

Antimicrobial resistance profile and detection of β -lactamase genes in *Klebsiella pneumoniae* isolated from sheep in Tuban, Indonesia

Dian A. Permatasari¹, Qorisu Savenda², Aswin R. Khairullah³, Muhammad S.R. Mahandaru², Mustofa H. Effendi¹, Neysa S. Mufti², Wiwiek Tyasningsih^{4*}, Nove Hidajati⁵, Yulianna Puspitasari⁴, Nnabuiife B. Agumah⁶, Liyana L. Zulfa⁷, Katty H.P. Riwu⁸, Budiastuti Budiastuti⁹, Sheila M. Yanestria¹⁰, Bima P. Pratama¹¹, Ricadonna Raissa¹²

¹Division of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Airlangga, Kampus C Mulyorejo, Jl. Dr. Ir. H. Soekarno, Surabaya, 60115, East Java, Indonesia.

²Profession Program of Veterinary Medicine, Faculty of Veterinary Medicine, Universitas Airlangga, Kampus C Mulyorejo, Jl. Dr. Ir. H. Soekarno, Surabaya, 60115, East Java, Indonesia.

³Research Center for Veterinary Science, National Research and Innovation Agency (BRIN), Jl. Raya Bogor Km. 46 Cibinong, Bogor, 16911, West Java, Indonesia.

⁴Division of Veterinary Microbiology, Faculty of Veterinary Medicine, Universitas Airlangga, Kampus C Mulyorejo, Jl. Dr. Ir. H. Soekarno, Surabaya, 60115, East Java, Indonesia.

⁵Division of Basic Veterinary Medicine, Faculty of Veterinary Medicine, Universitas Airlangga, Kampus C Mulyorejo, Jl. Dr. Ir. H. Soekarno, Surabaya, 60115, East Java, Indonesia.

⁶Department of Applied Microbiology, Faculty of Science, Ebonyi State University, Abakaliki Rd, Abakaliki, 481101, Ebonyi, Nigeria.

⁷Research Center for Polymer Technology, National Research and Innovation Agency (BRIN), KST BJ Habibie, Serpong, South Tangerang, 15314, Banten, Indonesia.

⁸Department of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Pendidikan Mandalika, Jl. Pemuda No. 59A, Dasan Agung Baru, Mataram, 83125, West Nusa Tenggara, Indonesia.

⁹Study Program of Pharmacy Science, Faculty of Health Science, Universitas Muhammadiyah Surabaya, Jl. Raya Sutorejo No.59, Dukuh Sutorejo, Mulyorejo, Surabaya, 60113, East Java, Indonesia.

¹⁰Department of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Wijaya Kusuma Surabaya, Jl. Dukuh Kupang XXV No.54, Dukuh Kupang, Dukuh Pakis, Surabaya, 60225, East Java, Indonesia.

¹¹Research Center for Process Technology, National Research and Innovation Agency (BRIN), KST BJ Habibie, Serpong, South Tangerang, 15314, Banten, Indonesia.

¹²Department of Pharmacology, Faculty of Veterinary Medicine, Universitas Brawijaya, Jl. Puncak Dieng, Kunci, Kalisongo, Dau, Malang, 65151, East Java, Indonesia.

ARTICLE INFO

Received: 01 July 2026

Accepted: 31 July 2026

*Correspondence:

Corresponding author: Wiwiek Tyasningsih

E-mail address: wiiwiek-t@fkh.unair.ac.id

Keywords:

Antimicrobial resistance, ESBL, *Klebsiella pneumoniae*, Public health, Sheep.

ABSTRACT

An opportunistic Gram-negative bacterium of veterinary and public health significance, *Klebsiella pneumoniae* is increasingly linked to the synthesis of extended-spectrum β -lactamase (ESBL) and multidrug resistance (MDR). The purpose of this study was to ascertain the distribution of β -lactamase genes (*bla*_{CTX-M}, *bla*_{SHV}, and *bla*_{TEM}), antibiotic susceptibility profile, and prevalence of *K. pneumoniae* isolated from sheep in Tuban Regency, Indonesia. Sheep in Ngrayung Village, Plumpang District, provided 150 rectal swab samples. These samples were cultured on MacConkey Agar, followed by Gram staining, biochemical identification (TSIA and IMViC), and antimicrobial susceptibility testing using the Kirby-Bauer disk diffusion method. PCR was subsequently performed on selected resistant isolates to detect ESBL-associated genes (*bla*_{CTX-M}, *bla*_{SHV}, and *bla*_{TEM}). *K. pneumoniae* was identified in 17 isolates (11.3%) out of the 150 samples. Testing for antimicrobial susceptibility showed 100% resistance to erythromycin and 88.2% resistance to ampicillin, but 100% susceptibility to aztreonam. No isolate fulfilled the international consensus definition of multidrug resistance (MDR) after exclusion of intrinsic resistance traits. Only one of the two selected resistant isolates (50%) carried the *bla*_{SHV} gene, according to PCR analysis, while no isolates had *bla*_{CTX-M} or *bla*_{TEM}. The detection of antimicrobial-resistant *K. pneumoniae* in sheep that appear to be in good condition suggests that animals could act as reservoirs for bacteria resistant to antibiotics. The limited detection of ESBL-associated genes suggests that additional resistance mechanisms may contribute to the observed phenotypic resistance. In order to reduce the spread of resistant bacteria, these results emphasize the necessity of ongoing surveillance and careful antibiotic usage in animal production under the One Health framework.

Introduction

Known for being an opportunistic pathogen in both humans and animals, *Klebsiella pneumoniae* is a Gram-negative, encapsulated bacterium that is a member of the *Enterobacteriaceae* family (Thesia *et al.*, 2025). *K. pneumoniae* has been more frequently identified in veterinary medicine as the cause of gastrointestinal issues, mastitis, respiratory infections, and urinary tract infections in animals (Putri *et al.*, 2026). In addition to its clinical significance, this bacterium is thought to be a significant part of the animal gut microbiota, where it may serve as a reservoir for antimicrobial resistance (AMR) determinants (Riwu *et al.*, 2022).

