Original Research

Journal of Advanced Veterinary Research
(2026) Volume 16, Issue 4, 514-518

Phenotypic and genotypic characterization of antibiotic-resistant *Klebsiella* spp. from chicken eggshells in traditional and modern markets of Surabaya

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ARTICLE INFO

Received: 03 April 2026

Accepted: 16 June 2026

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Keywords:

Klebsiella spp., Eggshell, Antibiotic resistance, Multidrug-resistant, Public health.

ABSTRACT

The emergence of antibiotic-resistant bacteria in food products is a growing public health concern. Among these, *Klebsiella* spp. are opportunistic pathogens capable of causing severe infections and disseminating antimicrobial resistance genes, including extended-spectrum β-lactamases (ESBLs). This study aimed to evaluate the phenotypic and genotypic profiles of antibiotic-resistant *Klebsiella* spp. isolated from chicken eggshells in traditional and modern markets of Surabaya, Indonesia. A total of 150 eggshell swab samples were collected across five administrative regions, with equal representation from traditional and modern markets. Presumptive *Klebsiella* isolates were enriched, cultured on MacConkey agar, and confirmed via Gram staining and biochemical tests. Antibiotic susceptibility was assessed using the Kirby–Bauer disk diffusion method against ampicillin, tetracycline, ciprofloxacin, gentamicin, and sulfamethoxazole–trimethoprim. Multidrug-resistant (MDR) isolates were further analyzed for ESBL-associated genes (bla_{SHV}, bla_{TEM}, bla_{CTX-M}) via PCR. From 150 samples, 28 isolates (18.7%) were confirmed as *Klebsiella* spp., with similar prevalence in traditional (20.0%) and modern (17.3%) markets. Phenotypic analysis revealed high resistance to ampicillin (78.6%) and lower resistance to other antibiotics. Four isolates (14.3%) were classified as MDR, all recovered from modern markets. Genotypic detection showed that all MDR isolates carried the bla_{SHV} gene, whereas bla_{TEM} and bla_{CTX-M} were absent, indicating SHV-type β-lactamase as the primary determinant of β-lactam resistance. These findings demonstrate that chicken eggs in Surabaya can serve as reservoirs of phenotypically and genotypically antibiotic-resistant *Klebsiella*, posing potential public health risks. Integrated phenotypic and genotypic surveillance, responsible antimicrobial use in poultry, and improved hygiene practices in both traditional and modern markets are essential to mitigate the spread of multidrug-resistant bacteria through the food chain.

Introduction

The global emergence of antibiotic-resistant bacteria in food products represents a significant threat to public health, challenging the effectiveness of therapeutic interventions and increasing the risk of disease outbreaks (Salam *et al*., 2023). Among these pathogens, *Klebsiella* species are opportunistic Gram-negative bacteria that frequently cause both nosocomial and community-acquired infections, including urinary tract infections, pneumonia, and septicemia (Riwu *et al*., 2022a). The ability of *Klebsiella* spp. to acquire, harbor, and disseminate antimicrobial resistance determinants, particularly extended-spectrum β-lactamases (ESBLs), has led to the development of multidrug-resistant (MDR) phenotypes, complicating clinical management and heightening morbidity and mortality risks (Putri *et al*., 2026). Consequently, surveillance of antibiotic resistance in foodborne *Klebsiella* is of paramount importance to safeguard food safety and inform public health strategies (Thesia *et al*., 2025).

Poultry products, including chicken eggs, are recognized as potential vehicles for the transmission of antibiotic-resistant bacteria (Srimaryanto *et al*., 2025). Eggshell contamination can arise at multiple points along the production and supply chain, such as during handling, storage, and retail sale, particularly under conditions of inadequate hygiene (Hemeda *et al*., 2025). In Indonesia, urban centers such as Surabaya feature a diverse market landscape where eggs are sold through traditional wet markets and modern retail outlets. These market types differ considerably in consumer density, sanitation standards, and handling practices, factors that can influence both the prevalence and dissemination of resistant bacteria on eggshell surfaces (Permatasari *et al*., 2020). Despite these risks, there remains a paucity of studies investigating the phenotypic and genotypic profiles of antibiotic-resistant *Klebsiella* spp. in eggs sold in Surabaya, underscoring the need for systematic monitoring.

