Original Research

Journal of Advanced Veterinary Research
(2026) Volume 16, Issue 4, 519-524

Phenotypic and genotypic characterization of antibiotic-resistant *Salmonella* spp. isolated from sheep rectal swabs in Tuban, Indonesia

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ARTICLE INFO

Received: 03 April 2026

Accepted: 24 June 2026

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Keywords:

Salmonella spp., Antimicrobial resistance, Multidrug resistance, Sheep, Public health.

ABSTRACT

Salmonella spp. are important zoonotic pathogens capable of colonizing the intestinal tract of livestock and contributing to the spread of antimicrobial resistance (AMR). This study aimed to investigate the phenotypic and genotypic characteristics of antibiotic-resistant *Salmonella* spp. isolated from sheep rectal swabs in Tuban, Indonesia. A total of 150 rectal swab samples were collected from sheep and processed using selective enrichment, culture on *Salmonella*-Shigella Agar, and biochemical identification. Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method, while multidrug-resistant (MDR) isolates were further analyzed by polymerase chain reaction (PCR) for the presence of ESBL-associated genes (bla_{TEM}, bla_{SHV}, and $bla_{CTX\text{-}M}$). Out of 150 samples, 20 isolates (13.33%) were confirmed as *Salmonella* spp. Phenotypic analysis revealed high resistance to erythromycin (100%) and tetracycline (95%), while all isolates were fully susceptible to aztreonam (100%). Moderate susceptibility was observed for ampicillin (80%) and cefoxitin (75%). A total of 5 isolates (25%) were classified as MDR. Genotypic analysis showed that only one MDR isolate (20%) harbored the bla_{TEM} gene, whereas bla_{SHV} and $bla_{CTX\text{-}M}$ were not detected in any isolates. These findings demonstrate that sheep can serve as reservoirs of antibiotic-resistant and MDR *Salmonella* spp., posing potential risks to animal health, food safety, and public health. The detection of bla_{TEM} highlights the presence of β-lactam resistance determinants, although other resistance mechanisms may also be involved. Continuous AMR surveillance, rational antibiotic use, and improved farm biosecurity are essential to mitigate the spread of resistant zoonotic pathogens within a One Health framework.

Introduction

Salmonella spp. are among the most important zoonotic bacterial pathogens affecting both animal health and public health worldwide (Galán-Relaño *et al*., 2023). These Gram-negative bacteria belonging to the family Enterobacteriaceae are widely distributed in livestock, poultry, wildlife, food products, and the environment (Ayuti *et al*., 2024; Shaji *et al*., 2023). In food-producing animals, *Salmonella* can colonize the intestinal tract asymptomatically, allowing infected animals to serve as reservoirs and continuously shed the bacteria through feces (Ehuwa *et al*., 2021). This fecal shedding plays a major role in the contamination of farm environments, water sources, animal products, and ultimately the food chain, posing a substantial risk of transmission to humans (Srimaryanto *et al*., 2025).

Sheep are recognized as potential carriers of *Salmonella* spp., particularly in intensive farming systems where close animal contact, environmental contamination, and antimicrobial exposure may facilitate bacterial persistence and dissemination (Khairullah *et al*., 2025). Fecal carriage in sheep is of particular concern because rectal shedding may contribute to cross-contamination among animals and increase the risk of zoonotic transmission to farm workers, slaughterhouse personnel, and consumers through meat contamination (Sillankorva *et al*., 2012). Reports from sheep farming systems have shown that *Salmonella* infection may occur subclinically, making surveillance based on rectal swabs essential for early detection and epidemiological monitoring (Kotton *et al*., 2006).

In recent years, antimicrobial resistance (AMR) in *Salmonella* spp. has emerged as a growing global challenge. The extensive and sometimes inappropriate use of antibiotics in veterinary medicine and livestock production has accelerated the selection of resistant bacterial strains (Salam *et al*., 2023). Resistance to commonly used antimicrobials, particularly tetracyclines, β-lactams, and macrolides, has been increasingly reported in Enterobacteriaceae isolated from animal sources (Zhou *et al*., 2023). This issue is especially important because resistant *Salmonella* strains can compromise treatment efficacy, increase morbidity, and contribute to the spread of resistance genes across bacterial populations through horizontal gene transfer (Ranjan *et al*., 2026).

