotics, revealing variable resistance patterns among markets (Table 2). Overall, the highest resistance rate was detected against ciprofloxacin (33.1%; 49/148), followed by tetracycline (22.2%; 33/148) and aztreonam (13.5%; 20/148). In contrast, lower resistance frequencies were observed for kanamycin (6.0%; 9/148) and chloramphenicol (4.7%; 7/148).

Market-specific analysis showed that ciprofloxacin resistance was most prevalent in isolates from Kupang (55.1%) and Benowo (41.3%). Resistance to tetracycline was also commonly detected, particularly in Benowo (31%) and Kupang (27.5%). The highest proportion of aztreonam-resistant isolates was found in Cemara Pabean (20%) and Benowo (17.2%). Resistance to kanamycin and chloramphenicol remained relatively low across all sampling locations.

Assessment of multidrug resistance among the 148 isolates indicated that four isolates (2.7%) fulfilled the criteria for multidrug-resistant (MDR) *E. coli* (Table 3). MDR isolates were identified in samples from the Turi (3.3%; 1/30), Cemara Pabean (6.6%; 2/30), and Kupang (3.4%; 1/29) markets, while no MDR strains were detected in Bratang or Benowo.

The MDR isolates exhibited distinct resistance profiles to the five tested antibiotics (Table 4). Two isolates (PTU29 and PKU21) shared an identical resistance pattern to aztreonam, ciprofloxacin, and tetracycline (ATM/CIP/TE). One isolate (PCP25) was resistant to aztreonam, ciprofloxacin, and kanamycin (ATM/CIP/K). The most extensive resistance pattern was observed in isolate PCP26, which demonstrated resistance to all five antibiotics tested (ATM/CIP/TE/K/C), representing the highest level of multidrug resistance identified in this study. Representative MDR *E. coli* isolates are illustrated in Figure 4.

Figure 4. Antibiotic sensitivity test of *E. coli* isolates. (1) Aztreonam; (2) Tetracycline; (3) Kanamycin; (4) Ciprofloxacin; (5) Chloramphenicol.

Table 2. Identification of antibiotic resistance against *E. coli* bacteria.

Traditional market	Number of samples	Number of *E. coli*	ATM		CIP		TE		K		C	
			R	%	R	%	R	%	R	%	R	%
Turi	30	30	5	16.6% (5/30)	8	26.6% (8/30)	7	23.3% (7/30)	1	3.3% (1/30)	1	3.3% (1/30)
Bratang	30	30	3	10% (3/30)	7	23.3% (7/30)	6	20% (6/30)	4	13.3% (4/30)	0	0% (0/30)
Cemara Pabean	30	30	6	20% (6/30)	6	20% (6/30)	3	10% (3/30)	3	10% (3/30)	2	6.6% (2/30)
Kupang	30	29	1	3.4% (1/29%)	16	55.1% (16/29)	8	27.5% (8/29)	1	3.4% (1/29%)	2	6.8% (2/29)
Benowo	30	29	5	17.2% (5/29)	12	41.3% (12/29)	9	31% (9/29)	0	0% (0/29)	2	6.8% (2/29)
Total	150	148	20	13.5% (20/148)	49	33.1% (49/148)	33	22.2% (33/148)	9	6% (9/148)	7	4.7% (7/148)

Note: Aztreonam (ATM), Ciprofloxacin (CIP), Tetracycline (TET), Kanamycin (K), Chloramphenicol (C), Resistant (R)

Table 3. Multidrug resistance test results on *E. coli*.

Traditional market	Number of *E. coli*	Multidrug resistant (MDR)	Percentage (%)
Turi	30	1	3.3% (1/30)
Bratang	30	0	0% (0/30)
Cemara Pabean	30	2	6.6% (2/30)
Kupang	29	1	3.4% (1/29)
Benowo	29	0	0% (0/29)
Total	148	4	2.7% (4/148)

Isolates exhibiting resistance to aztreonam were subsequently subjected to Polymerase Chain Reaction (PCR) analysis to detect the presence of the bla_{TEM} gene. Among the 20 aztreonam-resistant *E. coli* isolates, five were confirmed to harbor the bla_{TEM} gene. Positive amplification results are presented in Figures 5 and 6.

Table 4. Resistance patterns of MDR *E. coli*.