The public health implications of MDR bacteria in food products are profound. Exposure to antibiotic-resistant *Klebsiella* through contaminated eggs may facilitate the transfer of resistance genes to commensal or pathogenic bacteria in humans, potentially exacerbating the spread of antimicrobial resistance in the community (Sulaiman *et al*., 2025). β-lactam resistance, often mediated by ESBL enzymes such as bla_{SHV}, bla_{TEM}, and bla_{CTX-M} types, is particularly concerning given the reliance on β-lactam antibiotics as first-line treatment options (Aniokete *et al*., 2025). The occurrence of MDR *Klebsiella* in retail poultry products, therefore, constitutes a dual threat: direct infection and the broader dissemination of resistance determinants within microbial populations (El Baz *et al*., 2026).

Monitoring phenotypic and genotypic resistance patterns provides a holistic understanding of the antimicrobial resistance landscape. Phenotypic detection of resistance offers insight into the actual susceptibility of bacterial isolates to clinically relevant antibiotics, while genotypic analysis elucidates the underlying genetic mechanisms driving these resistance phenotypes (Muntean *et al*., 2022). Integrating both approaches is essential for accurate risk assessment, as isolates may harbor silent or transferable resistance genes that are not immediately evident from susceptibility profiles alone (Oliveira *et al*., 2024). Such comprehensive evaluation supports evidence-based interventions aimed at reducing contamination, limiting resistance transmission, and safeguarding consumer health.

Given the widespread consumption of chicken eggs and their potential as reservoirs of antibiotic-resistant *Klebsiella*, it is critical to understand the prevalence and molecular characteristics of these bacteria in urban market settings (Sajish *et al*., 2025). Differences in market types, handling practices, and sanitation conditions may influence the contamination risk and the distribution of resistance determinants. Insight into both phenotypic resistance patterns and genotypic profiles informs strategies for monitoring, controlling, and mitigating the dissemination of MDR bacteria in the food supply chain (He *et al*., 2026).

This study aimed to evaluate the phenotypic and genotypic profiles of antibiotic-resistant *Klebsiella* spp. isolated from chicken eggshells in traditional and modern markets of Surabaya, Indonesia. The findings are expected to provide scientific evidence to guide food safety policies, antimicrobial stewardship programs, and public health interventions aimed at reducing the impact of antibiotic-resistant bacteria in urban poultry supply chains in Indonesia.

Materials and methods

Study location and period

This study was conducted in Surabaya, Indonesia, involving both traditional and modern market settings across the five administrative regions of the city, namely East, West, North, South, and Central Surabaya. Sampling was carried out to ensure representative geographical coverage of the study area.

All microbiological and molecular analyses, including phenotypic identification, antibiotic susceptibility testing, and genotypic detection of antibiotic resistance genes, were performed at the Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga. The study was conducted from August to November 2024.

Sample collection and sampling strategy

A purposive sampling method was employed to select sampling sites that adequately represented the objectives of the study.

A total of 150 chicken eggshell swab samples were collected from traditional and modern markets across the five regions of Surabaya, consisting of 30 samples from each region, with an equal distribution of 15 samples from traditional markets and 15 samples from modern markets.

In traditional markets, samples were collected from three different egg vendors per market, with five eggs obtained from each vendor. In modern markets, five eggs were collected from each selected retail outlet.

The selection of sampling sites was based on consumer traffic density, market activity, and environmental sanitation conditions, including waste disposal proximity, clean water availability, and hygiene standard operating procedures (SOPs).

Eggshell swab samples were collected aseptically using sterile cotton swabs by thoroughly swabbing the entire outer surface of each eggshell. Each swab was immediately placed into sterile 1% Buffered Peptone Water (BPW) for transport and enrichment (Sulaiman *et al*., 2025).