Of particular concern is the emergence of multidrug-resistant (MDR) *Salmonella* isolates, defined as resistance to three or more classes of antimicrobials (Xu *et al*., 2020). MDR strains not only limit therapeutic options in veterinary and human medicine but also represent a significant One Health threat due to their ability to circulate among animals, humans, and the environment (Temesgen and Shiferaw, 2025). Recent studies have demonstrated high levels of resistance to tetracycline and ampicillin among Enterobacteriaceae from livestock, reflecting the widespread use of these drugs in animal production systems (Wiśniewski *et al*., 2024).

In addition to phenotypic resistance, molecular characterization of resistance determinants is essential to understand the genetic basis of AMR. Extended-spectrum β-lactamase (ESBL)-associated genes, such as bla_{TEM}, bla_{SHV}, and $bla_{CTX\text{-}M}$, are among the most frequently reported β-lactam resistance genes in Enterobacteriaceae (Dirar *et al*., 2020). These genes

ISSN: 2090-6277/2090-6269/

encode enzymes capable of hydrolyzing β-lactam antibiotics, thereby reducing the effectiveness of important antimicrobial agents (Pishtiwan and Khadija, 2019). Studies in Indonesia and other countries have highlighted the growing dissemination of these genes in animal-associated bacteria, emphasizing the need for continued surveillance (Widodo *et al*., 2023).

Despite the recognized public health significance of *Salmonella* and AMR, data on phenotypic and genotypic characteristics of antibiotic-resistant *Salmonella* spp. in sheep farms in Indonesia remain limited, particularly in East Java. Tuban Regency is one of the important livestock-producing areas, yet information regarding the prevalence of *Salmonella* carriage and resistance gene distribution in sheep is still scarce.

Therefore, this study aimed to investigate the phenotypic and genotypic characterization of antibiotic-resistant *Salmonella* spp. isolated from sheep rectal swabs in Tuban, Indonesia, including bacterial isolation, antimicrobial susceptibility profiling, identification of multidrug-resistant isolates, and detection of ESBL-associated resistance genes.

Materials and methods

Study location and period

This study was conducted at a sheep farm located in Ngrayung Village, Plumpang District, Tuban Regency, East Java, Indonesia.

Rectal swab samples were collected directly from the farm, while all bacteriological procedures, including bacterial isolation, identification, antimicrobial susceptibility testing, and molecular analysis, were carried out at the Division of Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga. The study was performed from May to June 2025, encompassing sample collection, laboratory examination, and data analysis.

Sample collection and sampling strategy

A total of 150 rectal swab samples were collected from sheep raised at the study location using a random sampling method to ensure representative sampling across the flock population.

Rectal specimens were collected aseptically using sterile cotton swabs inserted into the rectum and gently rotated to obtain fecal material. Each swab was immediately transferred into sterile Amies transport medium, properly labeled, and maintained in a cool box during transportation to the laboratory to preserve sample viability prior to processing.

Phenotypic detection and identification of Salmonella spp.

For selective enrichment, each rectal swab sample was inoculated into Tetrathionate Broth (TTB; HiMedia, India) and incubated aerobically at 37°C for 24 h.

Following enrichment, a loopful of the culture was streaked onto *Salmonella*-Shigella Agar (SSA; HiMedia, India) using the quadrant streak method and incubated at 37°C for 24 h. Colonies presumptively identified as *Salmonella* spp. were characterized by transparent or colorless colonies with black centers, indicating hydrogen sulfide (H_2S) production.

Presumptive colonies were subsequently subjected to Gram staining. Briefly, bacterial colonies were smeared onto clean glass slides, heat-fixed, and sequentially stained with crystal violet, Lugol’s iodine, 96% ethanol, and safranin. Microscopic observation was performed under oil immersion at 1000× magnification. Gram-negative short rod-shaped bacteria appearing red were considered consistent with *Salmonella* spp.

