

# Detection of antimicrobial-resistant *Escherichia coli* isolated from chicken eggshell swabs sold in traditional markets in Surabaya City

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## ARTICLE INFO

Received: 01 April 2026

Accepted: 27 April 2026

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Keywords:

*E. coli*, Eggshell contamination, Antimicrobial resistance, Public health, Food safety.

## ABSTRACT

Chicken eggs are widely consumed as an affordable and important source of animal protein; however, eggshell surfaces may become contaminated with pathogenic and antimicrobial-resistant bacteria, posing a potential public health risk. This study aimed to detect *Escherichia coli* contamination on chicken eggshell swabs collected from traditional and modern markets in Surabaya City, Indonesia, and to determine the antimicrobial susceptibility profiles of the confirmed isolates. A total of 150 eggshell swab samples were collected from five regions of Surabaya during August 2024. Isolation and identification of *E. coli* were performed using Buffered Peptone Water (BPW), Eosin Methylene Blue Agar (EMBA), Gram staining, IMViC biochemical tests, and Triple Sugar Iron Agar (TSIA). Antimicrobial susceptibility testing was conducted using the Kirby-Bauer disk diffusion method against aztreonam, chloramphenicol, gentamicin, tetracycline, and trimethoprim-sulfamethoxazole according to CLSI 2020 guidelines. Among the 150 samples, 17 isolates showed presumptive *E. coli* characteristics on EMBA, while only 8 isolates (5.33%) were biochemically confirmed as *E. coli*. The distribution of confirmed isolates was equal between traditional and modern markets, with 4 isolates (2.67%) from each market type. Antimicrobial susceptibility testing showed that all confirmed isolates were fully susceptible to aztreonam, chloramphenicol, and gentamicin. In contrast, resistance was highest against tetracycline (75%), followed by trimethoprim-sulfamethoxazole (37.5%). Three isolates exhibited multidrug resistance patterns. These findings indicate that chicken eggshells sold in Surabaya City may serve as reservoirs of antimicrobial-resistant *E. coli*, highlighting the need for improved hygienic handling practices and prudent antibiotic use in poultry production to reduce foodborne antimicrobial resistance risks.

## Introduction

Chicken eggs are among the most widely consumed animal-derived food products worldwide due to their high nutritional value, affordability, and accessibility (Mesas *et al.*, 2022). In Indonesia, eggs are commonly sold in both traditional and modern markets and serve as an essential source of protein for the general population (Sumiati *et al.*, 2025). However, despite the protective role of the eggshell as a physical barrier, its outer surface may become contaminated with various microorganisms during laying, handling, transportation, and retail display (Hemeda *et al.*, 2025). Among these microorganisms, *Escherichia coli* is one of the most important bacterial contaminants associated with food safety and public health concerns (Chen *et al.*, 2019; Widodo *et al.*, 2023).

*E. coli* is a Gram-negative bacterium commonly found as a normal inhabitant of the intestinal tract of humans and animals (Ansharieta *et al.*, 2021; Naidoo and Zishiri, 2025). Although many strains are considered commensal, certain pathogenic strains are capable of causing severe gastrointestinal and extraintestinal infections in humans and animals (Effendi *et al.*, 2018; Ramos *et al.*, 2020; Agustin *et al.*, 2025). The presence of *E. coli* on eggshell surfaces is generally considered an indicator of fecal contamination and poor hygienic handling practices during egg production and marketing (Risalvato *et al.*, 2025). Contaminated eggshells may serve as a potential vehicle for bacterial transmission through direct contact or cross-contamination during food preparation (Lien *et al.*, 2025). Previous studies have reported that shell eggs sold in markets may harbor *E. coli* and other Enterobacteriaceae, posing a significant foodborne risk to consumers (Kilonzo-Nthenge *et al.*, 2016).

The emergence of antimicrobial-resistant (AMR) *E. coli* has become a growing global concern under the One Health framework (Al-Khalifah *et al.*, 2025). Excessive and inappropriate use of antibiotics in poultry farming for therapeutic, prophylactic, and growth-promoting purposes has contributed substantially to the selection and dissemination of resistant bacterial strains (Sana *et al.*, 2025). These resistant bacteria may subsequently enter the food chain through contaminated poultry products, including eggs (Farrukh *et al.*, 2025). Studies from various countries have demonstrated the occurrence of multidrug-resistant *E. coli* isolates recovered from eggshells, with particularly high resistance rates reported for tetracycline and sulfonamide-based antibiotics (Al-Ghanayem, 2018; Rafiq *et al.*, 2024).