A significant public health concern is the growth and worldwide spread of AMR bacteria, especially in light of the One Health concept that connects environmental, animal, and human health (Widodo *et al.*, 2022). Because they are frequently exposed to antimicrobial drugs used for treatment, prevention, and growth promotion, livestock animals, especially sheep, can act as reservoirs and sources of transmission of resistant bacteria (Khairullah *et al.*, 2025). This selection pressure speeds up the emergence of resistant strains and makes it easier for resistance genes to spread throughout bacterial populations (Enshaie *et al.*, 2025).

One of the most important resistance mechanisms to β -lactam antibiotics is the formation of β -lactamase enzymes, especially extended-spectrum β -lactamases (ESBLs) (Khalifa *et al.*, 2021). The most commonly doc-

umented ESBL-associated determinants in *Enterobacteriaceae*, including *K. pneumoniae*, include genes like *bla*_{CTX-M}, *bla*_{SHV}, and *bla*_{TEM} (Alsheikh *et al.*, 2026). These genes are frequently found on mobile genetic elements like plasmids, which facilitate horizontal gene transfer between bacteria and hasten the spread of resistance in both agricultural and clinical contexts (Pishtiwan and Khadija, 2019).

Concerns about animal-to-human transmission and the possible effects on food safety and public health have been raised in recent years due to an increase in reports of ESBL-producing *K. pneumoniae* in livestock habitats (Li *et al.*, 2022). As one of the most common ruminants cultivated in Indonesia, sheep may serve as a potential reservoir for antimicrobial-resistant bacteria (Dewi *et al.*, 2025). Nevertheless, little is known about the molecular traits and prevalence of *K. pneumoniae* in sheep populations, especially in rural agricultural settings (Liu *et al.*, 2025).

Indonesia confronts difficulties in implementing biosecurity and antimicrobial stewardship because it is an agricultural nation with a large amount of small-scale livestock production (Sumiati *et al.*, 2025). *K. pneumoniae* and other resistant bacteria may arise as a result of the frequent and occasionally unchecked use of antibiotics in animal husbandry (Eissa *et al.*, 2025). Despite this, there is currently a lack of surveillance information on phenotypic resistance patterns and related resistance genes in isolates linked to livestock (Nasilele *et al.*, 2026).

Thus, the purpose of this investigation was to ascertain the frequency

of *K. pneumoniae* in sheep rectal swabs from Tuban, Indonesia, to describe its antimicrobial susceptibility profile, and to identify ESBL-associated β -lactamase genes among selected antimicrobial-resistant isolates. This study advances knowledge of antimicrobial resistance dynamics in livestock systems and provides insight into the occurrence of resistant *K. pneumoniae* in sheep by combining phenotypic and genotypic approaches.

Materials and methods

Study location and period

This study employed a descriptive design with an exploratory laboratory approach aimed at isolating and identifying *K. pneumoniae* and determining its antimicrobial resistance profile. Sample collection was conducted at sheep farms located in Ngrayung Village, Plumpang District, Tuban Regency, Indonesia. Laboratory procedures, including bacterial isolation, phenotypic identification, antimicrobial susceptibility testing, and molecular detection of resistance genes, were carried out at the Division of Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia, from May to June 2025.

Sample collection and sampling strategy

The study population comprised sheep reared in farms located in Ngrayung Village, Plumpang District, Tuban Regency. The minimum sample size was calculated using the Slovin formula with a 10% margin of error, based on a total population of 1,080 sheep, resulting in 92 animals. To increase representativeness and improve statistical robustness, the sample size was expanded by 50%, yielding a final total of 150 rectal swab samples.

Rectal swab samples were collected using sterile cotton swabs selected through a random sampling approach. Each swab was immediately placed into Amies transport medium, properly labeled, and transported in a cool box to preserve bacterial viability until processing in the laboratory.

Phenotypic detection and identification of *Klebsiella pneumoniae*

All rectal swab samples were cultured on MacConkey Agar (MCA) (HiMedia, India) and incubated at 37°C for 24 h to isolate Gram-negative enteric bacteria. Colonies suspected as *K. pneumoniae* were identified based on typical morphology, namely pink, mucoid colonies measuring approximately 2–5 mm, indicative of lactose fermentation. Suspected colonies were further purified through subculture on fresh MCA plates to obtain pure isolates.

Gram staining was performed to confirm microscopic morphology. Bacterial smears were heat-fixed and sequentially stained using crystal violet, iodine solution, alcohol decolorizer, and safranin. Microscopic examination was conducted under oil immersion at 1000 $\times$ magnification. Isolates showing Gram-negative short rod-shaped morphology were considered presumptive *K. pneumoniae* (Purbowati et al., 2025).

Biochemical confirmation was conducted using Triple Sugar Iron Agar (TSIA) and IMViC tests (Indole, Methyl Red, Voges–Proskauer, and Citrate). TSIA testing was performed using the stab-and-streak method followed by incubation at 37°C for 24 h, with typical results showing acid slant/acid butt (A/A), gas production, and absence of H₂S.

IMViC testing included indole production using Sulfide Indole Motility (SIM) medium with Kovac's reagent, methyl red (MR) and Voges–Proskauer (VP) reactions using MR–VP broth, and citrate utilization using Simmons Citrate Agar (SCA). Isolates exhibiting indole-negative, MR-negative, VP-positive, and citrate-positive profiles were confirmed as *K. pneumoniae* (Effendi et al., 2018).

Phenotypic detection of antimicrobial resistance

Antimicrobial susceptibility testing of confirmed *K. pneumoniae* isolates was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton Agar (MHA) (HiMedia, India).

A bacterial suspension was prepared from fresh colonies in 0.9% sterile physiological saline and adjusted to a 0.5 McFarland standard ($\approx 1.5 \times 10^8$ CFU/mL). The suspension was evenly inoculated onto MHA plates using sterile cotton swabs.

Antibiotic disks used in this study included erythromycin (15 μ g), ampicillin (10 μ g), tetracycline (30 μ g), ceftiofur (30 μ g), and aztreonam (30 μ g) (Oxoid, UK). Plates were incubated at 37°C for 24 h.