Biochemical confirmation was performed using Triple Sugar Iron Agar (TSIA), Sulfide Indole Motility (SIM) medium, Methyl Red–Voges Proskauer (MR-VP) test, Simmons Citrate Agar (SCA), and urease test. Isolates showing characteristic biochemical profiles of *Salmonella* spp. were considered confirmed isolates (Wibisono *et al*., 2025).

Phenotypic detection of antibiotic resistance

Antimicrobial susceptibility testing was conducted using the Kirby–Bauer disk diffusion method on Mueller-Hinton Agar (MHA; HiMedia, India).

Pure colonies grown on Nutrient Agar (Oxoid, UK) were suspended in sterile 0.9% NaCl solution and adjusted to a 0.5 McFarland turbidity standard, equivalent to approximately 1.5×10^8 CFU/mL. The standardized bacterial suspension was uniformly spread over the surface of MHA plates using sterile cotton swabs. Antibiotic disks (Oxoid, UK) used in this study included erythromycin (15 μg), ampicillin (10 μg), tetracycline (30 μg), cefoxitin (30 μg), and aztreonam (30 μg).

The inoculated plates were incubated at 37°C for 24 h. After incubation, inhibition zone diameters were measured in millimeters using a digital caliper. The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) 2024 guidelines (Clinical and Laboratory Standards Institute, 2024). Isolates resistant to three or more antimicrobial classes were categorized as multidrug-resistant (MDR).

Genotypic detection of antibiotic resistance genes by PCR

Genotypic characterization of antibiotic resistance genes was performed on confirmed MDR *Salmonella* spp. isolates using Polymerase Chain Reaction (PCR) to detect extended-spectrum β-lactamase (ESBL)-associated genes, namely bla_{SHV}, bla_{TEM}, and bla_{CTX-M}.

These genes were selected because they are widely recognized as important determinants of β-lactam resistance among Enterobacteriaceae, including *Salmonella* spp. The primer sequences used in this study are presented in Table 1.

Genomic DNA was extracted from overnight cultures of confirmed MDR isolates using a commercial bacterial DNA extraction kit according to the manufacturer’s instructions. The extracted DNA was used as the template for PCR amplification.

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

PCR amplification was carried out under the following cycling conditions: initial denaturation at 95°C for 5 min, followed by 35 cycles 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 at 72°C for 7 min.

The amplified PCR products were separated by electrophoresis on 1.5% agarose gel stained with nucleic acid dye and visualized under UV transillumination. Positive amplification was determined by comparing the observed DNA bands with the expected amplicon sizes for each target gene (Srimaryanto *et al*., 2025).

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

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

Data analysis

Data were analyzed descriptively and presented in tables and figures. Phenotypic resistance profiles were determined based on inhibition zone diameters and categorized as susceptible, intermediate, or resistant according to CLSI 2024 criteria.

The prevalence of MDR isolates was calculated as the percentage of isolates resistant to three or more antimicrobial classes. Genotypic results were interpreted based on the presence or absence of specific PCR amplicon bands corresponding to the target resistance genes. The frequency distribution of ESBL-associated genes among MDR *Salmonella* spp. isolates was presented descriptively.

Results

Isolation and phenotypic identification of Salmonella spp.

A total of 150 rectal swab samples collected from sheep at a farm located in Ngrayung Village, Plumpang District, Tuban Regency, were initially enriched in Tetrathionate Broth Base (TTB). Following 24 h incubation at 37°C, all samples (150/150; 100%) exhibited visible turbidity, indicating bacterial growth in the enrichment medium.

Subsequent subculture on *Salmonella*-Shigella Agar (SSA) yielded 61 presumptive isolates (40.67%) with colony morphology suggestive of *Salmonella* spp., characterized by transparent, slightly convex circular colonies with black centers, indicating hydrogen sulfide (H2S) production (Figure 1).