Recent evidence from poultry eggshell studies in Southeast Asia has highlighted the significant prevalence of resistant *E. coli* isolates, especially in eggs sold in traditional markets and local retail stores. For example, a study in Vietnam reported high levels of resistance to tetracycline and trimethoprim-sulfamethoxazole, with several isolates exhibiting multidrug resistance patterns (Lien *et al.*, 2025). These findings suggest that eggshell contamination may represent an overlooked route for the transmission of antimicrobial-resistant bacteria to humans.

Traditional markets in Indonesia, particularly in large metropolitan areas such as Surabaya City, often involve open-display systems, high human traffic, and variable hygiene standards (Wardhana *et al.*, 2021). Such conditions may increase the likelihood of microbial contamination during storage and sale. However, data regarding the prevalence of antimicrobial-resistant *E. coli* on chicken eggshells sold in traditional markets in Surabaya remain limited.

Therefore, this study aimed to detect *E. coli* contamination on chicken eggshell swabs collected from traditional and modern markets across Surabaya City and to determine the antimicrobial susceptibility profiles of the confirmed isolates against commonly used antibiotics, including aztreonam, chloramphenicol, gentamicin, tetracycline, and trimethoprim-sulfamethoxazole. The findings of this study are expected to provide important baseline data for food safety surveillance and contribute to the development of strategies to reduce the spread of antimicrobial resistance through poultry products.

## Materials and methods

### Study location and period

The study was conducted in August 2024. Sample collection was performed at selected traditional and modern markets across Surabaya City, while bacterial isolation, identification, and antimicrobial susceptibility testing were carried out at the Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga.

### Sample collection and sample size

A total of 150 chicken eggshell swab samples were collected from five regions of Surabaya City, Indonesia: Central, East, West, North, and South Surabaya. From each vendor, five eggs were randomly selected, resulting in 15 eggs per location. Samples were placed in sterile plastic bags, labeled, and transported in a cool box to the laboratory for immediate analysis.

### Isolation and identification of *E. coli*

Eggshell surfaces were swabbed using sterile cotton swabs moistened with 1% BPW. The swabs were incubated in BPW as a pre-enrichment step. Following incubation, samples were streaked onto EMBA plates and incubated at 37°C for 24 hours. Presumptive *E. coli* colonies were identified by their characteristic metallic green sheen (de Souza et al., 2024; Putra et al., 2019).

Suspected colonies were subjected to Gram staining. Smears were prepared, heat-fixed, and stained sequentially with crystal violet, iodine, alcohol, and safranin. Microscopic examination was performed under 1000× magnification using immersion oil to confirm Gram-negative rod morphology. Biochemical tests included Indole, Methyl Red (MR), Voges-Proskauer (VP), Citrate, and Triple Sugar Iron Agar (TSIA) tests (Zilon et al., 2026).

### Antimicrobial Susceptibility Testing

Antimicrobial susceptibility was assessed using the Kirby-Bauer disk diffusion method on Mueller Hinton Agar. The antibiotic disks used in this study included aztreonam (ATM, 30 µg), chloramphenicol (C, 30 µg), gentamicin (CN, 10 µg), tetracycline (TE, 30 µg), and trimethoprim-sulfamethoxazole (SXT, 1.25/23.75 µg). Bacterial suspensions were adjusted to McFarland 0.5 standard (approximately  $1.5 \times 10^8$  CFU/mL). The suspension was evenly spread on MHA plates using sterile swabs.

Antibiotic discs were placed on the agar surface and incubated at 37°C for 24 hours. The diameter of inhibition zones was measured and interpreted as susceptible, intermediate, or resistant according to Clinical and Laboratory Standards Institute (CLSI) 2020 standards (Clinical and Laboratory Standards Institute, 2020).

### Data Analysis

The data were analyzed descriptively and presented in tables and figures. The diameter of inhibition zones for each antibiotic was measured

and compared with CLSI standards to determine antimicrobial resistance patterns of *E. coli* isolates.

## Results

### Isolation and identification of *E. coli* from chicken eggshell swabs

Following pre-enrichment in Buffered Peptone Water (BPW), bacterial growth was observed as turbidity in the medium, indicating microbial proliferation. Subsequent inoculation onto Eosin Methylene Blue Agar (EMBA) resulted in the growth of colonies with different morphologies, including colorless, pink, and metallic green colonies (Figure 1).

