

Multidrug-resistant *Klebsiella* spp. and extended-spectrum β -lactamases-associated resistance genes in chicken eggshells marketed in Surabaya, Indonesia

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ABSTRACT

Antimicrobial-resistant bacteria have become a global health and food safety problem due to their appearance and spread in animal-derived foods. Extended-spectrum β -lactamase (ESBL)-associated genes are among the several antibiotic resistance determinants that *Klebsiella* spp. are known to acquire, making them significant opportunistic pathogens. The purpose of this work was to discover multidrug-resistant (MDR) isolates, assess the antimicrobial susceptibility profiles of *Klebsiella* spp. on chicken eggshells sold in Surabaya, Indonesia, and find ESBL-associated resistance genes (*bla*_{SHV}, *bla*_{TEM}, and *bla*_{CTX-M}). A total of 150 eggshell swab samples were gathered from five Surabaya administrative regions' traditional and contemporary retail markets. Gram staining, biochemical testing, and standard culture techniques were used for isolation and identification. The Kirby-Bauer disk diffusion method was used to test for antimicrobial susceptibility to ampicillin, gentamicin, azithromycin, enrofloxacin, and tetracycline. Polymerase chain reaction (PCR) was used to further screen MDR isolates for ESBL-associated genes. 28 (18.7%) of the 150 samples that were analyzed were identified as *Klebsiella* spp. According to antimicrobial susceptibility tests, ampicillin had the highest resistance rate (71.4%), followed by azithromycin (39.3%) and tetracycline (25.0%). At 78.6%, gentamicin showed the highest susceptibility rate. Due to their resistance to three or more antimicrobial classes, two isolates (7.1%) were categorized as MDR. While *bla*_{TEM} and *bla*_{CTX-M} were not found, molecular analysis revealed that both MDR isolates carried the *bla*_{SHV} gene. These results suggest that antimicrobial-resistant *Klebsiella* spp. and ESBL-associated resistance determinants may be present in chicken eggshells sold in Surabaya. To reduce the spread of antimicrobial resistance within a One Health framework, ongoing surveillance and better hygiene procedures are crucial across the chicken production chain.

Introduction

One of the biggest risks to food safety, animal welfare, and public health is the global spread of antimicrobial resistance (AMR) (Khairullah *et al.*, 2025). The growth and spread of resistant bacterial infections have been accelerated by the extensive and frequently improper use of antimicrobial drugs in human health, veterinary practice, and livestock agriculture (Trinchera *et al.*, 2025). Due to their capacity to acquire numerous resistance determinants and cause a variety of illnesses in both humans and animals, *Klebsiella* spp. have drawn the most interest among Gram-negative bacteria (Li *et al.*, 2025). Within the One Health perspective, which acknowledges the interdependence of human, animal, and environmental health, the spread of antimicrobial-resistant *Klebsiella* strains down the food chain is a serious concern (Al-Khalaifah *et al.*, 2025).

Klebsiella spp. are members of the family that are opportunistic (Thesia *et al.*, 2025). *Enterobacteriaceae* are frequently found in soil, water, plants, animals, and human digestive tracts (Paz-Calvache *et al.*, 2025). Despite being a natural component of the microbiota, these bacteria can cause serious infections, such as pneumonia, septicemia, urinary tract infections, and wound infections, especially in immunocompromised people (Paczosa and Meccas, 2016). *Klebsiella* spp. have been linked to respiratory infections, mastitis, and other opportunistic illnesses in animals raised for food (Wareth and Neubauer, 2021). Concerns about foodborne transmission and the transfer of antibiotic resistance genes to humans are raised by their presence in animal-derived foods (El Baz *et al.*, 2026).

One of the most popular foods derived from animals in the world is chicken eggs (Caffa *et al.*, 2025). Eggshells can become contaminated during manufacture, processing, transit, storage, and marketing, even if the inside contents of freshly laid eggs are typically sterile (Hsu *et al.*, 2023). Members of the *Enterobacteriaceae* family are among the bacterial infections that can be transported by contaminated eggshells (Hemeda *et al.*, 2025). Eggshells' porous shape makes it easier for bacteria to adhere to and survive on their surface, potentially contaminating areas used for food preparation (Chen *et al.*, 2019). As a result, eggshell contamination is a significant food safety concern, especially in underdeveloped nations where hygienic handling procedures may be uneven (De Reu *et al.*, 2006).

