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Genetic relatedness and antimicrobial resistance of *Staphylococcus aureus* in goats with subclinical mastitis, North Sumatra, Indonesia

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Abstract. *Manalu SMR, Lisnawita, Hanafi ND, Siregar LAM. 2026. Genetic relatedness and antimicrobial resistance of* Staphylococcus aureus *in goats with subclinical mastitis, North Sumatra, Indonesia. Biodiversitas 27 (2): d270233. https://doi.org/10.13057/biodiv/d270233. Staphylococcus aureus* is widely recognized as a dominant etiological agent of mastitis in dairy goats and is also regarded as a zoonotic pathogen capable of causing a broad spectrum of human infections, including severe and life-threatening conditions. Despite the routine use of antimicrobial agents to control *S. aureus* infections, increasing resistance to multiple antibiotic classes has substantially reduced treatment effectiveness. This study investigated the isolation and molecular characterization of *S. aureus* associated with subclinical mastitis in dairy goats (*Capra hircus*) from Deli Serdang and Serdang Bedagai Regencies, North Sumatra, and evaluated antimicrobial susceptibility against commonly used antibiotics in regional livestock production. Descriptive analysis supported by graphical visualization and phylogenetic reconstruction was applied to assess distribution patterns and genetic relatedness, while statistical comparisons of antimicrobial resistance proportions between regencies were performed using Fisher's exact test. This study confirms *S. aureus* as the most frequently bacterium isolated bacterium in CMT-positive samples based on phenotypic and molecular analyses. High resistance to β-lactam antibiotics was observed in both regions. In Deli Serdang, resistance to amoxicillin and penicillin G was detected in 73.8% (31/42) and 71.4% (30/42) of isolates, respectively. Similarly, in Serdang Bedagai, resistance to amoxicillin and penicillin G was found in 69.0% (20/29) and 58.6% (17/29) of isolates. In contrast, tetracycline showed a higher susceptibility profile in both regions. No statistically significant differences were observed between the two regencies for amoxicillin, penicillin G, or tetracycline resistance ($p>0.05$). Partial 16S rRNA analysis placed the isolates within *S. aureus* clade, showing high sequence similarity to reference strains reported from Brazil and India. Collectively, these findings provide essential baseline evidence supporting rational antimicrobial selection and continued resistance surveillance in caprine mastitis management.

Keywords: Antimicrobial susceptibility testing, dairy goats, molecular epidemiology, small ruminant mastitis, zoonotic pathogens

INTRODUCTION

Staphylococcus aureus is a principal bacterial agent of mastitis in dairy goats (*Capra hircus*), with subclinical cases predominating and frequently escaping detection. Despite the absence of overt symptoms, these infections markedly impair milk yield and quality, generating sustained economic losses in small ruminant systems (Bashabsheh et al. 2024). Its predominance is linked to its capacity for persistent intramammary colonization and immune evasion (Touaitia et al. 2025). Moreover, the production of heat-resistant toxins enables survival through standard processing, elevating the risk of foodborne exposure via contaminated dairy products (Francis et al. 2024), particularly in contexts with suboptimal milk hygiene.

Subclinical mastitis attributable to *S. aureus* constitutes both a veterinary burden and a zoonotic threat with significant public health ramifications. In humans, this pathogen is implicated in a wide clinical continuum, from minor cutaneous infections to critical, life threatening diseases (Lubna et al. 2023; Touaitia et al. 2025). The convergence of animal and human reservoirs highlights the necessity for vigilant surveillance in livestock systems, particularly where intensive human-animal interactions and exposure to raw animal products occur (Tibebu et al. 2025). This concern is heightened in developing regions, where goat milk remains integral to nutrition and livelihoods and is frequently consumed with minimal processing, thereby increasing the risk of zoonotic transmission (Miles and Huson 2021; Fayisa and Tuli 2023).

Antimicrobial agents remain a cornerstone for the control and treatment of *S. aureus* infections in both veterinary and human medicine. However, inappropriate antibiotic usage, including empirical treatment, subtherapeutic dosing, and inadequate withdrawal practices, has accelerated the emergence and dissemination of antimicrobial resistance (AMR) (Lianou and Fthenakis 2022). The growing prevalence of AMR constitutes a major challenge that compromises disease management strategies, threatens livestock productivity, and increases the risk of therapeutic failure in human health systems (Iraguha et al. 2024). Global surveillance studies have consistently documented

rising resistance rates in *S. aureus*, particularly to β-lactam antibiotics, a trend frequently associated with traditional husbandry practices and limited regulation of antimicrobial use in smallscale or low input farming systems (Yosyana et al. 2024).

Indonesia sustains a large small-ruminant sector in which goats play a central role in rural and peri-urban production systems (Sujarwanta et al. 2024). In regions such as North Sumatra, husbandry is largely traditional, with constrained biosecurity, limited veterinary input, and unregulated antimicrobial use (Dewi et al. 2024). Despite the acknowledged significance of mastitis and antimicrobial resistance, region-specific data on *S. aureus* prevalence in subclinical cases and its resistance profiles remain limited (Dhital et al. 2024), restricting the development of evidence-driven control and stewardship strategies (Ahmed et al. 2025).