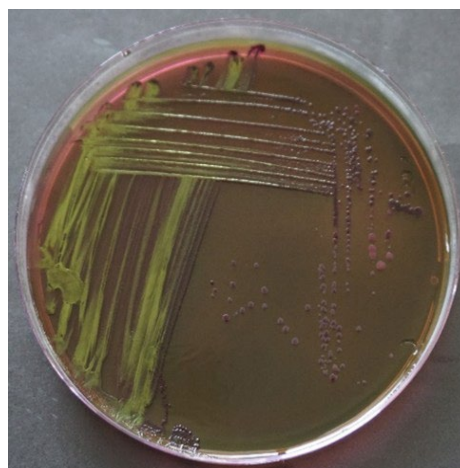


Figure 1. Representative colony morphology on EMBA showing metallic green sheen of presumptive *E. coli* isolates.

Among the 150 samples, 17 isolates exhibited the characteristic metallic green sheen suggestive of presumptive *E. coli* growth. These isolates were identified from both traditional and modern market samples (Table 1).

Table 1. Distribution of colony morphology on EMBA (n = 150).

Colony morphology	Number of samples	Percentage (%)
Metallic green	17	11.33
Pink	49	32.67
Colorless	84	56

All 17 presumptive isolates with metallic green colonies were subjected to Gram staining. Microscopic examination showed that all isolates were Gram-negative short rods (cocci/bacilli), characterized by red-stained bacterial cells (Figure 2).

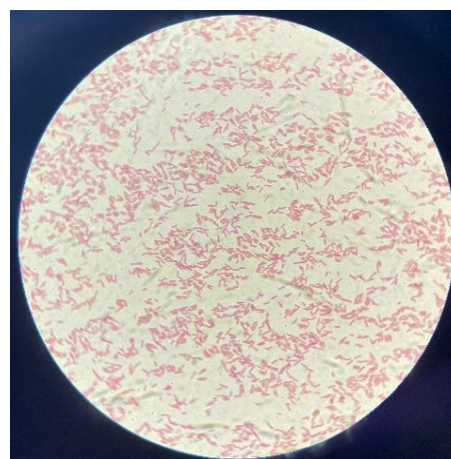


Figure 2. Gram staining of presumptive *E. coli* isolates showing Gram-negative short rod morphology.

Biochemical identification was performed using the IMViC panel and TSIA. All 17 presumptive isolates showed Indole positive (+), Methyl Red positive (+), Voges–Proskauer negative (–), TSIA acid/acid (A/A), gas positive, and H<sub>2</sub>S negative (Figure 3).

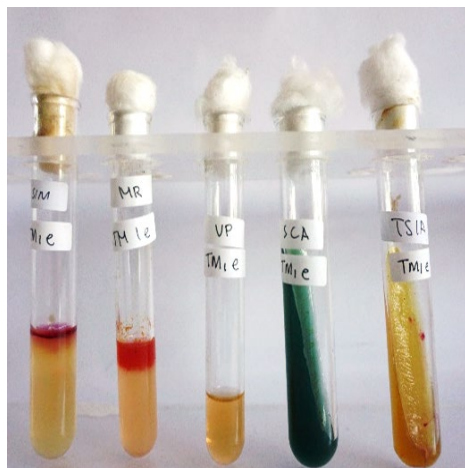


Figure 3. Biochemical confirmation of *E. coli*: (A) positive indole test; (B) positive methyl red and negative VP test; (C) negative citrate test; (D) TSIA A/A reaction with gas production and no H<sub>2</sub>S.

However, only 8 isolates showed negative citrate reactions, which is consistent with the typical biochemical profile of *E. coli*. Based on the final biochemical confirmation, 8 of 150 samples (5.33%) were confirmed positive for *E. coli*. Of these 4 isolates (2.67%) originated from traditional markets and 4 isolates (2.67%) originated from modern markets (Table 2).

Antimicrobial susceptibility test

Antimicrobial susceptibility testing was performed on the 8 con-

firmed *E. coli* isolates using the Kirby–Bauer disk diffusion method against five antibiotics (Figure 4).