Inhibition zone diameters were measured using a digital caliper with 0.02 mm precision. Results were interpreted as resistant (R), intermediate (I), or susceptible (S) according to Clinical and Laboratory Standards Institute (CLSI) guidelines (Clinical and Laboratory Standards Institute (CLSI), 2024). Multidrug resistance (MDR) was assessed according to the international consensus criteria proposed by Magiorakos et al. (2012), which define MDR as acquired non-susceptibility to at least one agent in three or more antimicrobial categories. Intrinsic resistance characteristics of *K. pneumoniae*, including resistance to ampicillin and non-susceptibility to erythromycin, were excluded from MDR classification.

Genotypic detection of antibiotic resistance genes by PCR

Molecular detection of antibiotic resistance genes was performed on selected resistant isolates *K. pneumoniae* isolates using Polymerase Chain Reaction (PCR) targeting β -lactamase genes, namely *bla*_{CTX-M} and *bla*_{SHV} and *bla*_{TEM} which are widely recognized as key determinants of extended-spectrum β -lactamase (ESBL) production in *Enterobacteriaceae*.

Genomic DNA was extracted from overnight bacterial cultures using a commercial DNA extraction kit following the manufacturer's protocol. The extracted DNA served as the template for PCR amplification.

Each PCR reaction was prepared in a final volume of 25 μ L, consisting of 12.5 μ L PCR master mix, 1 μ L forward primer, 1 μ L reverse primer, 2 μ L DNA template, and 8.5 μ L nuclease-free water.

Thermal cycling conditions were as follows: initial denaturation at 95°C for 5 min; 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; followed by a final extension step at 72°C for 7 min.

PCR products were separated by electrophoresis on 1.5% agarose gel stained with a nucleic acid dye and visualized under UV transillumination. Positive amplification was determined by comparison with expected amplicon sizes. Primer sequences used in this study are listed in Table 1.

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

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

Note: F = forward primer; R = reverse primer.

Data analysis

All data obtained from bacterial isolation, phenotypic identification, antimicrobial susceptibility testing, and molecular assays were analyzed descriptively. The prevalence of *K. pneumoniae*, distribution of resistance patterns, frequency of multidrug-resistant isolates, and detection rates of resistance genes were presented using tables and figures. Antibiotic susceptibility interpretations were based on CLSI 2024 standards.

Results

Isolation and phenotypic identification of *Klebsiella pneumoniae*

A total of 150 rectal swab samples were obtained from sheep reared in farms located in Ngrayung Village, Plumpang District, Tuban Regency, Indonesia. All samples were cultured on MacConkey Agar (MCA) and incubated at 37°C for 24 h to isolate Gram-negative enteric bacteria.

Out of the total samples, 43 isolates (28.6%) exhibited colony characteristics suspected as *K. pneumoniae* on MCA, represented by pink, mucoid colonies indicative of lactose fermentation (Figure 1). These presumptive isolates were further purified and subjected to Gram staining and biochemical confirmation.



Figure 1. Representative colony morphology of presumptive *K. pneumoniae* isolates on MacConkey Agar showing pink and mucoid colonies.

Microscopic examination revealed Gram-negative short rod-shaped bacteria consistent with the morphology of *K. pneumoniae* (Figure 2). Biochemical identification using Triple Sugar Iron Agar (TSIA) and IMViC tests demonstrated typical reactions of *K. pneumoniae*, including acid slant/acid butt (A/A), gas production, indole negativity, methyl red negativity, Voges–Proskauer positivity, and citrate utilization (Figure 3).

Based on combined phenotypic confirmation, 17 isolates (11.3%) were identified as *K. pneumoniae*, as summarized in Table 2.

Table 2. Isolation and phenotypic identification of *K. pneumoniae* from sheep rectal swabs.

Parameter	Number of isolates	Percentage (%)
Total samples examined	150	100
Suspected <i>K. pneumoniae</i> on MCA	43	28.6
Confirmed <i>K. pneumoniae</i> isolates	17	11.3

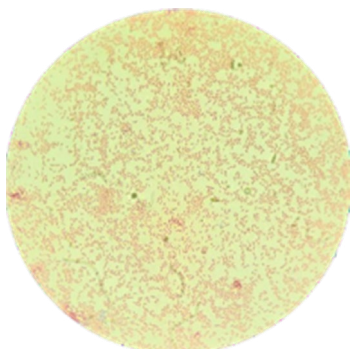


Figure 2. Gram staining of confirmed *K. pneumoniae* isolates observed under 1000× magnification showing Gram-negative short rods.

Table 4. Antibiotic resistance patterns of selected antimicrobial-resistant *K. pneumoniae* isolates.

Isolate code	Erythromycin	Ampicillin	Tetracycline	Cefoxitin	Aztreonam	Acquired resistance	Classification
S21	R	R	R	S	S	Tetracycline	Non-MDR
S28	R	S	R	R	S	Tetracycline + Cefoxitin	Non-MDR

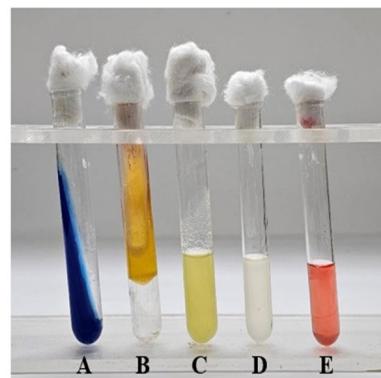


Figure 3. Representative biochemical identification results of *K. pneumoniae* including: (A) SCA, (B) TSIA, (C) SIM, (D) MR, and (E) VP tests.

Antibiotic susceptibility profile of *Klebsiella pneumoniae* isolates

All confirmed *K. pneumoniae* isolates (n = 17) were evaluated for antimicrobial susceptibility using the Kirby–Bauer disk diffusion method against five antibiotics: erythromycin, ampicillin, tetracycline, cefoxitin, and aztreonam.

Because *K. pneumoniae* is intrinsically resistant to ampicillin through chromosomally encoded β -lactamases and intrinsically non-susceptible to macrolides such as erythromycin due to the limited permeability of the Gram-negative outer membrane, resistance to these two antibiotics was reported only to describe the overall antimicrobial susceptibility profile and was not considered for MDR classification according to the international consensus criteria proposed by Magiorakos *et al.* (2012).