Previous studies on antimicrobial resistance (AMR) in mastitis associated bacteria have largely depended on phenotypic assays, particularly antibiotic susceptibility testing (Suwito et al. 2022; Fatmawati et al. 2023; Putra et al 2023; Yosyana et al. 2024; Aziz et al. 2025). Although informative, these approaches do not resolve genetic relationships, evolutionary trajectories, or transmission patterns. In contrast, phylogenetic analysis coupled with molecular identification provides deeper insight into population structure and enables alignment of local isolates with global lineages (Xu et al. 2023). Thus, integrating phenotypic and molecular frameworks is critical to situate AMR within an evolutionary context (Greening et al. 2021; Hackmann 2025). Nevertheless, such molecular and phylogenetic data for mastitis-associated *S. aureus* in goats from North Sumatra remain scarce or absent.

Integrated studies combining phenotypic characterization, AMR profiling, and molecular phylogenetic analysis of *S. aureus* isolated from goats with subclinical mastitis have not been systematically conducted in North Sumatra. Addressing this knowledge gap is crucial for improving mastitis management strategies and strengthening AMR surveillance in small ruminant production. Therefore, the present study aimed to isolate and molecularly characterize *S. aureus* from milk samples of goats with subclinical mastitis raised under traditional farming systems in Deli Serdang and Serdang Bedagai Regencies, North Sumatra, Indonesia. Specifically, this study sought to determine the occurrence of *S. aureus* among California Mastitis Test-positive goats, to evaluate antimicrobial susceptibility patterns against commonly used antibiotics in dairy goat health management, and to assess the phylogenetic placement of selected isolates based on 16S rRNA gene sequences in relation to reference strains reported globally.

MATERIALS AND METHODS

Sampling location

The research was carried out between April and October 2025, encompassing Deli Serdang and Serdang Bedagai Regencies which are the regencies with the highest goat populations in North Sumatra, Indonesia (BPS 2024). A total of 17 smallholder dairy goat farms operating under traditional management systems were included, comprising 10 farms in Deli Serdang Regency and 7 farms in Serdang Bedagai Regency (Figure 1).

Study sites were selected using databases from regency animal health services. Eligible farms required owner consent, actively lactating dairy goats, and willingness to join interviews and milk sampling. If a selected farm was inaccessible, a similar farm from the same area was used as a replacement. All farms used intensive backyard systems, with goat pens typically located near the house. All surveyed holdings operated household-centered confinement systems for dairy goats, with adjacent housing structures, applying largely standardized management practices encompassing hand milking, minimal preventive health protocols, and feed formulations derived predominantly from locally sourced vegetation and agricultural by-products.

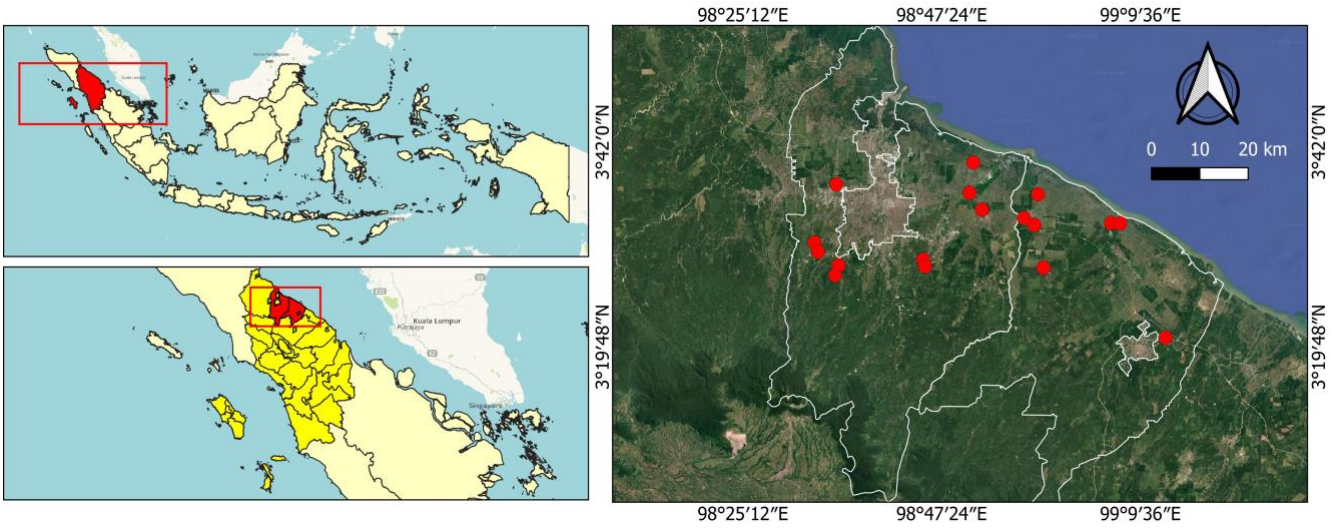


Figure 1. Map of milk sampling locations from traditional goat farms in Deli Serdang and Serdang Bedagai Regencies, North Sumatra Province, Indonesia