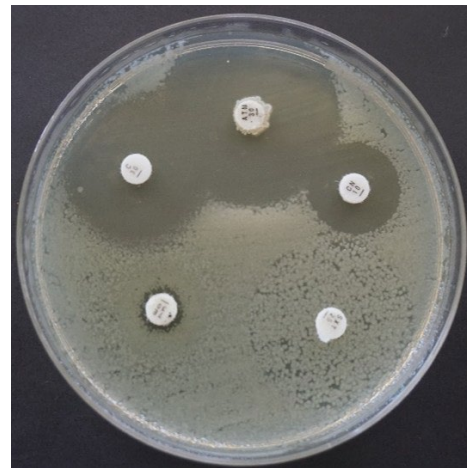


Figure 4. Representative Kirby–Bauer disk diffusion inhibition zones of *E. coli* isolates against five antibiotics on Mueller–Hinton agar.

The results showed that all confirmed isolates were fully susceptible to aztreonam, chloramphenicol, and gentamicin. In contrast, the highest resistance was observed against tetracycline, with 6 out of 8 isolates (75%) classified as resistant. Resistance to trimethoprim-sulfamethoxazole was observed in 3 isolates (37.5%) (Table 3).

Distribution of antimicrobial resistance by isolate

Detailed isolate-level analysis showed that tetracycline resistance was identified in isolates TT006, ST059, BT073, TM076, PM096, and PM100. Resistance to trimethoprim-sulfamethoxazole was detected in ST059, BT073, and PM100 (Table 4).

Table 2. Confirmed *E. coli* isolates based on biochemical tests.

Sample code	Market type	Indole	MR/VP	Citrate	TSIA	Final result
TT005	Traditional	+	+ / –	–	A/A gas + H <sub>2</sub> S–	Positive
TT006	Traditional	+	+ / –	–	A/A gas + H <sub>2</sub> S–	Positive
ST059	Traditional	+	+ / –	–	A/A gas + H <sub>2</sub> S–	Positive
BT073	Traditional	+	+ / –	–	A/A gas + H <sub>2</sub> S–	Positive
TM076	Modern	+	+ / –	–	A/A gas + H <sub>2</sub> S–	Positive
PM096	Modern	+	+ / –	–	A/A gas + H <sub>2</sub> S–	Positive
PM100	Modern	+	+ / –	–	A/A gas + H <sub>2</sub> S–	Positive
PM101	Modern	+	+ / –	–	A/A gas + H <sub>2</sub> S–	Positive

Table 3. Antibiotic susceptibility profile of confirmed *E. coli* Isolates (n = 8).

Antibiotic	Dose	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
Aztreonam (ATM)	30 µg	8 (100)	0 (0)	0 (0)
Chloramphenicol (C)	30 µg	8 (100)	0 (0)	0 (0)
Gentamicin (CN)	10 µg	8 (100)	0 (0)	0 (0)
Tetracycline (TE)	30 µg	2 (25)	0 (0)	6 (75)
Trimethoprim-sulfamethoxazole (SXT)	1.25/23.75 µg	5 (62.5)	0 (0)	3 (37.5)

Table 4. Resistant isolates and resistance pattern.

Sample code	Tetracycline	Trimethoprim-sulfamethoxazole	Resistance profile
TT006	R	S	Single-drug resistant
ST059	R	R	Multidrug resistant
BT073	R	R	Multidrug resistant
TM076	R	S	Single-drug resistant
PM096	R	S	Single-drug resistant
PM100	R	R	Multidrug resistant

These findings indicate that three isolates exhibited multidrug resistance (MDR) to both tetracycline and trimethoprim-sulfamethoxazole.

## Discussion

The present study demonstrated that *E. coli* contamination was detected in 5.33% (8/150) of chicken eggshell swab samples collected from both traditional and modern markets in Surabaya City. The detection of *E. coli* on eggshell surfaces confirms the potential for fecal contamination during egg production, handling, and distribution processes (Risalvato et al., 2025). Although the prevalence observed in this study is relatively low compared to reports from other regions, it still represents a significant food safety concern, considering that eggs are widely consumed and often handled directly prior to cooking (Bose et al., 2025).

The presence of 17 presumptive *E. coli* isolates based on characteristic metallic green colonies on EMBA, followed by confirmation of only 8 isolates through biochemical testing, highlights the importance of using a combination of phenotypic and biochemical methods for accurate bacterial identification. The IMViC profile (Indole positive, MR positive, VP negative, and citrate negative) together with TSIA results (A/A, gas production, H<sub>2</sub>S negative) confirmed the typical biochemical characteristics of *E. coli*. The discrepancy between presumptive and confirmed isolates may be attributed to the presence of other coliform bacteria that can exhibit similar colony morphology on EMBA but differ in biochemical properties (Zhang et al., 2015).