Erythromycin showed the highest resistance level, with all isolates (17/17; 100%) categorized as resistant. High resistance was also observed for ampicillin (15/17; 88.2%). In contrast, resistance to tetracycline and cefoxitin was detected in 2 (11.8%) and 1 (5.9%) isolates, respectively. All isolates (17/17; 100%) remained susceptible to aztreonam (Table 3, Figure 4). Therefore, the resistance frequencies observed for erythromycin and ampicillin represent the intrinsic antimicrobial resistance characteristics of *K. pneumoniae* rather than acquired resistance phenotypes.

Table 3. Antibiotic susceptibility profile of confirmed *K. pneumoniae* isolates (n = 17).

Antibiotic	Resistant n (%)	Intermediate n (%)	Susceptible n (%)
Erythromycin	17 (100)	0	0
Ampicillin	15 (88.2)	0	2 (11.8)
Tetracycline	2 (11.8)	0	15 (88.2)
Cefoxitin	1 (5.9)	0	16 (94.1)
Aztreonam	0	0	17 (100)

Note: Resistance to ampicillin and non-susceptibility to erythromycin are intrinsic characteristics of *K. pneumoniae* and were therefore excluded from MDR classification in accordance with the international consensus criteria of Magiorakos *et al.* (2012).

Antimicrobial-resistant *Klebsiella pneumoniae* isolates

MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial classes according to the international consensus criteria proposed by Magiorakos *et al.* (2012), excluding antibiotics to which *K. pneumoniae* is intrinsically resistant. Based on these criteria, none of the 17 isolates fulfilled the definition of MDR.

Isolate S21 exhibited acquired resistance to tetracycline, whereas isolate S28 exhibited acquired resistance to tetracycline and cefoxitin. All

though these isolates were selected for subsequent molecular characterization because they displayed resistance to clinically relevant antibiotics, neither fulfilled the international consensus definition of MDR (Table 4, Figure 4).

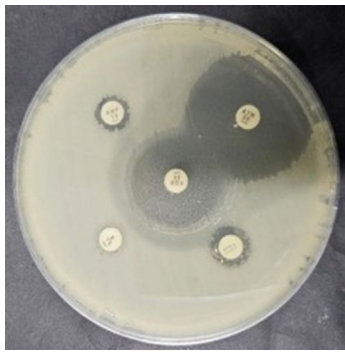


Figure 4. Representative antibiotic susceptibility test of *K. pneumoniae* isolates on Mueller-Hinton Agar using the disk diffusion method. ATM: aztreonam, TE = tetracycline; FOX = cefoxitin, AMP = ampicillin, E = erythromycin.

Genotypic detection of antibiotic resistance genes

To investigate the molecular basis of antimicrobial resistance, two selected antimicrobial-resistant *K. pneumoniae* isolates were analyzed using PCR targeting β -lactamase-associated genes (bla_{CTX-M} , bla_{SHV} and bla_{TEM}).

PCR results revealed that only one isolate (S21; 50%) carried the bla_{SHV} gene, whereas both isolates (100%) were negative for bla_{CTX-M} and bla_{TEM} (Table 5, Figures 5–7).

Table 5. Distribution of β -lactamase-associated genes among selected antimicrobial-resistant *K. pneumoniae* isolates

Isolate code	bla_{CTX-M}	bla_{SHV}	bla_{TEM}
S21	Negative	Positive	Negative
S28	Negative	Negative	Negative

Specifically, isolate S21 showed positive amplification of bla_{SHV} while isolate S28 did not harbor any of the investigated resistance genes.

Overall, bla_{SHV} was the only β -lactamase gene detected among the analyzed isolates in this study. Although isolate S28 exhibited phenotypic resistance to cefoxitin, no bla_{CTX-M} , bla_{SHV} or bla_{TEM} genes were detected. Because cefoxitin resistance is more commonly associated with AmpC β -lactamase production or alterations in outer membrane porins than with classical ESBL enzymes, the resistance phenotype observed in isolate

S28 may be explained by alternative mechanisms that were not investigated in the present study.

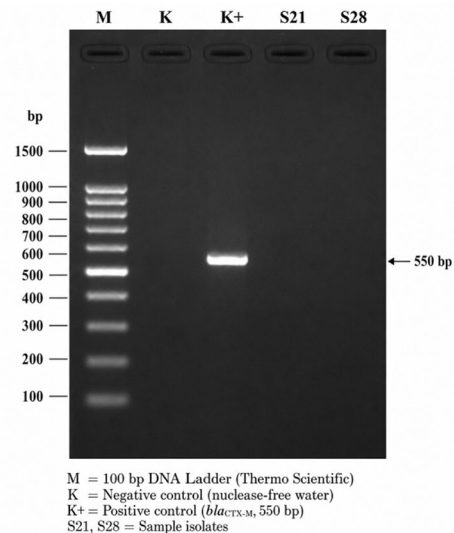


Figure 5. PCR amplification results for bla_{CTX-M} gene among selected antimicrobial-resistant *K. pneumoniae* isolates.

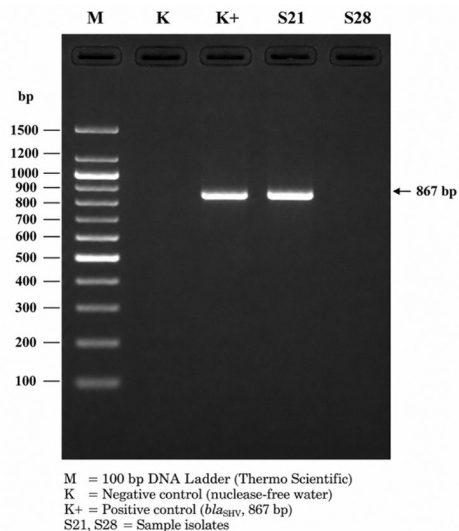


Figure 6. PCR amplification results for bla_{SHV} gene among selected antimicrobial-resistant *K. pneumoniae* isolates.