Milk samples were collected from 123 lactating does, including 72 goats from Deli Serdang and 51 goats from Serdang Bedagai. Sampling encompassed all dairy goats undergoing active lactation at each farm during the study period, from which milk samples were collected for analysis. Milk samples were collected from smallholder dairy goat farms located in multiple sub-regencies within Deli Serdang and Serdang Bedagai Regencies. The inclusion of farms from different sub-regencies was intended to capture the spatial distribution of traditional dairy goat production systems within the study area. All sampled animals were clinically asymptomatic for mastitis at the time of collection. Before milk sampling, teat ends were cleaned and disinfected using sterile cotton swabs soaked in 70% ethanol and allowed to air-dry prior to sample collection. Fresh milk was aseptically collected into sterile containers for subsequent analysis. The sampled goats constituted the initial population for screening of subclinical mastitis.

Ethical approval

The research protocol was evaluated and approved in advance by the Ethics Committee of Universitas Sumatera Utara, Indonesia (Approval No. 367/KEPK/USU/2025). All animal-related procedures were performed in compliance with applicable institutional and national regulations governing animal welfare, and milk sampling was carried out using non-invasive methods with prior permission obtained from the respective farm owners.

Implementation of the California Mastitis Test (CMT)

Screening for subclinical mastitis was performed using the California Mastitis Test (CMT) in accordance with established field-based protocols. An equal proportion of fresh milk and CMT reagent was combined on a four-well paddle and gently agitated to facilitate reaction development. Outcomes were evaluated through visual inspection of gel consistency and viscosity alterations, and reactions were graded on a scale ranging from 0 (negative, no reaction) to +3. A score of 0 (negative) indicated no visible change in the mixture; +1 indicated slight viscosity without gel formation; +2 indicated moderate gel formation; and +3 indicated strong gel formation with pronounced viscosity. Samples displaying reaction intensities of +1 to +3 were regarded as indicative of subclinical mastitis (Khasanah et al. 2025). Milk samples yielding positive CMT reactions were aseptically collected in sterile containers at a volume of 10 mL, individually labeled, and preserved under refrigerated conditions (approximately 4°C) using an insulated ice box (Suwito et al. 2022). For each CMT-positive goat, one representative isolate was selected for further analysis. All samples were transported to the Balai Veteriner Medan on the day of collection, within a maximum interval of six hours, for subsequent bacteriological isolation, antimicrobial susceptibility testing, and molecular identification.

Isolation and characterization of *Staphylococcus aureus*

The isolation of *S. aureus* was initiated by enrichment in buffered peptone water (pH 7.0), followed by sequential cultivation on blood agar, mannitol salt agar (MSA), and Baird-Parker agar (BPA). The use of selective media (MSA and BPA) targeted *Staphylococcus* spp., which may explain the exclusive recovery of *S. aureus* isolates. Presumptive isolates were subsequently subjected to phenotypic confirmation using Gram staining as well as catalase and coagulase assays. All laboratory procedures were performed in accordance with the Indonesian National Standard (SNI) 2897:2008 for microbiological testing of meat, eggs, milk, and related processed products (BSN 2008).

Antibiotic resistance test of *Staphylococcus aureus* isolates

Based on records from the Indonesian Animal Health Information System (iSIKHNAS), penicillin, tetracycline, and amoxicillin are among the antimicrobial agents most frequently administered for disease management in goats (Ministry of Agriculture of the Republic of Indonesia 2024). Therefore, the selected antibiotics reflect commonly used therapeutic agents in the study region rather than representing a comprehensive antimicrobial resistance profiling panel. Accordingly, susceptibility testing of *S. aureus* isolates in this study was performed using penicillin G (10 IU; CT0043B, Oxoid), tetracycline (30 µg; CT0054B, Oxoid), and amoxicillin (25 µg; CT0061B, Oxoid). Quality control was performed using *S. aureus* ATCC 25923 and antimicrobial susceptibility was evaluated following the Kirby-Bauer disc diffusion protocol in accordance with the Clinical and Laboratory Standards Institute guidelines (CLSI M100) (CLSI 2025).

Confirmed *S. aureus* isolates were revived on Mueller-Hinton agar (Oxoid) and incubated at 37°C for 18-24 h. Well-isolated colonies were then aseptically suspended in sterile distilled water and adjusted to a turbidity equivalent to the 0.5 McFarland standard. The standardized suspension was evenly inoculated onto Mueller-Hinton agar plates using a sterile cotton swab. Plates were allowed to air-dry briefly at ambient temperature (approximately 29°C) prior to the placement of antibiotic discs using sterile forceps. Following incubation at 37°C for 18-24 h, inhibition zone diameters were measured in millimeters with a digital caliper and documented for analysis (CLSI 2025). Each isolate was tested once under uniform experimental conditions.