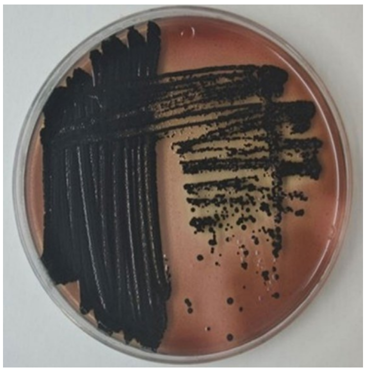

Figure 1. Representative colony morphology of presumptive *Salmonella* spp. on *Salmonella*-Shigella Agar showing transparent colonies with black centers.

Microscopic examination following Gram staining demonstrated short rod-shaped Gram-negative bacteria appearing red under 1000× magnification (Figure 2).

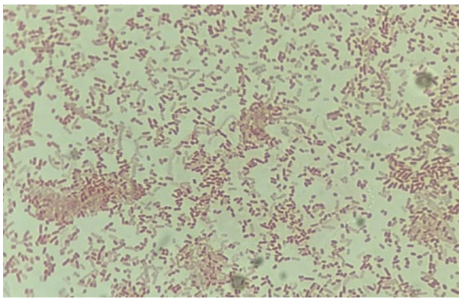

Figure 2. Gram staining of presumptive *Salmonella* spp. showing short rod-shaped Gram-negative bacteria under 1000× magnification.

Further biochemical confirmation was performed using urease, Simmons Citrate Agar (SCA), Triple Sugar Iron Agar (TSIA), Sulfide Indole Motility (SIM), and Methyl Red–Voges Proskauer (MR-VP) tests.

The confirmed isolates showed the typical biochemical characteristics of *Salmonella* spp., namely urease-negative, citrate-positive, alkaline slant/acid butt (B/A) on TSIA with H2S production and no gas formation, indole-negative, motility-positive, methyl red-positive, and Voges–Proskauer-negative.

Out of the 61 presumptive isolates, 20 isolates (13.33% of total samples; 32.79% of presumptive isolates) were biochemically confirmed as *Salmonella* spp. (Figure 3, Table 2).

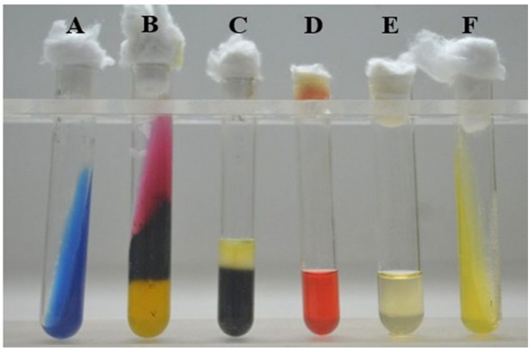

Figure 3. Representative biochemical test results of confirmed *Salmonella* spp. Isolates. (A) SCA test, (B) TSIA test, (C) SIM test, (D) MR test, (E) VP test, and (F) Urease.

Table 2. Biochemical characteristics of confirmed *Salmonella* spp. isolates (n = 20).

No	Sample code	Urease	SCA	TSIA (Slant/Butt)	H_2S	Gas	Indole	Motility	MR	VP
1	S17	–	+	B/A	+	–	–	+	+	–
2	S50	–	+	B/A	+	–	–	+	+	–
3	S57	–	+	B/A	+	–	–	+	+	–
4	S61	–	+	B/A	+	–	–	+	+	–
5	S62	–	+	B/A	+	–	–	+	+	–
6	S75	–	+	B/A	+	–	–	+	+	–
7	S77	–	+	B/A	+	–	–	+	+	–
8	S84	–	+	B/A	+	–	–	+	+	–
9	S85	–	+	B/A	+	–	–	+	+	–
10	S86	–	+	B/A	+	–	–	+	+	–
11	S92	–	+	B/A	+	–	–	+	+	–
12	S94	–	+	B/A	+	–	–	+	+	–
13	S96	–	+	B/A	+	–	–	+	+	–
14	S97	–	+	B/A	+	–	–	+	+	–
15	S99	–	+	B/A	+	–	–	+	+	–
16	S106	–	+	B/A	+	–	–	+	+	–
17	S118	–	+	B/A	+	–	–	+	+	–
18	S119	–	+	B/A	+	–	–	+	+	–
19	S121	–	+	B/A	+	–	–	+	+	–
20	S122	–	+	B/A	+	–	–	+	+	–

Note: B = alkaline; A = acid; + = positive; – = negative.