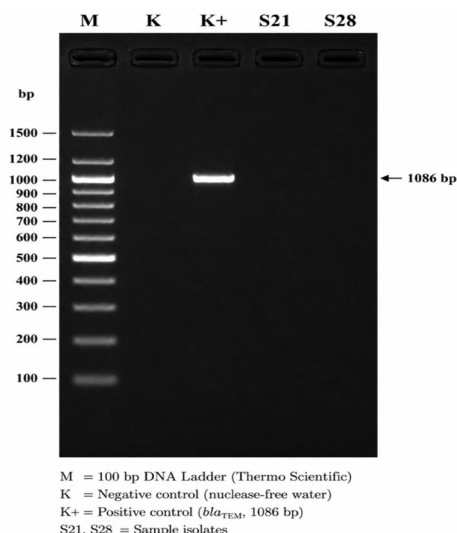


Figure 7. PCR amplification results for bla_{TEM} gene among selected antimicrobial-resistant *K. pneumoniae* isolates.

Discussion

Based on phenotypic confirmation, this study showed that rectal swab samples of sheep from Ngrayung Village, Tuban Regency, had *K. pneumoniae*, with a prevalence of 11.3%. *K. pneumoniae*'s importance as a component of the gut microbiota and as a possible reservoir for AMR is highlighted by its isolation from seemingly healthy livestock (De Maio *et al.*, 2025). Similar results have been documented in livestock systems, where *Enterobacteriaceae*, including *K. pneumoniae*, are regularly found in gastrointestinal samples. This is indicative of the bacteria's widespread presence in agricultural settings and their capacity to endure in animal hosts without manifesting symptoms (Tuhamize and Bazira, 2024).

The characteristic profile of *K. pneumoniae*, which includes lactose-fermenting mucoid colonies, Gram-negative short rods, and an IMViC pattern of indole-negative, methyl red-negative, Voges-Proskauer-positive, and citrate-positive reactions, was confirmed by the phenotypic identification approach used in this study, which combined culture on MacConkey Agar, Gram staining, and biochemical testing (TSIA and IMViC). These results support the validity of traditional diagnostic tech-

niques for first identification in veterinary diagnostic labs, especially in resource-constrained environments, and are in line with traditional microbiological descriptions of *K. pneumoniae* (Bermúdez-Fornos *et al.*, 2025).

Testing for antimicrobial susceptibility showed that the isolates had a worrying pattern of resistance. The poor effectiveness of widely used antibiotics in livestock systems is indicated by universal resistance to erythromycin and considerable resistance to ampicillin (Liu *et al.*, 2024). Even though erythromycin is not usually used as a first-line medication for *Enterobacteriaceae*, co-selection associated with mobile genetic elements or broader macrolide resistance determinants may be the cause of its resistance (Miklasínska-Majdanik, 2021). The high ampicillin resistance is in line with global trends in *K. pneumoniae*, where reports of both intrinsic and acquired β -lactam resistance are common (Zdarska *et al.*, 2026). On the other hand, total susceptibility to aztreonam indicates that this monobactam is still rather effective against the isolates in this group, possibly as a result of less selective pressure or restricted use in local veterinary practice (Sader *et al.*, 2021).

The detection of antimicrobial-resistant *K. pneumoniae* isolates is noteworthy from a One Health perspective. The observed resistance to tetracycline and ceftiofur indicates ongoing selective pressure within the livestock environment and highlights the circulation of resistant bacteria in sheep production systems (Rahayu *et al.*, 2025). The resistance patterns observed, particularly resistance to tetracycline and ceftiofur, may reflect the circulation of resistant clones or horizontal dissemination of resistance determinants among *Enterobacteriaceae* in sheep farms (Bucur *et al.*, 2025). Livestock production systems with weak biosecurity and routine antibiotic usage are known to enhance the establishment and maintenance of antimicrobial-resistant organisms (Widodo *et al.*, 2023).

PCR analysis of β -lactamase genes showed that *bla*_{SHV} was detected in one of the two antimicrobial-resistant isolates analyzed, whereas *bla*_{CTX-M} and *bla*_{TEM} were not detected (Twinomujuni *et al.*, 2026). Interestingly, isolate S28 demonstrated phenotypic resistance to ceftiofur despite testing negative for *bla*_{CTX-M}, *bla*_{SHV}, and *bla*_{TEM}. Since ceftiofur belongs to the cephamycin group, resistance to this antibiotic is generally more indicative of *AmpC* β -lactamase production or alterations in outer membrane porins than of classical ESBL-mediated resistance (Jacoby, 2009; Tamma *et al.*, 2019). Therefore, the absence of the investigated ESBL genes does not necessarily indicate the absence of β -lactam resistance mechanisms in isolate S28.

Alternative mechanisms, including plasmid-mediated or chromosomal *AmpC* β -lactamases, reduced outer membrane permeability caused by porin loss (e.g., *OmpK35/OmpK36* alterations), or other resistance determinants not evaluated in this study, may explain the observed ceftiofur-resistant phenotype. Because *AmpC*-associated genes were not included in the PCR panel, their contribution could not be determined and should be regarded as a limitation of the present study.

The discrepancy between the ceftiofur-resistant phenotype and the absence of *bla*_{CTX-M}, *bla*_{SHV}, and *bla*_{TEM} further highlights the complexity of antimicrobial resistance in *K. pneumoniae*. This finding suggests that screening only the three classical ESBL genes is insufficient to explain all β -lactam resistance phenotypes in livestock-associated isolates (Pires *et al.*, 2024; Wibisono *et al.*, 2025). Future investigations should include *AmpC* β -lactamase genes and comprehensive genomic approaches, such as whole-genome sequencing, to better characterize the molecular mechanisms underlying ceftiofur resistance.

Since animals can act as reservoirs for resistant bacteria that may spread through direct contact, environmental contamination, or the food chain, the detection of antimicrobial-resistant *K. pneumoniae* in sheep is significant from a public health standpoint within the One Health framework (Wareth and Neubauer, 2021). The existence of resistant *Enterobacteriaceae* in agricultural settings emphasizes the necessity of using antibiotics carefully, maintaining better hygiene, and routinely observing resistance patterns in animals raised for food (Abraham *et al.*, 2025).