Sample code	Antibiotic resistance					Resistance pattern
	ATM	CIP	TE	K	C	
PTU29	R	R	R	S	S	ATM/CIP/TE
PKU21	R	R	R	S	S	ATM/CIP/TE
PCP25	R	R	S	R	S	ATM/CIP/K
PCP26	R	R	R	R	R	ATM/CIP/TE/K/C

Note: Aztreonam (ATM), Ciprofloxacin (CIP), Tetracycline (TET), Kanamycin (K), Chloramphenicol (C), Resistant (R), Sensitive (S)

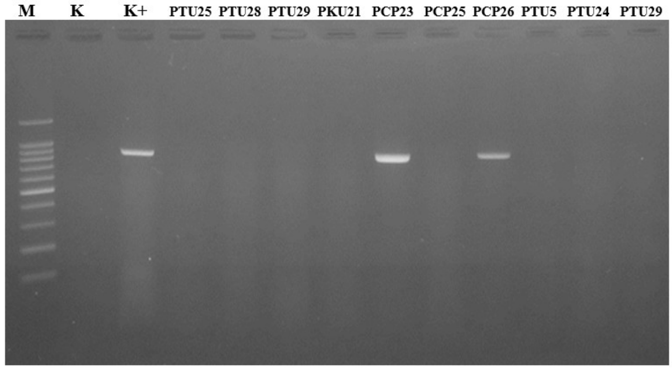

Figure 5. PCR results of the bla_{TEM} gene in Aztreonam-resistant *E. coli* isolates.

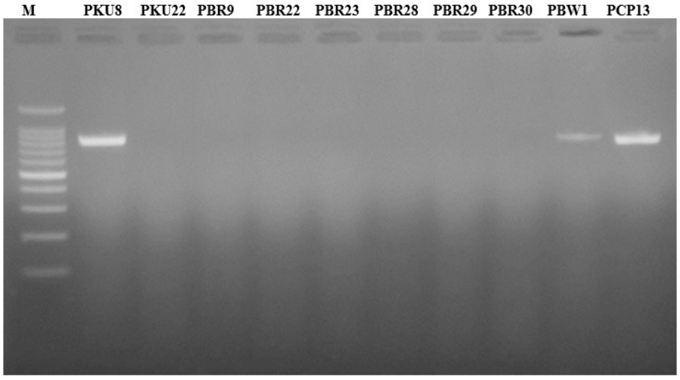

Figure 6. PCR results of the bla_{TEM} gene in Aztreonam-resistant *E. coli* isolates.

Discussion

The present study demonstrates a remarkably high prevalence of *E. coli* isolated from cloacal swabs of quails obtained from traditional markets in Surabaya, with a detection rate of 99%. This finding is consistent with the biological nature of *E. coli* as a dominant commensal bacterium inhabiting the intestinal tract of poultry (Abdelhamid *et al*., 2024). Similar high isolation rates of *E. coli* from cloacal samples have been reported in various poultry species, reflecting its ubiquitous presence in the avian gastrointestinal system (Agusi *et al*., 2024; Nguyen *et al*., 2021). However, the near-universal detection observed in this study may also reflect suboptimal hygienic conditions during quail handling, transport, and marketing in traditional market settings (Martinez-Laorden *et al*., 2023).

The consistent isolation of *E. coli* across all sampled markets suggests widespread bacterial colonization among quails sold in Surabaya. Traditional markets often involve close contact between live birds, shared cages, and limited sanitation, which may facilitate fecal contamination and bacterial transmission (Rahayu *et al*., 2025). These conditions not only promote the persistence of commensal bacteria but may also contribute to the dissemination of antimicrobial-resistant strains, posing potential risks to both animal and public health (Sparaciari *et al*., 2025).

Biochemical characterization confirmed that all isolates exhibited typical *E. coli* phenotypes, including lactose fermentation, indole production, and mixed-acid fermentation. The uniformity of biochemical profiles across isolates supports the reliability of the identification process and indicates that the recovered bacteria represent classical *E. coli* rather than atypical coliforms (Ema *et al*., 2022). This confirmation is important, as accurate species identification is essential for interpreting antimicrobial resistance data and subsequent molecular findings (Boulmaiz *et al*., 2025).