All samples were transported to the laboratory in a cool box under controlled temperature conditions for immediate processing.

Phenotypic detection and identification of Klebsiella sp.

For phenotypic detection, eggshell swab samples in BPW were incubated at 37°C for 24 h for bacterial enrichment. Following incubation, enriched samples showing turbidity were streaked onto MacConkey Agar (MCA) plates and incubated at 37°C for 24 h.

Presumptive *Klebsiella* sp. isolates were identified phenotypically based on their characteristic pink, mucoid, lactose-fermenting colonies on MCA. Further confirmation was performed by Gram staining and biochemical characterization using Indole, Methyl Red, Voges-Proskauer, Citrate (IMViC) and Triple Sugar Iron Agar (TSIA) tests following standard microbiological procedures (Riwu *et al*., 2022b).

Phenotypic detection of antibiotic resistance

Phenotypic detection of antibiotic resistance was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton Agar (MHA). Pure colonies of confirmed *Klebsiella* sp. isolates were suspended in sterile 0.9% physiological saline and adjusted to a 0.5 McFarland standard (approximately 1.5 × 108 CFU/mL).

The bacterial suspension was evenly spread on the surface of MHA plates using sterile cotton swabs. After 5–10 min of absorption, antibiotic disks were placed aseptically on the agar surface. The plates were incubated at 37°C for 24 h.

The antibiotics tested were: ampicillin (10 µg), tetracycline (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), and sulfamethoxazole–trimethoprim (1.25/23.75 µg). After incubation, inhibition zone diameters were measured using a digital caliper with a precision of 0.02 mm.

The results were interpreted as susceptible, intermediate, or resistant according to CLSI 2020 guidelines (Clinical and Laboratory Standards Institute, 2020). Isolates resistant to three or more antimicrobial classes were classified as multidrug-resistant (MDR) and selected for genotypic analysis.

Genotypic detection of antibiotic resistance genes by PCR

Genotypic detection of antibiotic resistance genes was performed on multidrug-resistant (MDR) *Klebsiella* sp. isolates using the Polymerase Chain Reaction (PCR) method to identify the presence of extended-spectrum β-lactamase (ESBL)-associated genes, namely bla_{SHV}, bla_{TEM}, and $bla_{\text{CTX-M}}$. These genes were selected because they are among the most commonly reported determinants associated with β-lactam antibiotic resistance in *Klebsiella* species. The primers used are listed in Table 1.

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

Gene	Primer sequence (5’–3’)	Amplicon size	Reference
bla_{SHV}	F: GGTTATGCGTTATATTCGCC R: TTAGGTTGCCAGTGCTC	867 bp	(Sulaiman *et al*., 2025)
bla_{TEM}	F: ATAAAATTCTTGAAGACGAAA R: GACAGTTACCAATGCTTAATC	1086 bp	(Ansharieta *et al*., 2021)
$bla_{\text{CTX-M}}$	F: CGCTTTGCGATGTGCAG R: ACCGCGATATCGTTGGT	550 bp	(Prayudi *et al*., 2023)

Genomic DNA was extracted from overnight cultures of confirmed MDR isolates using a standard bacterial DNA extraction kit according to the manufacturer’s instructions. The extracted DNA served as the template for PCR amplification. Each PCR reaction was prepared in a total volume of 25 µL, consisting of 12.5 µL of PCR master mix, 1 µL of forward primer, 1 µL of reverse primer, 2 µL of DNA template, and 8.5 µL of nuclease-free water.

Amplification was carried out using specific primers targeting bla_{SHV}, bla_{TEM}, and $bla_{\text{CTX-M}}$, as presented in Table 1, with expected amplicon sizes of 867 bp, 1086 bp, and 550 bp, respectively. The PCR cycling conditions included an initial denaturation at 95°C for 5 min, followed by 35 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, with a final extension step at 72°C for 7 min.

The amplified PCR products were separated by electrophoresis using a 1.5% agarose gel stained with an appropriate nucleic acid stain and subsequently visualized under UV transillumination. The presence of antibiotic resistance genes was confirmed by comparing the observed DNA bands with the expected amplicon sizes for each target gene (Elkady *et al*., 2024).