Interestingly, the distribution of confirmed *E. coli* isolates was equal between traditional and modern markets (2.67% each). This finding suggests that contamination is not exclusively associated with traditional market conditions, which are often perceived as less hygienic due to open display systems, high environmental exposure, and limited sanitation control (Wibisono et al., 2023). Instead, it indicates that contamination may occur at multiple stages along the supply chain, including at the farm level, during transportation, or within retail environments regardless of market type. This observation aligns with previous studies indicating that eggshell contamination can occur before reaching the point of sale (De Reu et al., 2006).

The antimicrobial susceptibility results revealed that all *E. coli* isolates were fully susceptible to aztreonam, chloramphenicol, and gentamicin. This finding suggests that these antibiotics remain effective against *E. coli* isolates associated with eggshell contamination in the study area (Miles et al., 2006). The high susceptibility may reflect limited exposure of these antibiotics in poultry production systems or controlled usage practices in the region. However, continuous monitoring is necessary to ensure that susceptibility patterns are maintained over time (Yassin et al., 2017).

In contrast, a high level of resistance was observed against tetracycline (75%), followed by trimethoprim-sulfamethoxazole (37.5%). The high resistance to tetracycline is consistent with numerous studies conducted in poultry production systems worldwide, where tetracycline has been extensively used for therapeutic and growth-promoting purposes (Bebora et al., 1994). Prolonged and often unregulated use of tetracycline in animal husbandry exerts selective pressure, facilitating the emergence and persistence of resistant bacterial strains (Abate and Birhanu, 2025; Prayudi et al., 2023). Similarly, resistance to trimethoprim-sulfamethoxazole may be associated with its widespread use in veterinary and human medicine, contributing to cross-resistance development (Sevilla-Navarro et al., 2022).

At the isolate level, six isolates (TT006, ST059, BT073, TM076, PM096, and PM100) exhibited resistance to tetracycline, while three isolates (ST059, BT073, and PM100) were resistant to trimethoprim-sulfamethoxazole. Notably, three isolates demonstrated multidrug resistance (MDR), defined as resistance to at least two classes of antibiotics. The presence of MDR *E. coli* isolates on eggshell surfaces is of particular concern, as it indicates the potential for transmission of resistant bacteria to humans through direct contact or cross-contamination during food preparation

(Hussein et al., 2025; Khairullah et al., 2025).

The detection of antimicrobial-resistant and MDR *E. coli* in this study supports the growing body of evidence emphasizing the role of poultry products as reservoirs of antimicrobial resistance within the One Health framework (Chousalkar et al., 2010). Eggs, although typically cooked before consumption, can act as vehicles for the spread of resistant bacteria through improper handling, inadequate cooking, or contamination of kitchen surfaces and utensils (Dorn-In et al., 2024).

Several factors may contribute to the occurrence of antimicrobial-resistant *E. coli* in eggshells, including antibiotic use practices at the farm level, environmental contamination, biosecurity measures, and hygiene practices during egg handling and distribution (Lien et al., 2025). In traditional markets, exposure to dust, flies, and frequent human contact may increase the risk of contamination (Thongratsakul et al., 2023). However, the similar prevalence observed in modern markets suggests that additional factors, such as upstream contamination during production and processing, also play a critical role (Faridah et al., 2023; Ferasyi et al., 2025).

Despite these findings, this study has some limitations. The relatively small number of confirmed *E. coli* isolates (n = 8) may limit the generalizability of antimicrobial resistance patterns. In addition, molecular characterization of resistance genes was not performed, which could provide deeper insights into the mechanisms of resistance and potential transmission pathways.

## Conclusion

This study highlights that chicken eggshells sold in Surabaya City can harbor antimicrobial-resistant *E. coli*, including multidrug-resistant strains. These findings underscore the importance of improving hygiene practices along the egg production and distribution chain, implementing prudent antibiotic use in poultry farming, and strengthening surveillance programs to monitor antimicrobial resistance in foodborne bacteria.

## Acknowledgments

The authors would like to express their sincere gratitude to Prof. Dr. Mustofa Helmi Effendi, DTAPH for his generous financial support and valuable contribution to the completion of this research.

## Conflict of interest

The authors have no conflict of interest to declare.

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