Antimicrobial-resistant bacteria can be isolated from retail eggs and chicken products, according to an increasing amount of evidence (Hinson *et al.*, 2025). The rise of multidrug-resistant (MDR) *Klebsiella* spp., which are isolates resistant to at least one antimicrobial agent in three or more antimicrobial classes, is especially concerning (Drakul *et al.*, 2025). MDR *Klebsiella* bacteria restrict available treatments and raise morbidity, death, and medical expenses (Riwu *et al.*, 2022). Moreover, extended-spectrum β -lactamases (ESBLs), which can hydrolyze a variety of β -lactam medicines, including third-generation cephalosporins, are produced by a large number of MDR isolates (Farizqi *et al.*, 2023). *bla*_{SHV}, *bla*_{TEM}, and *bla*_{CTX-M} which are frequently found on mobile genetic elements that promote horizontal gene transfer between bacterial populations, are among the most widely documented ESBL-associated genes in *Klebsiella* spp. (Yartey *et al.*, 2025).

The prevalence of MDR and ESBL-producing *Klebsiella* spp. in retail eggs in Indonesia is still poorly understood, despite the growing recognition of foodborne AMR as a global health concern (Sulaiman *et al.*, 2025). One of Indonesia's biggest cities, Surabaya, has a vast distribution network that includes both traditional and contemporary retail markets, which increases the risk of bacterial contamination and spread across the food supply chain (Keytumu *et al.*, 2026). Therefore, monitoring antimicrobial-resistant bacteria in consumer-marketed eggs is crucial for evaluating possible threats to public health (Owusu-Doubreh *et al.*, 2023).

The current study's objectives were to identify and isolate *Klebsiella* spp. from chicken eggshells sold in Surabaya, Indonesia, ascertain their antimicrobial susceptibility profiles, find isolates that were resistant to multiple drugs, and look into the existence of ESBL-associated resistance genes (*bla_{SHV}*, *bla_{TEM}*, and *bla_{CTX-M}*). The results of this study support ongoing initiatives to improve food safety monitoring and antibiotic resistance surveillance within the One Health framework and offer useful baseline data on the prevalence of antimicrobial-resistant *Klebsiella* spp. in retail eggs.

Materials and methods

Study design and sample collection

A laboratory-based cross-sectional study was conducted to investigate the occurrence of antimicrobial-resistant *Klebsiella* spp. on commercial chicken eggshells marketed in Surabaya, Indonesia. The study was conducted from August to September 2025. A total of 150 eggshell swab samples were collected from five administrative regions of Surabaya, including East, West, North, South, and Central Surabaya. From each region, 30 samples were obtained, comprising 15 samples from traditional markets and 15 samples from modern retail markets.

Samples were selected using a purposive sampling approach, in which only eggs exhibiting visible dirt or surface contamination were included. Each egg was handled aseptically using sterile gloves, placed individually in sterile plastic bags, and transported in a cool box to the Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga, for microbiological analysis.

Isolation and identification of *Klebsiella* spp.

Eggshell surfaces were swabbed using sterile cotton swabs, and the swabs were inoculated into 1% Buffered Peptone Water (BPW; Merck KGaA, Darmstadt, Germany) as a pre-enrichment medium. The cultures were incubated at 37°C for 24h. Subsequently, enriched samples were streaked onto MacConkey Agar (MCA; HiMedia Laboratories, India) and incubated at 37°C for an additional 24h.

Presumptive *Klebsiella* colonies were identified based on their characteristic colonial morphology on MCA, namely large, smooth, mucoid, pink-to-red colonies indicative of lactose fermentation. Representative colonies were selected for further phenotypic characterization (Putri *et al.*, 2026).