Data analysis

Data were analyzed descriptively and presented in tables and figures. Phenotypic resistance profiles were determined based on inhibition zone diameters according to CLSI criteria.

Genotypic results were interpreted based on the presence or absence of specific PCR amplicon bands corresponding to the target resistance genes. Comparisons between isolates from traditional and modern markets were described descriptively to evaluate differences in antibiotic resistance patterns.

Results

Isolation and phenotypic identification of Klebsiella sp.

A total of 150 chicken eggshell swab samples were collected from both traditional and modern markets in Surabaya. Initial culture on MacConkey agar produced 57 presumptive *Klebsiella* sp. isolates (38.0%), which appeared as pink, mucoid, convex colonies, characteristic of lactose-fermenting Gram-negative bacteria (Figure 1). Gram staining of these isolates revealed short rod-shaped Gram-negative bacilli, consistent with *Klebsiella* morphology.

Figure 1. Morphology of *Klebsiella* colonies on MacConkey agar.

Subsequent biochemical confirmation using IMViC and TSIA tests identified 28 isolates (18.7%; 28/150) as positive for *Klebsiella* sp. (Figures 2 and 3). These confirmed isolates exhibited the typical biochemical profile of *Klebsiella* sp., including indole-negative, methyl red-negative, Voges–Proskauer-positive, citrate-positive, non-motile, and H2S-negative reactions, with A/A and gas production on TSIA.

Regarding market distribution, 15 isolates (53.6%) were recovered from traditional markets, while 13 isolates (46.4%) originated from modern markets, as summarized in Table 2. This indicates a comparable prevalence of *Klebsiella* sp. between market types.

Table 2. Prevalence and distribution of *Klebsiella* sp. isolates according to market type.

Market type	Total samples	Positive isolates	Prevalence (%)
Traditional market	75	15	20
Modern market	75	13	17.3
Total	150	28	18.7

Antibiotic susceptibility profile of Klebsiella sp. isolates

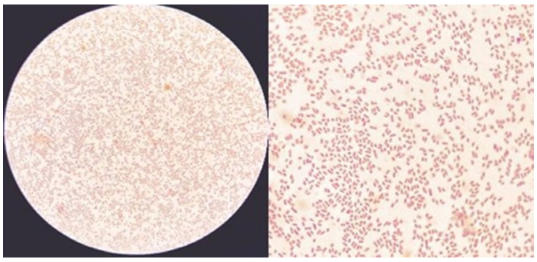

Figure 2. Gram staining of presumptive *Klebsiella* isolates showing short rod-shaped Gram-negative bacilli.

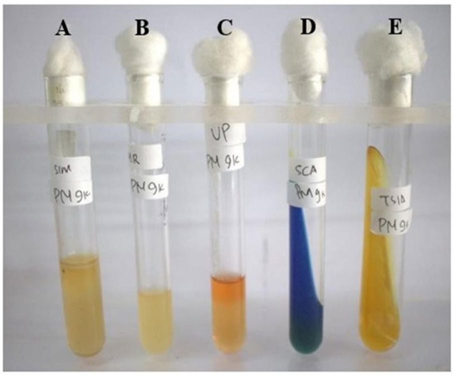

Figure 3. Biochemical confirmation of *Klebsiella* sp.: (A) SIM test, (B) MR test, (C) VP test, (D) Citrate test, (E) TSIA test.

Phenotypic antibiotic resistance of the 28 confirmed isolates was assessed using the Kirby–Bauer disk diffusion method against five antibiotics: ampicillin (AMP), tetracycline (TE), ciprofloxacin (CIP), gentamicin (CN), and sulfamethoxazole–trimethoprim (SXT).