Antimicrobial susceptibility testing revealed variable resistance patterns among the isolates, with the highest resistance observed against ciprofloxacin, followed by tetracycline and aztreonam. Resistance to fluoroquinolones such as ciprofloxacin is of particular concern, as these antibiotics are classified as critically important antimicrobials for human medicine (Cardoso *et al*., 2025). The relatively high ciprofloxacin resistance detected in this study may reflect the frequent or inappropriate use of fluoroquinolones in poultry production, either for therapeutic or prophylactic purposes (Oxinou *et al*., 2025). Similar resistance trends have been widely reported in poultry-associated *E. coli*, indicating a global pattern of fluoroquinolone resistance in food-producing animals (Zhang *et al*., 2025).

Tetracycline resistance was also common among the isolates, which is not surprising given the long-standing and widespread use of tetracyclines in livestock production due to their broad spectrum and low cost. The persistence of tetracycline resistance suggests ongoing selective pressure and highlights the challenge of reducing resistance to older antimicrobials that remain widely accessible (Semenova *et al*., 2025). In contrast, resistance to kanamycin and chloramphenicol was relatively low across all markets, possibly reflecting reduced usage of these antibiotics in contemporary poultry management (Abreu *et al*., 2023).

Although the overall prevalence of multidrug-resistant (MDR) *E. coli* was relatively low (2.7%), the detection of MDR isolates in multiple markets remains epidemiologically significant. The presence of MDR strains indicates that quails can serve as reservoirs for bacteria resistant to multiple antimicrobial classes (Liu *et al*., 2020). The absence of MDR isolates in some markets suggests variability in antimicrobial exposure or management practices, underscoring the influence of local factors on resistance development (Muteeb *et al*., 2023).

Notably, one MDR isolate exhibited resistance to all five tested antibiotics, representing an extreme resistance phenotype. Such isolates are of particular concern, as they may severely limit therapeutic options and have an increased potential for dissemination of resistance determinants (Chiș *et al*., 2022). The diversity of MDR resistance patterns observed in this study suggests that resistance in *E. coli* is not confined to a single antibiotic combination but may arise through multiple selective pathways (Dolou *et al*., 2025).

Molecular analysis revealed the presence of the bla_{TEM} gene in a subset of aztreonam-resistant *E. coli* isolates. This finding confirms that β-lactam resistance in quail-derived *E. coli* is, at least in part, mediated by ESBL-associated genes (Kendek *et al*., 2025). The detection of bla_{TEM} aligns with previous studies reporting this gene as one of the most prevalent ESBL determinants in both human and animal bacterial isolates (Rahaman *et al*., 2025). However, not all aztreonam-resistant isolates carried the bla_{TEM} gene, suggesting that other resistance mechanisms or ESBL genes, such as bla_{SHV} or $bla_{\mathrm{CTX\text{-}M}}$, may also be involved (Effendi *et al*., 2022).

From a One Health perspective, the identification of bla_{TEM}-harboring *E. coli* in quails marketed for human consumption is particularly concerning (Rahayu *et al*., 2025). Traditional markets represent critical interfaces where animals, humans, and the environment interact closely, increasing the risk of zoonotic transmission of antimicrobial-resistant bacteria (Hasan *et al*., 2025). The potential spread of ESBL-producing *E. coli* through direct contact, food contamination, or environmental exposure underscores the need for integrated surveillance and improved biosecurity measures (Prayudi et al., 2023; Ribeiro *et al*., 2024).

This study provides important baseline data on antimicrobial resistance and ESBL-associated genes in quail-derived *E. coli* in Surabaya. The findings highlight the role of quails as potential reservoirs of resistant bacteria and emphasize the need for prudent antimicrobial use, enhanced hygiene practices in traditional markets, and continuous AMR monitoring within the poultry sector (Hedman *et al*., 2020). Further studies incorporating additional resistance genes, molecular typing, and environmental sampling are recommended to better understand transmission dynamics and to inform effective control strategies.

Conclusion

This study demonstrates a high prevalence of *E. coli* in quails marketed in traditional markets in Surabaya, with varying levels of antimicrobial resistance. Resistance was most frequently observed against ciprofloxacin, tetracycline, and aztreonam, and a small proportion of isolates exhibited multidrug-resistant phenotypes. Molecular analysis confirmed the presence of the bla_{TEM} gene among aztreonam-resistant isolates, indicating the circulation of ESBL-associated resistance in quail-derived *E. coli*. These findings highlight the role of quails as potential reservoirs of antimicrobial-resistant bacteria and underscore the importance of prudent antimicrobial use, improved hygiene practices in traditional markets, and continuous surveillance within a One Health framework to mitigate the spread of ESBL-producing bacteria.

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Conflict of interest

The authors have no conflict of interest to declare.

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