Overall, the results of this study demonstrate the presence of *K.*

pneumoniae isolates exhibiting clinically relevant antimicrobial resistance characteristics, including ceftiofur resistance and the detection of a *bla*_{SHV} β -lactamase-associated gene, in sheep from Tuban Regency. These findings demonstrate the need for ongoing monitoring and integrated antimicrobial stewardship techniques to stop the spread of resistant bacteria in animal production systems and lower possible health hazards (Liza *et al.*, 2024).

A limitation of this study is that molecular screening was restricted to three ESBL-associated genes (*bla*_{CTX-M}, *bla*_{SHV}, and *bla*_{TEM}). *AmpC* β -lactamase genes and other mechanisms associated with ceftiofur resistance, such as porin alterations or efflux pump overexpression, were not investigated. Consequently, the molecular basis of ceftiofur resistance observed in isolate S28 could not be fully elucidated.

Conclusion

Based on phenotypic identification, this study reveals that *K. pneumoniae* is present in sheep from Tuban Regency, Indonesia, with a prevalence of 11.3%. A small proportion of isolates exhibited acquired resistance to tetracycline and ceftiofur, whereas resistance to ampicillin and erythromycin reflected intrinsic characteristics of *K. pneumoniae*.

Overall, these findings indicate that sheep may serve as reservoirs of antimicrobial-resistant *K. pneumoniae*, underscoring their potential role in the dissemination of antimicrobial resistance within the One Health framework. Therefore, to prevent the emergence of resistant bacteria, careful antibiotic use in livestock production and ongoing surveillance are crucial.

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.

References

- Abraham, A., Mtewa, A.G., Chiutula, C., Mvula, R.L.S., Maluwa, A., Eregno, F.E., Njalam'mano, J., 2025. Prevalence of Antibiotic Resistance Bacteria in Manure, Soil, and Vegetables in Urban Blantyre, Malawi, from a Farm-to-Fork Perspective. *Int. J. Environ. Res. Public Health* 22, 1273. doi: 10.3390/ijerph22081273.
- Alsheikh, A., Shanabla, R., Badawi, A., Al-Momani, H., Nasser-Ali, M., Rjoub, Y., Al-Najjar, M.A.A., Al-Mansi, M., Aolymat, I., Al-Shoubaki, L., Al-Zaa'q, N., 2026. Epidemiology and Molecular Profiles of ESBL-Producing *Klebsiella pneumoniae* in Urinary Tract Infections Across Jordanian Hospitals. *Microorganisms* 14, 1142. doi: 10.3390/microorganisms14051142.
- Ansharieta, R., Ramandinianto, S.C., Effendi, M.H., Plumeriastuti, H., 2021. Molecular identification of *bla*_{CTX-M} and *bla*_{TEM} genes encoding extended-spectrum β -lactamase (ESBL) producing *Escherichia coli* isolated from raw cow's milk in East Java, Indonesia. *Biodiversitas* 22, 1600–1605. doi: 10.13057/biodiv/d220402.
- Bermúdez-Fornos, I., Cepeda, A., Garrido-Maestu, A., Lamas, A., 2025. Detection of *Klebsiella pneumoniae* in Veterinary and Food Matrices Using Loop-Mediated Isothermal Amplification. *Pathogens* 14, 296. doi: 10.3390/pathogens14030296.
- Bucur, I.M., Rus, A., Imre, K., Tirziu, A., Iancu, I., Ivan, A.A., Moza, A.C., Popa, S.A., Hotea, I., Tirziu, E., 2025. A One Health Comparative Study of MDR *Escherichia coli* Isolated from Clinical Patients and Farm Animals in Satu Mare, Romania. *Antibiotics (Basel)* 14, 1157. doi: 10.3390/antibiotics14111157.
- Clinical and Laboratory Standards Institute (CLSI), 2024. Performance standards for antimicrobial susceptibility testing (34th ed., CLSI supplement M100). Wayne, PA: Clinical and Laboratory Standards Institute.
- De Maio, F., Bianco, D.M., Santarelli, G., Rosato, R., Monzo, F.R., Fiori, B., Sanguinetti, M., Posteraro, B., 2025. Profiling the gut microbiota to assess infection risk in *Klebsiella pneumoniae*-colonized patients. *Gut Microbes* 17, 2468358. doi: 10.1080/19490976.2025.2468358.
- Dewi, R.R., Sihombing, J.M., Jajere, S.M., Purba, A., Ma'arif, I., Hafid, H., 2025. Knowledge, attitudes and practices regarding antimicrobial resistance, usage and residues among livestock farmers in North Sumatra, Indonesia. *BMC Agric.* 1, 19. doi: 10.1186/s44399-025-00019-5.
- Effendi, M.H., Bintari, I.G., Aksono, E.B., Hermawan, I.P., 2018. Detection of *bla*_{TEM} Gene of *Klebsiella pneumoniae* Isolated from Swab of Food-Producing Animals in East Java. *Trop. Anim. Sci. J.* 41, 174–178. doi: 10.5398/tasj.2018.41.3.174.
- Eissa, N., Salman, M.B., Younes, A.M., Mohamed, E.S.A., Abu-seida, A.M., Abdulkarim, A., Eldin, A.I.Z., 2025. One health approach on zoonotic multidrug-resistant *Klebsiella pneumoniae* isolated from Egyptian cattle, horses, and humans. *Open Vet. J.* 15, 4219–4234. doi: 10.5455/OVJ.2025.v15.i9.28.
- Enshaie, E., Nigam, S., Patel, S., Rai, V., 2025. Livestock Antibiotics Use and Antimicrobial Resistance. *Antibiotics (Basel)* 14, 621. doi: 10.3390/antibiotics14060621.
- Jacoby, G.A., 2009. *AmpC* beta-lactamases. *Clin. Microbiol. Rev.* 22, 161–182. doi: 10.1128/CMR.00036-08.
- Khairullah, A.R., Moses, I.B., Yanestria, S.M., Dameanti, F.N.A.E.P., Effendi, M.H., Tang, J.Y.H., Tyas-