Descriptive analysis was used to present antimicrobial resistance patterns as frequencies and percentages. Inferential analysis was subsequently performed to compare resistance proportions between regencies using Fisher's exact test, selected due to small sample sizes. Effect size measures, including odds ratios and 95% confidence intervals, were computed to facilitate epidemiological interpretation. Statistical analyses were conducted using SPSS Statistics.

Molecular identification of *Staphylococcus aureus* isolates

The selection of bacterial isolates for PCR-based molecular confirmation and sequencing was guided by phenotypic resistance patterns identified through the Kirby-Bauer disk diffusion assay. Isolates displaying multidrug resistance to penicillin G, amoxicillin, and tetracycline were prioritized. Focusing on resistant phenotypes was intended to enhance the clinical and epidemiological relevance of the molecular analysis, as resistance to β-lactam and tetracycline

classes has been increasingly linked to the emergence and regional circulation of resistant *S. aureus* lineages (Gharaibeh et al. 2025; Moawad et al. 2025; Narwal et al. 2025; Rodrigues et al. 2025; Yaovi et al. 2025). The entire molecular characterization of *S. aureus* bacteria in this study followed the standard protocol of the 16S rRNA gene Sanger sequencing service provided by Macrogen Inc. Seoul, Korea. The procedure included DNA extraction, 16S rRNA gene amplification, DNA sequencing, and analysis of the sequence results.

DNA extraction was performed by aseptically transferring pure bacterial colonies into 100 µL of sterile distilled water, followed by centrifugation at 10,000 rpm for 10 min to concentrate the cells. After removal of the supernatant, the pellet was treated with 50 µL of InstaGene Matrix (Bio-Rad, USA) and incubated at 56°C for 30 min, then subjected to thermal disruption at 100°C for 10 min to ensure complete cell lysis. The samples were subsequently cooled on ice for 5 min and centrifuged at 12,000 rpm for 5 min, after which the DNA-containing supernatant was collected and stored at -20°C until Polymerase Chain Reaction (PCR) analysis.

The 16S rRNA gene was amplified using universal primers 27F and 1492R in a 20 µL PCR mixture containing Taq buffer, dNTPs, primers, genomic DNA template, DNA polymerase, and nuclease-free water. Amplification was performed under standard thermal cycling conditions, and PCR products were confirmed by 1% agarose gel electrophoresis using GelRed staining and ultraviolet visualization. DNA sequencing was carried out using the Sanger dideoxy termination approach with BigDye Terminator Cycle Sequencing Kit v3.1 and internal primer 785F/907R to obtain high-quality bidirectional reads. The sequencing products were analyzed on an Applied Biosystems 3730XL automated sequencer at Macrogen Inc. (Seoul, Korea).

Sequence alignment was conducted using the ClustalW algorithm implemented in MEGA. The evolutionary history was inferred using the Maximum Likelihood method under the Tamura-Nei nucleotide substitution model. Initial trees for the heuristic search were generated automatically using Neighbor-Joining and BioNJ algorithms, and the topology with the highest log likelihood was selected. The robustness of the inferred tree was assessed by bootstrap analysis with 1,000 replicates, and bootstrap values were indicated at the corresponding branch nodes. The final phylogenetic tree was visualized and edited using Molecular Evolutionary Genetics Analysis version X (MEGA X) software. Isolates demonstrating nucleotide identity of at least 97% were regarded as belonging to the same or closely related species (Riva et al. 2023).

RESULTS AND DISCUSSION

California Mastitis Test and characterization of *S. aureus*

The geographic distribution of sampled farms and screened animals across Deli Serdang and Serdang Bedagai Regencies is illustrated in Figure 1. Milk samples were obtained from smallholder goat farms located in multiple sub-regencies within both regencies, reflecting the spatial spread of traditional dairy goat production systems in the study area. California Mastitis Test positive cases were identified in farms from each Regency, indicating that subclinical mastitis occurred across the sampled locations rather than being confined to a single cluster. Out of 123 goats subjected to examination, 71 (57.7%) were identified as positive for the California Mastitis Test (CMT). A total of 71 *S. aureus* isolates were recovered from these samples, including 42 isolates from Deli Serdang and 29 from Serdang Bedagai from each Regency. All isolates were further evaluated for antimicrobial susceptibility, and 4 isolates exhibiting resistance to all tested antibiotics were selected as representatives for 16S rRNA gene-based sequencing analysis.

Based on culture results on Blood Agar (BA), Mannitol Salt Agar (MSA), and Baird Parker Agar (BPA), all isolates recovered from CMT-positive samples were confirmed as *S. aureus* (Figure 2). The results of the bacterial culture test showed the characteristics of *S. aureus* and were confirmed by Gram staining (Figure 3). The Gram stain results indicated that the morphology of the bacterial colonies was round, purplish, and arranged in clusters, a typical morphological characteristic of *S. aureus* bacteria.

The results of the catalase and coagulase tests on each bacterial isolate, such as *S. aureus*, all showed positive results for both catalase and coagulase tests (Figure 4). A positive result for the catalase test indicated the formation of bubbles on the bacterial colony when H_2O_2 was added to the slide, while a positive result for the coagulase test was demonstrated by plasma clot formation in the solution in the tube. These results confirmed the findings of the previous series of identification tests.