Gram staining and biochemical identification

Presumptive isolates were subjected to Gram staining using crystal violet, Lugol's iodine, 96% ethanol, and safranin. Microscopic examination was performed under oil immersion at 1000× magnification. Isolates appearing as short Gram-negative bacilli were further analyzed using conventional biochemical tests.

Biochemical characterization included Sulfide–Indole–Motility (SIM), Methyl Red (MR), Voges–Proskauer (VP), Simmons Citrate Agar (SCA), and Triple Sugar Iron Agar (TSIA) assays. Identification of *Klebsiella* spp. was based on a characteristic biochemical profile consisting of negative reactions for hydrogen sulfide production, indole production, motility,

and methyl red tests, together with positive citrate utilization and Voges–Proskauer reactions. On TSIA medium, confirmed isolates typically exhibited an acid slant/acid butt (A/A) reaction with gas production and no hydrogen sulfide formation (Permatasari *et al.*, 2020).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was carried out using the Kirby–Bauer disk diffusion method on Mueller–Hinton Agar (MHA; HiMedia Laboratories, India). Fresh bacterial colonies were suspended in sterile 0.9% physiological saline and adjusted to the turbidity of a 0.5 McFarland standard, corresponding to approximately 1.5×10^8 CFU/mL.

The standardized bacterial suspension was evenly spread onto MHA plates using sterile cotton swabs. Antimicrobial disks (Oxoid, United Kingdom) were placed on the inoculated agar surface and incubated at 37°C for 18–24 h. The antimicrobial agents tested were ampicillin (10 µg), gentamicin (10 µg), azithromycin (15 µg), enrofloxacin (5 µg), and tetracycline (30 µg).

After incubation, inhibition zone diameters were measured using a digital caliper. The results were interpreted according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2020). Isolates showing resistance to at least one antimicrobial agent in three or more antimicrobial classes were categorized as MDR.

Detection of ESBL-associated resistance genes

Multidrug-resistant *Klebsiella* isolates were further analyzed by polymerase chain reaction (PCR) to detect ESBL-associated genes, including *bla_{SHV}*, *bla_{TEM}*, and *bla_{CTX-M}*. These genes were selected because they represent the most frequently reported β-lactam resistance determinants in *Klebsiella* spp.

Genomic DNA was extracted from overnight bacterial cultures using a commercial bacterial DNA extraction kit according to the manufacturer's instructions. PCR amplification was performed in a final reaction volume of 25 µL consisting of 12.5 µL PCR master mix, 1 µL of each forward and reverse primers (Table 1), 2 µL DNA template, and 8.5 µL nuclease-free water.

Table 1. Primers used for amplification of ESBL-associated resistance genes.

Gene	Primer sequence (5'–3')	Amplicon size	Reference
<i>bla_{SHV}</i>	F: GGTTATGCGTTATATTCGCC R: TTAGGTTGCCAGTGCTC	867 bp	Srimaryanto <i>et al.</i> (2025)
<i>bla_{TEM}</i>	F: ATAAAATTCTTGAAGACGAAA R: GACAGTTACCAATGCTTAATC	1086 bp	Prayudi <i>et al.</i> (2023)
<i>bla_{CTX-M}</i>	F: CGCTTTGCGATGTGCAG R: ACCGCGATATCGTTGGT	550 bp	Ansharieta <i>et al.</i> (2021)

The amplification protocol included an initial denaturation step at 95°C for 5 min, followed by 35 amplification cycles consisting of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min. A final extension step was performed at 72°C for 7 min.

PCR products were resolved by electrophoresis on 1.5% agarose gels stained with a nucleic acid dye and visualized under ultraviolet illumination. The presence of target genes was determined by comparing the observed amplicon sizes with the expected fragment lengths.

Data analysis

All data obtained from bacterial isolation, phenotypic identification, antimicrobial susceptibility testing, and molecular detection of resistance genes were analyzed descriptively. The prevalence of *Klebsiella* spp., antimicrobial susceptibility profiles, MDR occurrence, and distribution of ESBL-associated genes were expressed as frequencies and percentages. Results were summarized using tables and figures, while antimicrobial

susceptibility interpretations were based on CLSI criteria.