- ningsih, W., Budiastuti, B., Kusala, M.K.J., Kurniasih, D.A.A., Wardhani, B.W.K., Wibowo, S., Ma'ruf, I.F., Fauziah, I., Ahmad, R.Z., Latifah, L., 2025. Potential of the livestock industry environment as a reservoir for spreading antimicrobial resistance. *Open Vet. J.* 15, 504–518. doi: 10.5455/OVJ.2025.v15.i2.2.
- Khalifa, S.M., Abd El-Aziz, A.M., Hassan, R., Abdelmegeed, E.S., 2021. β -lactam resistance associated with β -lactamase production and porin alteration in clinical isolates of *E. coli* and *K. pneumoniae*. *PLoS One* 16, e0251594. doi: 10.1371/journal.pone.0251594.
- Li, Z., Xin, L., Peng, C., Liu, C., Wang, P., Yu, L., Liu, M., Wang, F., 2022. Prevalence and antimicrobial susceptibility profiles of ESBL-producing *Klebsiella pneumoniae* from broiler chicken farms in Shandong Province, China. *Poult. Sci.* 101, 102002. doi: 10.1016/j.psj.2022.102002.
- Liu, H.C., Tang, H.L., Chiou, C.S., Lin, Y.C., Chiang, M.K., Tung, K.C., Lai, Y.C., Lu, M.C., 2025. Prevalence and Virulence Profiles of *Klebsiella pneumoniae* Isolated from Different Animals. *Vet. Med. Sci.* 11, e70243. doi: 10.1002/vms3.70243.
- Liu, Y., Duan, H., Yang, L., Chen, H., Wu, R., Li, Y., Zhu, Y., Li, J., 2024. Antimicrobial Resistance Profiling of Pathogens from Cooked Donkey Meat Products in Beijing Area in One Health Context. *Vet. Sci.* 11, 645. doi: 10.3390/vetsci11120645.
- Liza, N.A., Hossain, H., Chowdhury, M.S.R., Al Naser, J., Lasker, R.M., Rahman, A., Haque, M.A., Al Mamun, M., Hossain, M.M., Rahman, M.M., 2024. Molecular Epidemiology and Antimicrobial Resistance of Extended-Spectrum β -Lactamase (ESBL)-Producing *Klebsiella pneumoniae* in Retail Cattle Meat. *Vet. Med. Int.* 2024, 3952504. doi: 10.1155/2024/3952504.
- Magiorakos, A.P., Srinivasan, A., Carey, R.B., Carmeli, Y., Falagas, M.E., Giske, C.G., Harbarth, S., Hindler, J.F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D.L., Rice, L.B., Stelling, J., Struelens, M.J., Vatopoulos, A., Weber, J.T., Monnet, D.L., 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin. Microbiol. Infect.* 18, 268–281. doi: 10.1111/j.1469-0691.2011.03570.x.
- Miklasinska-Majdanik, M., 2021. Mechanisms of Resistance to Macrolide Antibiotics among *Staphylococcus aureus*. *Antibiotics (Basel)* 10, 1406. doi: 10.3390/antibiotics10111406.
- Nasilele, S.J., Shawa, M., Kamboyi, H.K., Phiri, B.S.J., Lundu, T., Nundwe, M., Lungu, A., Moonga, L., Ogata, S., Kajihara, M., Sawa, H., Suzuki, Y., Higashi, H., Mudenda, N.B., Hang ombe, M.B., Muzandu, K., 2026. Detection of carbapenem resistance among third-generation cephalosporin-resistant Enterobacterales from small-scale poultry farms in peri-urban Lusaka, Zambia. *Access Microbiol.* 8, 001108.v4. doi: 10.1099/acmi.0.001108.v4.
- Pires, A.J., Pereira, G., Fangueiro, D., Bexiga, R., Oliveira, M., 2024. When the solution becomes the problem: a review on antimicrobial resistance in dairy cattle. *Future Microbiol.* 19, 903–929. doi: 10.2217/fmb-2023-0232.
- Pishtiwan, A.H., Khadija, K.M., 2019. Prevalence of bla_{TEM} , bla_{SHV} , and bla_{CTX-M} Genes among ESBL-Producing *Klebsiella pneumoniae* and *Escherichia coli* Isolated from Thalassemia Patients in Erbil, Iraq. *Mediterr. J. Hematol. Infect. Dis.* 11, e2019041. doi: 10.4084/MJHID.2019.041.
- Prayudi, S.K.A., Effendi, M.H., Lukiswanto, B.S., Az Zahra, R.L., Benjamin, M.I., Kurniawan, S.C., Khairullah, A.R., Silaen, O.S.M., Lisnanti, E.F., Baihaqi, Z.A., Widodo, A., Riwu, K.H.P., 2023. Detection of Genes on *Escherichia coli* Producing Extended Spectrum β -lactamase Isolated from the Small Intestine of Ducks in Traditional Markets Surabaya City, Indonesia. *J. Adv. Vet. Res.* 13, 1600–1608.
- Purbowati, T.E., Effendi, M.H., Puspitasari, Y., Kurniawan, M.A., Aksono, E.B., Rahardjo, D., Tyasningsih, W., Istiana, I., Sari, F.K., Rahmandari, D.A., Budiastuti, B., Rehman, S., Tang, J.Y.H., Khairullah, A.R., Ahmad, R.Z., Agustin, A.L.D., Kurniasih, D.A.A., 2025. Molecular identification of *Klebsiella pneumoniae* that produces extended spectrum β -lactamase (ESBL) in canaries (*Serinus canaria*) imported from Malaysia. *J. Adv. Vet. Res.* 15, 469–475.
- Putri, L.R., Keytumu, M.O., Rahayu, U., Tyasningsih, W., Effendi, M.H., Tang, J.Y.H., Khairullah, A.R., Rehman, S., Kurniasih, D.A.A., Pratama, B.P., Afrani, D.A., Ahmad, R.Z., 2026. Detection of FimH gene of *Klebsiella pneumoniae* isolate from quail cloacal swab in Surabaya city market. *J. Adv. Vet. Res.* 16, 223–226.