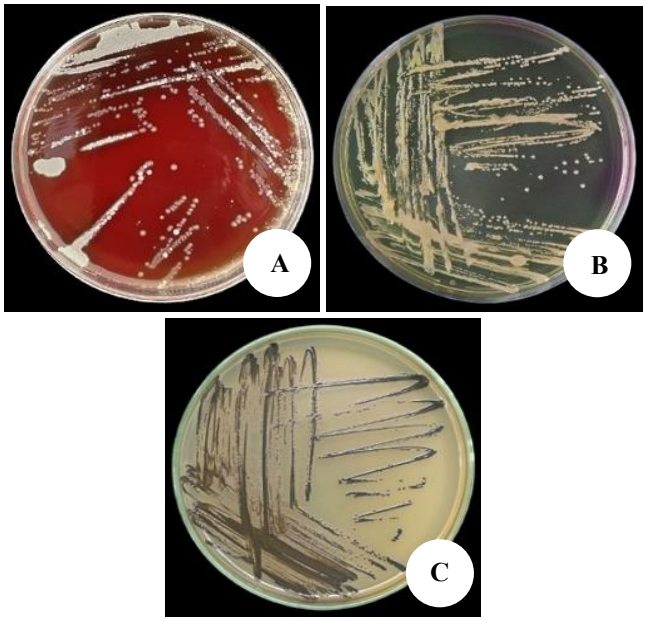


Figure 2. Results of bacterial culture of *S. aureus* isolates from goat milk with subclinical mastitis in Deli Serdang and Serdang Bedagai. Note: A: colony morphology on Blood Agar media; B: colony morphology on Mannitol Salt Agar media; C: colony morphology on Baird-Parker Agar media

Antimicrobial susceptibility tests

Antimicrobial susceptibility tests were performed on all *S. aureus* isolates to evaluate their responses to amoxicillin, penicillin G, and tetracycline. Susceptibility profiling indicated reduced β-lactam effectiveness across isolates from both regions, with intermediate responses to all tested antibiotics occurring at comparable levels between Regencys (Figure 5). In Deli Serdang Regency, 31 of 42 isolates (73.8%) were resistant to amoxicillin, while resistance to penicillin G was detected in 30 isolates (71.4%) (Table 1). A comparable resistance pattern was observed in Serdang Bedagai Regency, where amoxicillin and penicillin G resistance were identified in 20 (69.0%) and 17 (58.6%) of 29 isolates, respectively. By comparison, tetracycline exhibited a more favorable activity profile across both Regencys. The majority of isolates remained susceptible to tetracycline, accounting for 31 isolates (73.8%) in Deli Serdang and 17 isolates (58.6%) in Serdang Bedagai, whereas resistance to this antibiotic was less frequently observed. These findings provide insight into resistance patterns against commonly administered antibiotics in the region but do not represent an exhaustive antimicrobial resistance profile. Fisher's exact test revealed no statistically significant differences ($p>0.05$) in resistance prevalence between Deli Serdang and Serdang Bedagai Regencies for amoxicillin ($p = 0.789$), penicillin G ($p = 0.460$), or tetracycline ($p = 0.782$) (Table 2). Estimation of effect magnitude yielded expansive confidence intervals across all antimicrobial agents, reflecting insufficient statistical precision to reliably discern regional disparities. Accordingly, the present findings do not substantiate definitive inferences regarding geographic variation, and investigations incorporating larger sample frameworks are required to generate more stable and conclusive estimates.

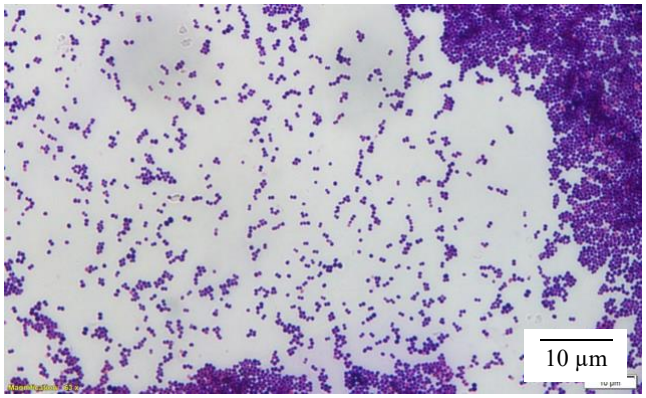
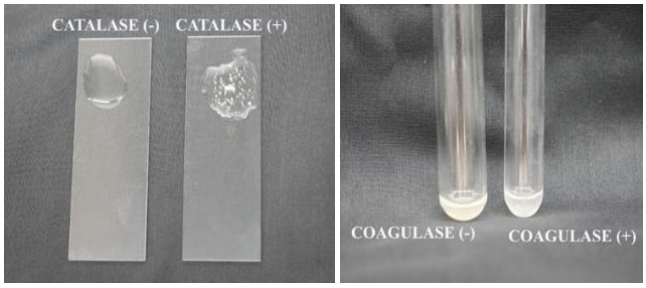