Antibiotic susceptibility profile of Salmonella spp. isolates

All 20 confirmed *Salmonella* spp. isolates were subjected to antimicrobial susceptibility testing using the Kirby–Bauer disk diffusion method on Mueller-Hinton Agar (Table 3, Figure 4).

Aztreonam demonstrated complete susceptibility, with all isolates (20/20; 100%) classified as sensitive. A very high resistance rate was observed for erythromycin, where all isolates (20/20; 100%) were resistant.

Similarly, tetracycline resistance was detected in 19 isolates (95%), with only one isolate (5%) remaining susceptible. Ampicillin showed 80% susceptibility, with 16 isolates sensitive, 2 intermediate, and 2 resistant. Cefoxitin demonstrated 75% susceptibility, with 15 isolates sensitive and 5 resistant.

Table 3. Antibiotic susceptibility profile of *Salmonella* spp. isolates (n = 20).

Antibiotic	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
Aztreonam	20 (100)	0 (0)	0 (0)
Tetracycline	1 (5)	0 (0)	19 (95)
Erythromycin	0 (0)	0 (0)	20 (100)
Ampicillin	16 (80)	2 (10)	2 (10)
Cefoxitin	15 (75)	0 (0)	5 (25)

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

Multidrug-resistant Salmonella spp. isolates

Analysis of antimicrobial resistance combinations revealed that 5 out of 20 confirmed isolates (25%) met the criteria for multidrug resistance (MDR), defined as resistance to three or more antimicrobial classes. The identified MDR resistance patterns are summarized in Table 4.

Table 4. Multidrug-resistant (MDR) profiles of *Salmonella* spp. isolates.

No	Sample code(s)	Resistance profile
1	S86, S92, S94	TE, E, FOX
2	S96	TE, E, AMP
3	S17	TE, E, AMP, FOX

Note: TE = tetracycline; E = erythromycin; AMP = ampicillin; FOX = cefoxitin.

Genotypic detection of antibiotic resistance genes

To further characterize the molecular basis of antimicrobial resistance, all multidrug-resistant (MDR) *Salmonella* spp. isolates (n = 5) were subjected to PCR-based detection of β-lactamase-associated resistance genes, including $bla_{\text{CTX-M}}$, bla_{SHV}, and bla_{TEM} (Figures 5–7).

Among the five MDR isolates tested, only one isolate (S17; 20%) was found to carry the bla_{TEM} gene, whereas all isolates (100%) were negative for $bla_{\text{CTX-M}}$ and bla_{SHV} (Table 5).

Specifically, isolate S17, which exhibited resistance to tetracycline, erythromycin, ampicillin, and cefoxitin, showed positive amplification of bla_{TEM}.

In contrast, the remaining MDR isolates (S86, S92, S94, and S96) did not harbor any of the tested genes.

These findings indicate that bla_{TEM} was the only detected β-lactamase gene among the MDR *Salmonella* spp. isolates, while resistance in the remaining isolates may be associated with alternative resistance determinants not examined in this study.

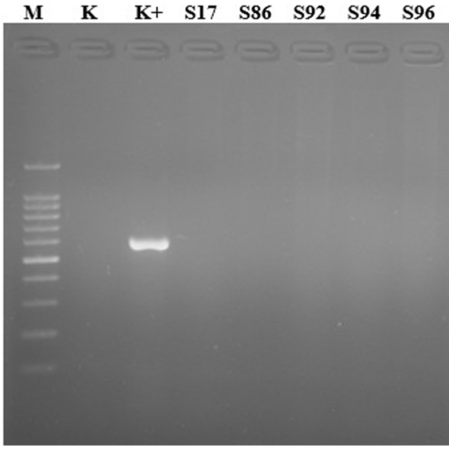

Figure 5. PCR amplification results for $bla_{\text{CTX-M}}$ gene among MDR *Salmonella* spp. isolates.