Results

Isolation and identification of *Klebsiella* spp.

A total of 150 chicken eggshell swab samples were collected from traditional and modern retail markets located in five administrative regions of Surabaya (East, West, North, South, and Central Surabaya), Indonesia. Primary isolation on MCA yielded 51 presumptive *Klebsiella* spp. isolates, representing 34.0% (51/150) of all samples examined. Of these presumptive isolates, 28 (18.7%) originated from traditional markets, whereas 23 (15.3%) were recovered from modern markets.

On MCA, presumptive *Klebsiella* colonies exhibited typical morphological characteristics, including large, smooth, mucoid, pink-to-red colonies resulting from lactose fermentation (Figure 1). Gram staining revealed short Gram-negative bacilli when examined under oil immersion at 1000× magnification (Figure 2).



Figure 1. Morphological appearance of presumptive *Klebsiella* spp. colonies on MacConkey agar showing pink, mucoid, and circular lactose-fermenting colonies.

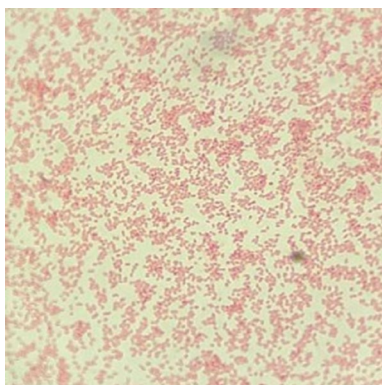


Figure 2. Gram staining of presumptive *Klebsiella* spp. isolates showing short rod-shaped Gram-negative bacilli under 1000× magnification.

Further confirmation was performed using conventional biochemical tests, including SIM, SCA, MR-VP, and TSIA. Confirmed isolates showed biochemical profiles consistent with *Klebsiella* spp., characterized by negative reactions for hydrogen sulfide production, indole production, motility, and methyl red tests, while positive reactions were observed for citrate utilization and the Voges–Proskauer test. On TSIA medium, isolates produced an acid slant/acid butt (A/A) reaction with gas formation but without hydrogen sulfide production (Figure 3).

Following phenotypic confirmation, 28 isolates were identified as *Klebsiella* spp., corresponding to an overall prevalence of 18.7% (28/150). Among the confirmed isolates, 15 were recovered from traditional markets and 13 from modern markets. The confirmed isolates were distributed across the five administrative regions of Surabaya as follows: Tambaksari and Tenggilis Mejoyo (East Surabaya), Tegalsari and Genteng (Central Surabaya), Pabean Cantian, Pabean, and Bulak (North Surabaya), Wonocolo (South Surabaya), and Tandes (West Surabaya) (Table 2).



Figure 3. Biochemical characterization of *Klebsiella* spp. isolate PT11: (A) SIM test; (B) Simmons citrate agar test; (C) Methyl red test; (D) Voges–Proskauer test; and (E) Triple sugar iron agar test.

Table 2. Distribution of confirmed *Klebsiella* spp. isolated from chicken eggshells marketed in Surabaya.

Market type	Location	Positive isolates (n)	Isolate codes
Traditional	Tambaksari (East Surabaya)	6	TT1, TT2, TT3, TT7, TT9, TT11
	Tegalsari (Central Surabaya)	4	PT1, PT3, PT10, PT11
	Pabean Cantian (North Surabaya)	5	UT1, UT3, UT9, UT11, UT12
Subtotal		15	
Modern	Pabean (North Surabaya)	1	UM4
	Genteng (Central Surabaya)	3	PM7, PM8, PM9
	Tenggilis Mejoyo (East Surabaya)	5	TM2, TM3, TM4, TM5, TM6
	Bulak (North Surabaya)	2	UM7, UM8
	Wonocolo (South Surabaya)	1	SM3
	Tandes (West Surabaya)	1	BM6
Subtotal		13	
Total		28	

Antimicrobial susceptibility profiles of *Klebsiella* spp.

The antimicrobial susceptibility of the 28 confirmed *Klebsiella* isolates was evaluated using the Kirby–Bauer disk diffusion method against five antimicrobial agents, namely ampicillin (AMP), gentamicin (CN), azithromycin (AZN), enrofloxacin (ENR), and tetracycline (TE) (Figure 4).