Figure 3. Gram staining results of *S. aureus* isolates from goat milk with subclinical mastitis in Deli Serdang and Serdang Bedagai Regencies, North Sumatra Province, Indonesia



A **B**

Figure 4. Results of catalase and coagulase tests on *S. aureus* isolates from goat milk with subclinical mastitis in Deli Serdang and Serdang Bedagai Regencies, North Sumatra Province, Indonesia. Note: A: catalase test results; B: coagulase test results

Table 1. Antimicrobial susceptibility profiles of *S. aureus* isolates by district

Regency	Antibiotics	Interpretation			Total (n)
		Susceptible (%)	Intermediate (%)	Resistant (%)	
Deli Serdang	Amoxicillin	4 (9.52)	7 (16.67)	31 (73.81)	42
	Penicillin G	5 (11.90)	7 (16.67)	30 (71.43)	42
	Tetracycline	31 (73.81)	2 (4.67)	9 (21.43)	42
Serdang Bedagai	Amoxicillin	3 (10.34)	6 (20.69)	20 (68.97)	29
	Penicillin G	6 (20.69)	6 (20.69)	17 (58.62)	29
	Tetracycline	17 (58.62)	5 (17.24)	7 (24.14)	29

Table 2. Comparison of resistance prevalence to selected antibiotics between Regencies

Antibiotic	Regency	Resistant (%)	Non-resistant (%)	Odds ratio (95% CI)	Fisher's exact test (p-value)
Amoxicillin	Deli Serdang	31 (73.8)	11 (26.2)	0.79 (0.28-2.24)	0.789
	Serdang Bedagai	20 (69.0)	9 (31.0)	-	-
Penicillin G	Deli Serdang	30 (71.4)	12 (28.6)	0.57 (0.21-1.54)	0.312
	Serdang Bedagai	17 (58.6)	12 (41.4)	-	-
Tetracycline	Deli Serdang	9 (21.4)	33 (78.6)	1.17 (0.38-3.60)	0.782
	Serdang Bedagai	7 (24.1)	22 (75.9)	-	-

Note: Values are presented as number of isolates followed by percentages within each regency. Odds ratios (OR) represent the likelihood of antimicrobial resistance in isolates from Deli Serdang relative to Serdang Bedagai. Statistical differences in resistance prevalence between regencies were assessed using Fisher's exact test due to limited sample sizes. CI: confidence interval

Molecular identification

Among the 71 recovered isolates, 4 fulfilled the operational criteria for multidrug resistance, exhibiting concurrent resistance to penicillin G, amoxicillin, and tetracycline. These 4 MDR isolates were consequently subjected in their entirety to PCR-based molecular verification and subsequent 16S rRNA gene sequencing. The PCR test results showed that the colonies were confirmed to be *S. aureus* with a match rate of 99.5-99.7% for the samples tested from each district. Phylogenetic comparison based on partial 16S rRNA gene sequences showed that all *S. aureus* isolates obtained in this study shared similarity with reference *S. aureus* sequences available in the GenBank database including strains reported from Brazil and India (Figure 6). The Maximum Likelihood tree placed the study isolates within the main *S. aureus* cluster, confirming their species-level identity without resolving finer genetic differentiation among geographic lineages. The 16S rRNA gene sequences generated in this study have been deposited in the NCBI GenBank database under accession numbers PX998243-PX998246.

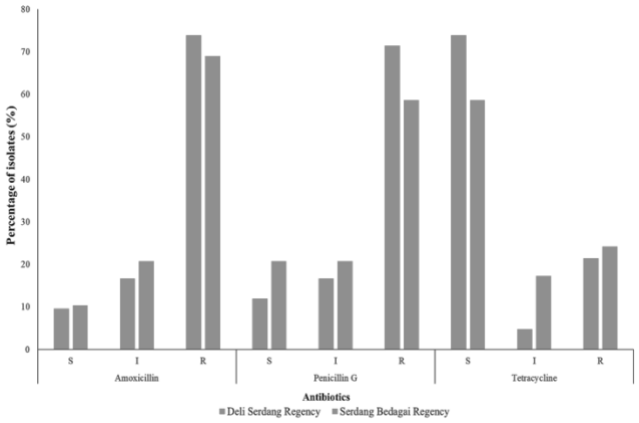


Figure 5. Graph of antibiotic resistance test results of *S. aureus* isolates from goat milk with subclinical mastitis in Deli Serdang and Serdang Bedagai Regencies, North Sumatra Province, Indonesia. S: Sensitive, I: Intermediate, R: Resistant