- Rahayu, U., Keytumu, M.O., Wibisono, F.J., Effendi, M.H., Tang, J.Y.H., Kendek, I.A., Budiastuti, B., Khairullah, A.R., Rehman, S., Moses, I.B., Ahmad, R.Z., Agustin, A.L.D., 2025. Detection of iss virulence gene in Avian Pathogenic *Escherichia coli* multidrug resistance from quail cloacal swabs in traditional markets in Surabaya, Indonesia. *J. Adv. Vet. Res.* 15, 334–339.
- Riwu, K.H.P., Rantam, F.A., Effendi, M.H., Khairullah, A.R., Widodo, A., Kurniawan, S.C., Ugbo, E.N., 2022. Molecular detection of extended spectrum β -lactamase producing *Klebsiella pneumoniae* from wild deer. *Biodiversitas* 23, 4256–4262. doi: 10.13057/biodiv/d230849.
- Sader, H.S., Carvalhaes, C.G., Arends, S.J.R., Castanheira, M., Mendes, R.E., 2021. Aztreonam/avibactam activity against clinical isolates of Enterobacterales collected in Europe, Asia and Latin America in 2019. *J. Antimicrob. Chemother.* 76, 659–666. doi: 10.1093/jac/dkaa504.
- Sulaiman, A.A., Effendi, M.H., Srimaryanto, L.R., Khairullah, A.R., Kurniasari, P., Tyasningsih, W., Moses, I.B., Ahmad, R.Z., Rehman, S., Budiastuti, B., Afrani, D.A., Yanestria, S.M., Riwu, K.H.P., Widodo, A., 2025. Molecular detection of bla_{SHV} gene in multidrug resistance of *Klebsiella pneumoniae* isolated from chicken egg shell swab from a traditional market in Surabaya. *Open Vet. J.* 15, 2193–2205. doi: 10.5455/OVJ.2025.v15.i5.37.
- Sumiati, Fadilah, R., Darmawan, A., Nadia, R., 2025. Challenges and constraints to the sustainability of poultry farming in Indonesia. *Anim. Biosci.* 38, 802–817. doi: 10.5713/ab.24.0678.
- Tamma, P.D., Doi, Y., Bonomo, R.A., Johnson, J.K., Simner, P.J., Antibacterial Resistance Leadership Group., 2019. A Primer on AmpC β -Lactamases: Necessary Knowledge for an Increasingly Multidrug-resistant World. *Clin. Infect. Dis.* 69, 1446–1455. doi: 10.1093/cid/ciz173.
- Thesia, N.L.N., Khairullah, A.R., Effendi, M.H., Tyasningsih, W., Puspitasari, Y., Ahadini, S.N., Moses, I.B., Yanestria, S.M., Riwu, K.H.P., Wasito, W., Baihaqi, Z.A., Ahmad, R.Z., 2025. Detection of multidrug-resistant *Klebsiella pneumoniae* isolated from duck cloacal swab in Jombang, Indonesia. *Open Vet. J.* 15, 300–306. doi: 10.5455/OVJ.2025.v15.i1.28.
- Tuhamize, B., Bazira, J., 2024. Carbapenem-resistant *Enterobacteriaceae* in the livestock, humans and environmental samples around the globe: a systematic review and meta-analysis. *Sci. Rep.* 14, 16333. doi: 10.1038/s41598-024-64992-8.
- Twinomujuni, M., Musunguzi, B., Asiimwe, M., Mpiima, S.S., Zamarano, H., Orikushaba, I., Muhanguzi, D., Twinamatsiko, C., Mallya, S.P., Samiri, J., Kamugisha, J., Nalumaga, P.P., Kabanda, T., Kasaza, K., Bagenda, C.N., Tuhamize, B., Bazira, J., Ricciardelli, R., Mpeirwe, M., 2026. Molecular characterization of extended-spectrum beta-lactamase-producing bacteria isolated from pregnant women's urine at Itojo Hospital, South Western Uganda. *Access Microbiol.* 8, 001045.v5. doi: 10.1099/acmi.0.001045.v5.
- Wareth, G., Neubauer, H., 2021. The Animal-foods-environment interface of *Klebsiella pneumoniae* in Germany: an observational study on pathogenicity, resistance development and the current situation. *Vet. Res.* 52, 16. doi: 10.1186/s13567-020-00875-w.
- Wibisono, F.J., Effendi, M.H., Tyasningsih, W., Rahmaniari, R.P., Khairullah, A.R., Kendek, I.A., Budiastuti, B., Rianto, V., Nico, D.C., Kurniasih, D.A.A., Salwa, S., Diningrum, D.P., Moses, I.B., Ahmad, R.Z., 2025. Antibiotic resistance profiles of *Escherichia coli* and *Salmonella* spp. isolated from chicken meat sold in traditional markets in Gresik District, East Java, Indonesia. *Open Vet. J.* 15, 2160–2170. doi: 10.5455/OVJ.2025.v15.i5.34.
- Widodo, A., Lamid, M., Effendi, M.H., Khairullah, A.R., Riwu, K.H.P., Yustinasari, L.R., Kurniawan, S.C., Ansori, A.N.M., Silaen, O.S.M., Dameanti, F.N.A.E.P., 2022. Antibiotic sensitivity profile of multidrug-resistant (MDR) *Escherichia coli* isolated from dairy cow's milk in Probolinggo, Indonesia. *Biodiversitas* 23, 4971–4976. doi: 10.13057/biodiv/d231002.
- Widodo, A., Lamid, M., Effendi, M.H., Tyasningsih, W., Raharjo, D., Khairullah, A.R., Kurniawan, S.C., Yustinasari, L.R., Riwu, K.H.P., Silaen, O.S.M., 2023. Molecular identification of bla_{TEM} and bla_{CTX-M} genes in multidrug-resistant *Escherichia coli* found in milk samples from dairy cattle farms in Tulungagung, Indonesia. *J. Vet. Res.* 67, 381–388. doi: 10.2478/jvetres-2023-0052.
- Zdarska, V., Arcari, G., Kolar, M., Mlynarcik, P., 2026. Antibiotic Resistance in *Klebsiella pneumoniae* and Related Enterobacterales: Molecular Mechanisms, Mobile Elements, and Therapeutic Challenges. *Antibiotics (Basel)* 15, 37. doi: 10.3390/antibiotics15010037.