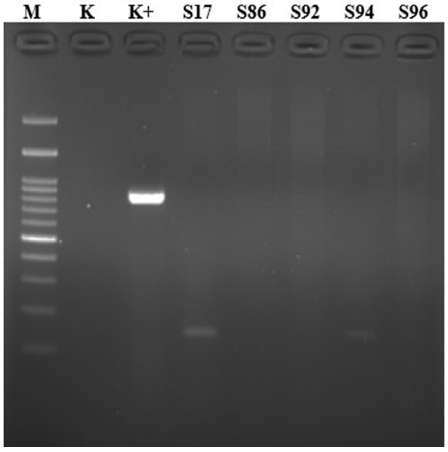

Figure 6. PCR amplification results for bla_{SHV} gene among MDR *Salmonella* spp. isolates.

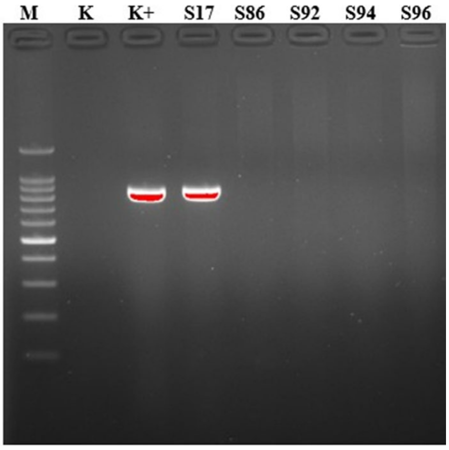

Figure 7. PCR amplification results for bla_{TEM} gene showing positive amplification in isolate S17.

Table 5. Distribution of antibiotic resistance genes among MDR *Salmonella* spp. isolates.

Isolate code	$bla_{\text{CTX-M}}$	bla_{SHV}	bla_{TEM}
S17	Negative	Negative	Positive
S86	Negative	Negative	Negative
S92	Negative	Negative	Negative
S94	Negative	Negative	Negative
S96	Negative	Negative	Negative

Discussion

The present study demonstrated that *Salmonella* spp. were isolated from sheep rectal swabs collected from a farm in Tuban, East Java, with a confirmed prevalence of 13.33% (20/150) following biochemical identification. This finding confirms that sheep can serve as intestinal carriers of *Salmonella* spp. and act as a potential source of environmental contamination and zoonotic transmission (Hawwas *et al*., 2022). The ability of *Salmonella* to colonize the gastrointestinal tract without necessarily causing overt clinical signs has been widely recognized, particularly in ruminant livestock (García-Díez *et al*., 2024). Fecal shedding from apparently healthy animals is epidemiologically important because it facilitates dissemination through feces, contaminated feed, water, bedding, and direct contact between animals (Penakalapati *et al*., 2017). From a One Health perspective, this carriage status increases the risk of transmission to farm workers, slaughterhouse personnel, and consumers through contaminated meat products (Aguilar *et al*., 2025).

The proportion of presumptive isolates on *Salmonella*-Shigella Agar (40.67%) that decreased to 13.33% after biochemical confirmation highlights the importance of confirmatory testing. Although SSA is highly useful for selective isolation, colony morphology alone may not be sufficiently specific because other Enterobacteriaceae may also produce H2S-positive colonies (Lee *et al*., 2020). Therefore, the use of Gram staining and a complete biochemical panel, including TSIA, SIM, citrate, urease, and MR-VP, was essential to improve diagnostic accuracy. The biochemical characteristics observed in this study, namely urease-negative, citrate-positive, H2S-positive, motility-positive, and MR-positive profiles, are consistent with the classical phenotypic characteristics of *Salmonella* spp., supporting the reliability of the identification method used (Olsen *et al*., 1992).

A major finding of this study was the very high level of phenotypic resistance to erythromycin (100%) and tetracycline (95%). These results suggest strong antimicrobial selection pressure within the farm environment (Urban-Chmiel *et al*., 2022). Tetracycline resistance is commonly reported in livestock-associated Enterobacteriaceae and is frequently linked to its extensive use in veterinary practice for therapeutic, prophylactic, and growth-related purposes in many farming systems (Pazra *et al*., 2023). Similar observations have been reported in ruminant-associated *Salmonella* isolates, where tetracycline remains one of the most common resistance phenotypes (Pavelquesi *et al*., 2021).