The highest resistance rate was observed against ampicillin (78.6%; 22/28), followed by sulfamethoxazole–trimethoprim (32.1%; 9/28), ciprofloxacin (21.4%; 6/28), and gentamicin (21.4%; 6/28). Resistance to tetracycline was lowest at 14.3% (4/28). In contrast, the highest susceptibility was observed for tetracycline (85.7%; 24/28), followed by ciprofloxacin and sulfamethoxazole–trimethoprim (both 64.3%; 18/28) (Table 3, Figure 4). These results highlight that β-lactam resistance, particularly to ampicillin, is predominant among *Klebsiella* sp. isolates from chicken eggshells in Surabaya.

Table 3. Antibiotic susceptibility pattern of *Klebsiella* sp. Isolates.

Antibiotic	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
AMP	3 (10.7)	3 (10.7)	22 (78.6)
TE	24 (85.7)	0 (0.0)	4 (14.3)
CIP	18 (64.3)	4 (14.3)	6 (21.4)
CN	7 (25.0)	15 (53.6)	6 (21.4)
SXT	18 (64.3)	1 (3.6)	9 (32.1)

Note: AMP: ampicillin; TE: tetracycline; CIP: ciprofloxacin; CN: gentamicin; SXT: sulfamethoxazole–trimethoprim.

Multidrug-resistant Klebsiella sp. isolates

Among the 28 confirmed isolates, four isolates (14.3%; 4/28) were classified as multidrug-resistant (MDR), defined as resistance to three or more antibiotic classes (Figure 4). The MDR isolates (PM7, PM8, PM9), and UM7 were all recovered from modern markets.

The most severe resistance profile was observed in isolates PM7, PM8, and PM9, which were resistant to all five tested antibiotics, while UM7 showed resistance to four antibiotics (AMP, TE, CIP, SXT) (Table 4). These findings suggest a higher risk of MDR *Klebsiella* sp. in modern market eggs, which may have implications for public health.

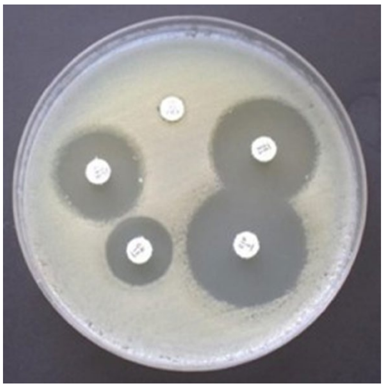

Figure 4. Antibiotic susceptibility testing of *Klebsiella* sp. isolates.

Table 4. MDR *Klebsiella* sp. isolates and resistance patterns.

Isolate code	Market type	Resistance pattern	MDR
PM9	Modern	AMP, TE, CIP, CN, SXT	Yes
PM7	Modern	AMP, TE, CIP, CN, SXT	Yes
PM8	Modern	AMP, TE, CIP, CN, SXT	Yes
UM7	Modern	AMP, TE, CIP, SXT	Yes

Note: AMP: ampicillin; TE: tetracycline; CIP: ciprofloxacin; CN: gentamicin; SXT: sulfamethoxazole–trimethoprim.

Genotypic detection of antibiotic resistance genes

PCR amplification was conducted on the four MDR *Klebsiella* sp. isolates to detect β-lactamase genes. All four isolates were positive for *bla*$_{\text{SHV}}$, whereas *bla*$_{\text{TEM}}$ and *bla*$_{\text{CTX-M}}$ were not detected in any isolate (Table 5, Figures 5–7).

These results indicate that SHV-type β-lactamase is the predominant genotypic determinant of β-lactam resistance in MDR *Klebsiella* sp. from chicken eggshells. The genotypic findings align with the observed phenotypic resistance patterns, confirming the utility of combining phenotypic and genotypic approaches for detecting antibiotic-resistant *Klebsiella* sp.

Table 5. Distribution of resistance genes among MDR *Klebsiella* sp. Isolates.