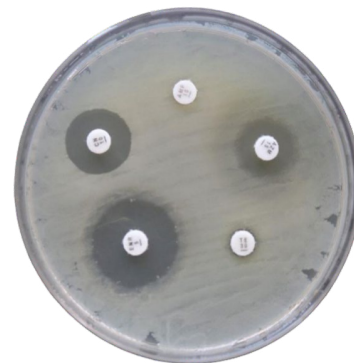


Figure 4. Representative Kirby–Bauer disk diffusion assay showing antimicrobial susceptibility patterns of *Klebsiella* spp. isolates against ampicillin, gentamicin, azithromycin, enrofloxacin, and tetracycline.

Among the tested antimicrobials, ampicillin demonstrated the highest resistance rate, with 20 of 28 isolates (71.4%) classified as resistant. In contrast, gentamicin exhibited the highest level of activity, with 22 isolates (78.6%) categorized as susceptible. Resistance to azithromycin and

tetracycline was observed in 39.3% (11/28) and 25.0% (7/28) of isolates, respectively, whereas only 10.7% (3/28) of isolates were resistant to enrofloxacin. Detailed antimicrobial susceptibility profiles are presented in Table 3.

Table 3. Antimicrobial susceptibility patterns of *Klebsiella* spp. isolates (n = 28).

Antibiotic	Susceptible n (%)	Intermediate n (%)	Resistant n (%)
Ampicillin (AMP)	4 (14.3)	4 (14.3)	20 (71.4)
Gentamicin (CN)	22 (78.6)	4 (14.3)	2 (7.1)
Azithromycin (AZN)	17 (60.7)	0 (0.0)	11 (39.3)
Enrofloxacin (ENR)	20 (71.4)	5 (17.9)	3 (10.7)
Tetracycline (TE)	21 (75.0)	0 (0.0)	7 (25.0)

Two isolates (7.1%) fulfilled the criteria for multidrug resistance (MDR), defined as resistance to at least one antimicrobial agent in three or more antimicrobial classes. Both MDR isolates originated from modern markets. Isolate TM2 showed resistance to ampicillin, azithromycin, and enrofloxacin (AMP–AZN–ENR), whereas isolate UM7 displayed a broader resistance profile involving ampicillin, azithromycin, enrofloxacin, and tetracycline (AMP–AZN–ENR–TE) (Table 4).

Table 4. MDR *Klebsiella* spp. isolates recovered from chicken eggshells.

Isolate code	Market type	Resistance pattern
TM2	Modern	AMP–AZN–ENR
UM7	Modern	AMP–AZN–ENR–TE

Note: Ampicillin (AMP), Gentamicin (CN), Azithromycin (AZN), Enrofloxacin (ENR), Tetracycline (TE).

The high prevalence of resistance to ampicillin indicates widespread β -lactam resistance among *Klebsiella* spp. recovered from retail eggshells. Moreover, the detection of MDR isolates highlights the potential role of eggshell contamination as a source of antimicrobial-resistant bacteria within the food supply chain.

Detection of ESBL-associated resistance genes

To investigate the molecular basis of antimicrobial resistance, MDR isolates were screened for three ESBL-associated β -lactamase genes, namely *bla*_{SHV}, *bla*_{TEM}, and *bla*_{CTX-M}. The two MDR isolates identified in this study (TM2 and UM7) were subjected to PCR analysis.

PCR amplification revealed the presence of the *bla*_{SHV} gene in both isolates, whereas neither *bla*_{TEM} nor *bla*_{CTX-M} was detected (Table 5). Agarose gel electrophoresis showed distinct amplification bands corresponding to the expected size of the *bla*_{SHV} amplicon (Figure 5). In contrast, no amplification products were observed for *bla*_{CTX-M} or *bla*_{TEM} (Figures 6 and 7).