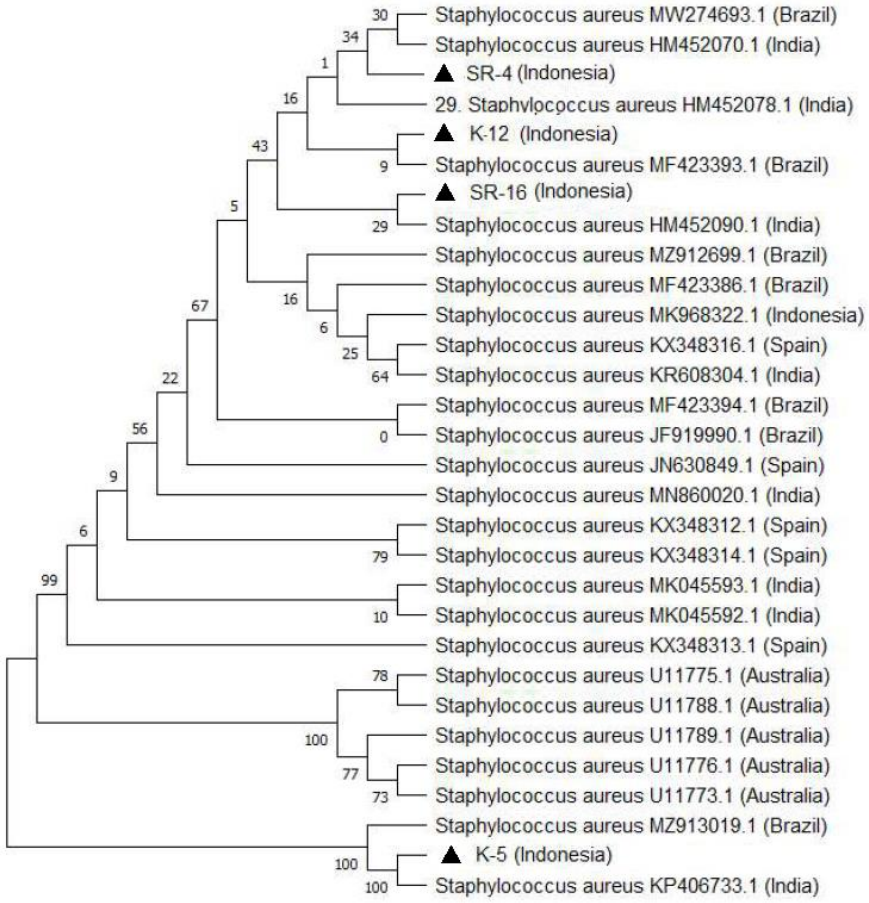


Figure 6. Phylogenetic tree based on partial 16S rRNA gene sequences of *Staphylococcus aureus* isolates from goats with subclinical mastitis in North Sumatra (SR-4, SR-16, K-12, and K-5 indicated by ▲) and reference sequences obtained from GenBank. The tree was constructed using the Maximum Likelihood method under the Tamura-Nei model in MEGA X. Bootstrap values (1,000 replicates) are shown at branch nodes

Discussion

The isolates obtained in this study exhibited phenotypic and genotypic characteristics consistent with *S. aureus* circulating in Indonesia. Culture results on BA agar media revealed round, convex bacterial colonies with flat and smooth edges, displaying a white to yellowish color. This finding is supported by studies conducted by Tang et al. (2024) and Barua et al. (2024), which stated that *S. aureus* bacteria form round, convex colonies with a white to golden yellow color on culture media. *S. aureus* colonies on MSA media were able to change the color of the MSA media from red to yellow. The color change in MSA media is due to the ability of *S. aureus* to ferment mannitol and produce phenolic compounds, which lower the pH and subsequently change the color of the media from red to yellow. In BPA media, the growth of blackish bacterial colonies was observed, as these bacteria produce lipase enzymes consisting of glycerol-ester-hydrolase compounds that can lyse fat from egg yolk tellurite added to the media (Gebremedhin et al. 2022; Nocera et al. 2022; Touaitia et al. 2025).The purple color of Gram-positive bacterial colonies results from the bacterial colonies having cell walls composed of thicker peptidoglycan, allowing them to absorb more crystal violet dye than Gram-negative bacteria (Ariyadi et al. 2023; Xu et al. 2023; Touaitia et al. 2025). *S. aureus* secretes the catalase enzyme, which catalyzes the decomposition of hydrogen peroxide (H_2O_2) into H_2O and O_2 compounds. Meanwhile, coagulase is an enzyme that reacts with prothrombin and its derivatives. This enzyme is secreted extracellularly on the surface of bacterial cells and subsequently binds to plasma fibrinogen to form a clot. The ability of *S. aureus* to produce catalase and coagulase enzymes serves as protection against phagocytosis by immune cells during the bacterial infection process, as well as being a virulence factor for *S. aureus* (Fayisa and Tuli 2023; Bashabsheh et al. 2024).

Another important virulence factor of *S. aureus* is its ability to form biofilms. *S. aureus* biofilms consist of an extracellular polysaccharide matrix, including Polysaccharide intercellular adhesin (PIA), an essential component of the extracellular polymeric substances (EPS) matrix. This matrix not only provides structural integrity and protection to the biofilm but also facilitates bacterial adhesion to the surfaces of body tissue cells (Peng et al. 2022; Aziz et al. 2025). *S. aureus* biofilms protect the bacteria from adverse environmental conditions, but they can also inhibit antibiotic penetration, leading to bacterial resistance to certain antibiotics and complicating treatment in cases of *S. aureus* infections (Kou et al. 2021). Although biofilm production has been implicated in the enhancement of antimicrobial resistance in *S. aureus* across prior research, the current study did not incorporate analyses aimed at evaluating this phenomenon.