Isolate code	Market type	*bla*$_{\text{SHV}}$	*bla*$_{\text{TEM}}$	*bla*$_{\text{CTX-M}}$
PM7	Modern	Positive	Negative	Negative
PM8	Modern	Positive	Negative	Negative
PM9	Modern	Positive	Negative	Negative
UM7	Modern	Positive	Negative	Negative

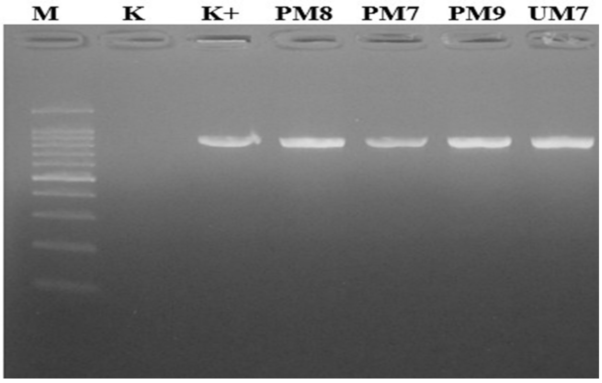

Figure 5. PCR results showing presence of *bla*$_{\text{SHV}}$ in MDR *Klebsiella* sp. isolates.

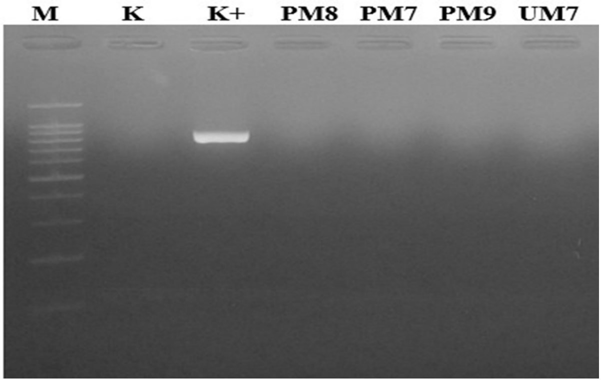

Figure 6. PCR results showing absence of *bla*$_{\text{TEM}}$ in MDR *Klebsiella* sp. isolates.

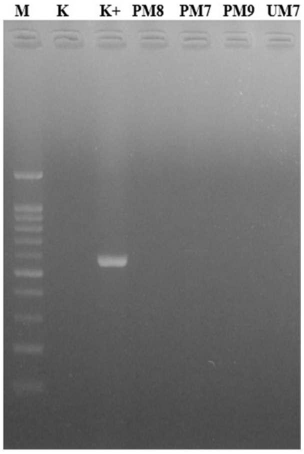

Figure 7. PCR results show absence of *bla*$_{\text{CTX-M}}$ in MDR *Klebsiella* sp. isolates.

Discussion

The present study provides a comprehensive assessment of both phenotypic and genotypic antibiotic resistance in *Klebsiella* sp. isolated from chicken eggshells in traditional and modern markets of Surabaya. The overall prevalence of *Klebsiella* sp. in eggshell samples was 18.7% (28/150), with a slightly higher occurrence in traditional markets (20.0%) compared to modern markets (17.3%). This finding aligns with previous studies indicating that eggs from environments with limited hygiene practices, such as traditional markets, may harbor opportunistic pathogens, although the difference observed in this study was relatively small (Song *et al.*, 2026).

Phenotypic antibiotic susceptibility testing revealed that β-lactam resistance, particularly to ampicillin, was the most prominent, affecting 78.6% of the isolates. Resistance to other antibiotics, including sulfamethoxazole–trimethoprim, ciprofloxacin, and gentamicin, was observed at lower rates, while tetracycline resistance was least frequent (14.3%). The predominance of ampicillin resistance corresponds with the widespread use of β-lactams in poultry production, which exerts selective pressure favoring β-lactamase-producing strains (Jung *et al.*, 2023). The detection of multidrug-resistant (MDR) isolates in 14.3% (4/28) of confirmed *Klebsiella* sp., all from modern markets, is notable, suggesting that MDR strains can persist even in retail environments with presumably higher hygiene standards. These findings underscore the risk of foodborne transmission of MDR *Klebsiella* through eggs, which may serve as vehicles for human exposure (Thesia *et al.*, 2025).