The exclusive detection of *bla*_{SHV} among the analyzed MDR isolates suggests that SHV-type β -lactamase is the predominant genetic determinant associated with β -lactam resistance in *Klebsiella* spp. recovered from chicken eggshells. The absence of *bla*_{TEM} and *bla*_{CTX-M} indicates that these ESBL-associated genes were not involved in the resistance phenotypes observed in the present study.

Collectively, these findings demonstrate the presence of *bla*_{SHV}-harboring MDR *Klebsiella* strains within the retail egg distribution chain and reinforce the importance of continuous surveillance of antimicrobial resistance in foodborne bacteria.

Table 5. Distribution of ESBL-associated resistance genes among MDR *Klebsiella* spp. isolates.

Isolate code	Market type	<i>bla</i> _{SHV}	<i>bla</i> _{TEM}	<i>bla</i> _{CTX-M}
UM7	Modern	Positive	Negative	Negative
TM2	Modern	Positive	Negative	Negative

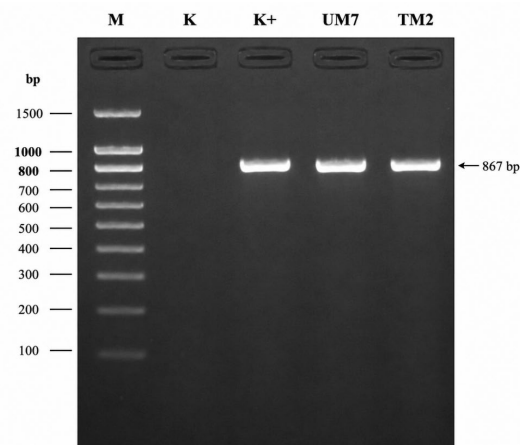


Figure 5. Agarose gel electrophoresis showing amplification of the *bla*_{SHV} gene in MDR *Klebsiella* spp. isolates. Lane M: DNA marker; K–: negative control; K+: positive control; UM7 and TM2: MDR isolates.

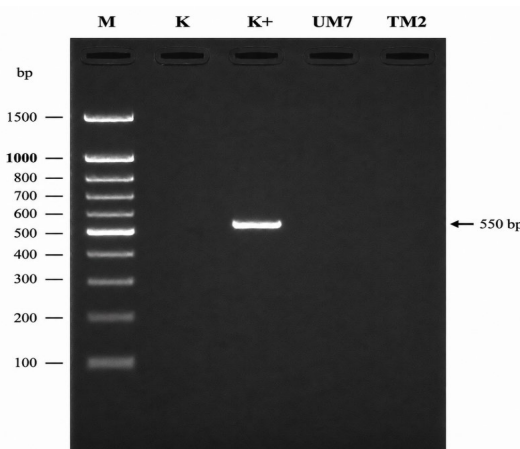


Figure 6. Agarose gel electrophoresis showing PCR amplification results for the *bla*_{CTX-M} gene. No amplification products were detected in MDR *Klebsiella* spp. isolates.

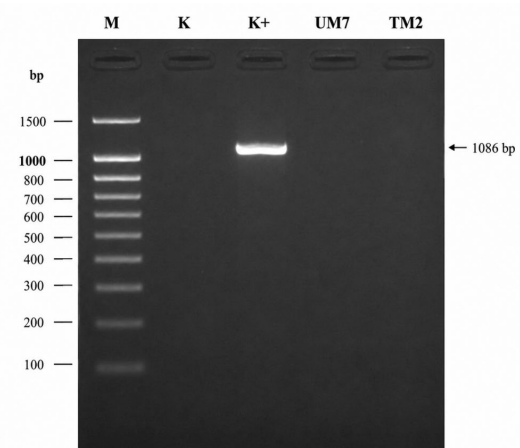


Figure 7. Agarose gel electrophoresis showing PCR amplification results for the *bla*_{TEM} gene. No amplification products were detected in MDR *Klebsiella* spp. isolates.

Discussion

Antimicrobial-resistant and multidrug-resistant isolates with ESBL-associated resistance genes were found in this investigation, which also showed that chicken eggshells sold in Surabaya, Indonesia, were contaminated with *Klebsiella* spp. The finding of *Klebsiella* spp. in retail eggshells emphasizes how poultry products may act as carriers and reservoirs for opportunistic pathogens and factors that contribute to antibiotic resistance in the food chain.