The complete resistance to erythromycin should be interpreted cautiously. Because *Salmonella* is a Gram-negative bacterium, intrinsic low permeability of the outer membrane and efflux mechanisms may naturally contribute to reduced susceptibility to macrolides (Li *et al*., 2015). Thus, this high resistance rate may reflect both intrinsic and acquired mechanisms. In contrast, the 100% susceptibility to aztreonam and relatively high susceptibility to ampicillin (80%) and cefoxitin (75%) indicate that not all β-lactam agents have been compromised in this farm population (Aydin *et al*., 2024). These findings may suggest limited exposure to certain β-lactam classes or lower selective pressure for these antibiotics compared with tetracycline (Urban-Chmiel *et al*., 2022).

Importantly, 25% of confirmed isolates were classified as multidrug-resistant (MDR). The presence of MDR *Salmonella* in apparently healthy sheep is a significant public health concern because these strains may disseminate through the food chain and the farm environment (García-Díez *et al*., 2024). MDR phenotypes involving tetracycline, erythromycin, ampicillin, and cefoxitin indicate the coexistence of resistance across multiple antimicrobial classes (Li *et al*., 2022). Such patterns are often associated with mobile genetic elements, including plasmids, integrons, and transposons, which facilitate horizontal gene transfer between bacteria in the gastrointestinal tract (Tokuda and Shintani, 2024).

At the molecular level, only one MDR isolate (S17) harbored the bla_{TEM} gene, whereas $bla_{\text{CTX-M}}$ and bla_{SHV} were not detected in any isolate. The detection of bla_{TEM} is particularly important because this gene is one of the most widely distributed β-lactamase determinants among Enterobacteriaceae and has been frequently associated with resistance to ampicillin and other β-lactam antibiotics (Widodo *et al*., 2023). The positive amplification of bla_{TEM} in isolate S17 is consistent with its resistance phenotype, particularly to ampicillin and cefoxitin.

Interestingly, four MDR isolates did not carry any of the tested ESBL-associated genes. This suggests that alternative resistance mechanisms may be involved, such as other β-lactamase genes (e.g., bla_{OXA}, bla_{CMY}, or AmpC-type genes), porin loss, efflux pump overexpression, or chromosomal mutations affecting antibiotic targets (Bajpai *et al*., 2017). This finding indicates that phenotypic resistance cannot always be fully explained by the limited panel of genes tested and highlights the need for broader molecular screening in future studies (Bard and Lee, 2018).

The findings of this study emphasize that sheep may act as reservoirs of antibiotic-resistant and multidrug-resistant *Salmonella* spp. in livestock production systems in Indonesia. The combination of fecal carriage, high tetracycline resistance, and detection of bla_{TEM} underscores the importance of routine AMR surveillance, prudent antibiotic use, and strengthened farm biosecurity measures (Enshaie *et al*., 2025). These efforts are essential to reduce the spread of resistant zoonotic pathogens and support integrated One Health strategies for antimicrobial stewardship (Velazquez-Meza *et al*., 2022).

Conclusion

This study demonstrated that sheep in Tuban, Indonesia, can serve as carriers of antibiotic-resistant *Salmonella* spp., with a confirmed prevalence of 13.33% from rectal swab samples. Phenotypic analysis revealed a high level of resistance to erythromycin and tetracycline, while 25% of the confirmed isolates were classified as multidrug-resistant (MDR). Molecular characterization identified the bla_{TEM} gene in one MDR isolate, whereas $bla_{\text{CTX-M}}$ and bla_{SHV} were not detected.

These findings indicate that sheep may act as a potential reservoir for the dissemination of antibiotic-resistant and MDR *Salmonella* spp., posing risks to animal health, food safety, and public health. Therefore, routine antimicrobial resistance surveillance, prudent antibiotic use, and improved farm biosecurity measures are essential to minimize the spread of resistant zoonotic pathogens within a One Health framework.

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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