Genotypic analysis of the MDR isolates revealed that all four isolates carried the *bla*$_{\text{SHV}}$ gene, whereas *bla*$_{\text{TEM}}$ and *bla*$_{\text{CTX-M}}$ were not detected. The universal presence of *bla*$_{\text{SHV}}$ among MDR isolates highlights the central role of SHV-type β-lactamases in mediating resistance to β-lactam antibiotics in *Klebsiella* sp. from poultry sources (Dameanti *et al.*, 2023). These results are consistent with previous reports indicating that *bla*$_{\text{SHV}}$ is frequently associated with multidrug resistance in *Klebsiella* spp. isolated from food animals (Sulaiman *et al.*, 2025). The absence of *bla*$_{\text{TEM}}$ and *bla*$_{\text{CTX-M}}$ genes suggests a limited distribution of these ESBL determinants in the sampled market environments, which may reflect local antimicrobial usage patterns or differences in plasmid-mediated gene transfer (Peymani *et al.*, 2017).

The correlation between phenotypic resistance to β-lactams and the presence of bla_{SHV} confirms that molecular detection complements phenotypic testing, providing a more accurate understanding of the mechanisms underlying antibiotic resistance (Pazos *et al*., 2025). Integrating both approaches is particularly relevant in food safety surveillance, as phenotypic susceptibility alone may not detect the potential for rapid dissemination of resistance genes among bacterial populations (He *et al*., 2026).

Interestingly, MDR isolates were only recovered from modern markets, despite expectations that traditional markets might harbor more resistant strains due to poorer sanitation. Although the underlying reasons remain unclear and were not directly assessed in this study, differences in distribution practices, handling conditions, or upstream antibiotic exposure during poultry farming may contribute to this pattern (Tawyabur *et al*., 2020). Therefore, these findings should be interpreted cautiously and warrant further investigation incorporating supply-chain and storage-related variables. It also emphasizes that MDR *Klebsiella* contamination is not limited to environments with low hygiene and highlights the need for monitoring across all retail contexts (Quintelas *et al*., 2024).

From a public health perspective, the presence of MDR *Klebsiella* sp. on eggshell surfaces poses a potential risk for horizontal gene transfer to other bacteria, including commensals and human pathogens (Stercz *et al*., 2024). Consumers handling contaminated eggs without adequate hygiene may inadvertently contribute to the spread of antibiotic resistance (Manyi-Loh *et al*., 2018). Therefore, surveillance programs targeting both phenotypic and genotypic resistance profiles in foodborne bacteria are essential for risk assessment and the development of evidence-based interventions in urban poultry supply chains (Xu *et al*., 2026).

In summary, this study demonstrates that *Klebsiella* sp. from chicken eggshells in Surabaya exhibit high β-lactam resistance phenotypically and are genotypically characterized by the presence of bla_{SHV}. The findings highlight the need for integrated monitoring of antibiotic resistance in food products, appropriate antimicrobial stewardship in poultry production, and improved hygiene practices at both traditional and modern markets to mitigate the dissemination of MDR pathogens to consumers.

Conclusion

This study demonstrates that *Klebsiella* spp. are present on chicken eggshells from both traditional (20.0%) and modern markets (17.3%) in Surabaya. Phenotypic analysis revealed high resistance to β-lactam antibiotics, particularly ampicillin (78.6%), while resistance to tetracycline, ciprofloxacin, gentamicin, and sulfamethoxazole–trimethoprim was lower. Multidrug-resistant (MDR) isolates (14.3%) were detected exclusively in modern markets. Genotypic analysis showed that all MDR isolates carried the bla_{SHV} gene, whereas bla_{TEM} and $bla_{\text{CTX-M}}$ were absent, indicating SHV-type β-lactamase as the main determinant of β-lactam resistance.

These findings highlight that chicken eggs can act as a reservoir for phenotypically and genotypically antibiotic-resistant *Klebsiella*, posing potential public health risks. Integrated phenotypic and genotypic surveillance, prudent antibiotic use in poultry production, and improved hygiene practices in both market types are essential to mitigate the dissemination of MDR bacteria through the food chain.

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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