The total prevalence of confirmed *Klebsiella* spp. isolates was 18.7% (28/150), meaning that this bacterium was present in almost one-fifth of

the eggshell samples that were tested. Laying, handling, transportation, storage, and marketing are just a few of the stages of manufacturing and distribution where contamination may occur and result in the presence of *Klebsiella* spp. on eggshell surfaces (Junaid *et al.*, 2022). Because of their porous nature, which encourages bacterial attachment and persistence on the shell surface, eggshells are extremely vulnerable to bacterial contamination (Vale *et al.*, 2024). *Enterobacteriaceae* can also be introduced into eggshells through poor sanitation, environmental pollution, and direct contact with poultry excrement (Pace *et al.*, 2025). *Klebsiella* spp. have been recovered from poultry products, eggs, and farm settings in similar studies, highlighting the bacterium's extensive dissemination throughout poultry production systems (Deleza *et al.*, 2025).

It's interesting to note that verified *Klebsiella* isolates were found in both conventional and contemporary markets, indicating that contamination is not limited to a particular retail setting. Bacterial survival may be encouraged by the open-air selling settings and inadequate refrigeration found in traditional markets (Vu *et al.*, 2025). However, the discovery of *Klebsiella* spp. in contemporary retail marketplaces suggests that microbiological contamination cannot be totally eradicated by improved infrastructure alone (Davis *et al.*, 2015). These results imply that rather than starting only at the retail level, contamination most likely starts earlier in the production and supply chain (Junaid *et al.*, 2022).

A high degree of ampicillin resistance was found by antimicrobial susceptibility testing; 71.4% of isolates were found to be resistant. Because *Klebsiella* spp. produce chromosomally encoded β -lactamases, they are inherently resistant to ampicillin, so this finding is not surprising (Zdarska *et al.*, 2026). However, the high frequency of resistance found in this study might also be a result of ongoing selection pressure brought on by the extensive usage of β -lactam antibiotics in both human and veterinary contexts (Caneschi *et al.*, 2023). There have been reports of similar patterns of increased ampicillin resistance in *Klebsiella* isolates from food-producing animals and food products all over the world (Kot and Witeska, 2024).

Conversely, 78.6% of isolates were still sensitive to gentamicin, which demonstrated the strongest antibacterial efficacy. Similarly, compared to ampicillin, resistance rates to tetracycline and enrofloxacin stayed comparatively high. These results imply that many *Klebsiella* isolates found in retail eggs may still be susceptible to the effects of aminoglycosides and fluoroquinolones (Chreim *et al.*, 2025). The existence of resistant isolates, however, suggests that resistance to these antimicrobial groups is already developing and necessitates ongoing observation. 39.3% of isolates showed resistance to azithromycin, which could be linked to the development of macrolide resistance mechanisms including ribosome target alterations or efflux pumps (Chiou *et al.*, 2023).

From the standpoint of public health, the discovery of two MDR isolates (7.1%) is quite concerning. Both MDR isolates showed resistance to at least three antimicrobial classes and came from contemporary markets. While isolate UM7 showed an even more extensive resistance profile involving ampicillin, azithromycin, enrofloxacin, and tetracycline, isolate TM2 shown resistance to ampicillin, azithromycin, and enrofloxacin. The presence of MDR *Klebsiella* spp. in retail eggshells suggests that food items could operate as reservoirs of germs that, if spread to people, could restrict treatment options (Olanami *et al.*, 2026). Despite the relatively low frequency of MDR isolates found in this investigation, their identification shows that clinically significant resistance bacteria are circulating across the food supply chain (Glass *et al.*, 2026).

MDR isolates were screened for the ESBL-associated genes *bla*_{SHV}, *bla*_{TEM}, and *bla*_{CTX-M} in order to further explore the molecular basis of resistance. *bla*_{TEM} and *bla*_{CTX-M} were not found, however PCR analysis showed that both MDR isolates harbored the *bla*_{SHV} gene. Since SHV-type β -lactamases were first identified in *Klebsiella pneumoniae* and are still among the most common ESBL determinants in this genus, the identification of *bla*_{SHV} is consistent with the biology of *Klebsiella* spp. (Hammond *et al.*, 2005). The presence of *bla*_{SHV} indicates that SHV-mediated hydrolysis of

β -lactam antibiotics is probably linked to the β -lactam resistance seen in the MDR isolates (Liakopoulos *et al.*, 2016).

The lack of *bla*_{TEM} and *bla*_{CTX-M} in the isolates under investigation could be a sign of restricted spread of these resistance determinants among *Klebsiella* communities linked to eggshell contamination in Surabaya or geographical variations in the distribution of ESBL genes. *bla*_{CTX-M} is the predominant ESBL gene among clinical *Enterobacteriaceae* isolates globally, according to numerous investigations (Eskandari-Nasab *et al.*, 2018; Park *et al.*, 2019; Zhang *et al.*, 2021). The prevalence of *bla*_{SHV} found in this study, however, raises the possibility that various ecological niches may have unique resistance gene profiles. The identification of a single ESBL-associated gene further suggests that the reported antimicrobial resistance traits may be influenced by other resistance mechanisms not examined in this investigation (Pishtiwan and Khadija, 2019).

Food safety and public health are significantly impacted by the presence of *bla*_{SHV}-positive MDR *Klebsiella* isolates in retail eggshells. Antimicrobial resistance can spread throughout microbial communities because resistance genes on plasmids or other mobile genetic components can be horizontally transferred between bacterial species (Sulaiman *et al.*, 2025). Therefore, infected eggshells may serve as repositories of transferable resistance determinants that can spread to other pathogens in addition to being carriers of resistant bacteria (Hinson *et al.*, 2025).

This study has a number of limitations that should be noted. Initially, traditional phenotypic techniques were the main basis for bacterial identification without species-level genetic confirmation (Clarridge, 2004). Second, the reported resistance profiles may possibly be influenced by additional resistance determinants, however only three ESBL-associated genes were examined (Carvalho *et al.*, 2016). Third, it was difficult to thoroughly assess the molecular epidemiology of resistance genes due to the very small number of MDR isolates (Rahman *et al.*, 2022). A more thorough understanding of antibiotic resistance in foodborne *Klebsiella* populations might be possible with future research utilizing bigger sample numbers, whole-genome sequencing, and increased resistance gene screening (Huang *et al.*, 2025).

Overall, our investigation shows that antimicrobial-resistant *Klebsiella* spp., including MDR isolates with the *bla*_{SHV} gene, are present in chicken eggshells sold in Surabaya. These results highlight the significance of expanding antimicrobial stewardship programs, improving surveillance of antimicrobial resistance in foods of animal origin, and putting appropriate hygiene measures into place throughout the poultry production and distribution chain (Farrukh *et al.*, 2025). In order to prevent the spread of resistant microorganisms and resistance genes in the food supply and to support One Health initiatives to fight antimicrobial resistance (Widodo *et al.*, 2023).

Conclusion

This study showed that 18.7% of chicken eggshells sold in Surabaya, Indonesia, had *Klebsiella* spp. contamination. Gentamicin continued to be the most effective antimicrobial agent against the isolates, however antimicrobial susceptibility testing showed a high degree of ampicillin resistance (71.4%). There are clinically significant resistant bacteria in the retail egg supply chain, as evidenced by the identification of two isolates (7.1%) as multidrug-resistant (MDR). All MDR isolates had the *bla*_{SHV} gene, according to molecular analysis, while *bla*_{TEM} and *bla*_{CTX-M} were not found. These results demonstrate how contaminated eggshells may serve as reservoirs for ESBL-associated resistance determinants and antimicrobial-resistant *Klebsiella* spp. To promote food safety and One Health-based antimicrobial resistance reduction initiatives, ongoing surveillance of antimicrobial resistance in foods of animal origin, together with better hygiene procedures and cautious antimicrobial usage in chicken production, is crucial.

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Conflict of interest

The authors have no conflict of interest to declare.

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