The antibiotic resistance pattern of *S. aureus* found in this study differs from previous studies. In Indonesia, Yosyana et al. (2024) reported resistance to penicillin of 50%, tetracycline of 21%, and amoxicillin of 13% in Boyolali. Research by Purnamasari et al. (2023) indicated that *S. aureus* resistance to penicillin was 7.3% and tetracycline was 7.3% in Pasuruan. In Asia, Lubna et al. (2023) reported resistance values of *S. aureus* to penicillin of 100% and tetracycline of 72.72% in Pakistan, and Ul Haq et al. (2024) reported resistance to amoxicillin of 40%. In Ethiopia, Tegegne et al. (2024) reported a resistance value of *S. aureus* to tetracycline of 25%, while Maksimović et al. (2022) reported a resistance of 41% to amoxicillin in Bosnia and Herzegovina. These findings provide new insights into the adaptability of *S. aureus* through the emergence of resistance to multiple antibiotics commonly used in veterinary practice.

Despite the fact that isolates originating from Deli Serdang demonstrated higher resistance proportions to amoxicillin and penicillin G in numerical terms, no statistically significant differences were detected when compared with isolates from Serdang Bedagai. The breadth of the odds ratio confidence intervals suggests insufficient statistical power, most plausibly attributable to the limited sample size rather than genuine uniformity between the two regions (Hemming and Taljaard 2021; Gan 2025).

From a public health perspective, the emergence of resistance to β-lactam and tetracycline antibiotics in *S. aureus* isolates in this research warrants careful consideration. These antimicrobial classes remain integral to the clinical management of both community-acquired and livestock-associated staphylococcal infections in humans (Lianou and Fthenakis 2022). The presence of resistant *S. aureus* strains in food-producing animals raises the potential for indirect human exposure through occupational contact, environmental dissemination, or the food chain, particularly in regions where raw milk handling and informal dairy consumption persist (Vinayamohan et al. 2022; Ahmed et al. 2025). Such transmission pathways may contribute to the broader reservoir of antimicrobial resistance, thereby complicating therapeutic outcomes in human medicine (Elbehiry and Marzouk 2025).

The resistance profiles to β-lactam and tetracycline identified in this investigation may plausibly correspond to the routine empirical administration of these agents within Indonesian small ruminant production settings, aligning with resistance patterns described more broadly across Southeast Asia (Dankar et al. 2022). Nevertheless, because antibiotic usage was not systematically documented at the farm level, a definitive cause-and-effect linkage cannot be inferred. Disparities in resistance prevalence across geographically distinct isolates may be indicative of differential antimicrobial exposure or localized ecological selection forces (Ahmed et al. 2025). These selective influences could encompass environmental contributors, including disinfectant utilization and heavy metal residues (Elbehiry and Marzouk 2025). Moreover, inherent genomic attributes of the bacterial populations may further shape variability in resistance expression (Okaiyeto et al. 2024).

Phylogenetic reconstruction based on partial 16S rRNA gene sequences demonstrated that *S. aureus* isolates recovered from goats with subclinical mastitis in North Sumatra (SR-4, SR-16, K-12, and K-5) clustered within the *S. aureus* clade represented by reference sequences available in GenBank (Figure 6). The inclusion of the Indonesian isolates within this clade, alongside sequences originating from diverse geographic regions, supports their species-

level identification as *S. aureus*. Within the tree topology, isolates K-12, SR-4, and SR-16 were grouped near reference sequences reported from Brazil and India, while isolate K-5 clustered with an Indian reference sequence supported by high bootstrap values. These topological associations reflect sequence similarity at the 16S rRNA level and do not imply direct epidemiological linkage or transmission between regions. As 16S rRNA sequencing provides resolution primarily at the species level, it does not permit discrimination among strains or clonal complexes.

This branching pattern reflects the highly conserved nature of the 16S rRNA gene among *S. aureus* isolates and the broad global distribution of closely related lineages in livestock-associated environments. *S. aureus* is known to possess the ability to initiate a horizontal gene transfer (HGT) mechanism facilitated by biofilms. This mechanism allows for the rapid spread of antibiotic resistance, directly impacting the ability to maintain its survival (Vidovic and Vidovic 2020; Vinayamohan et al. 2022; Francis et al. 2024). This also contributes to the persistence and proliferation of resistant strains while explaining similarities in the resistance capabilities of bacteria from different countries (Vidovic and Vidovic 2020; Tao et al. 2022; Vinayamohan et al. 2022).

It is important to acknowledge that the analytical resolution of 16S rRNA is inherently limited for discriminating genetic variation at the strain or population level. More discriminatory molecular approaches, such as multilocus sequence typing or whole-genome sequencing, would be necessary to investigate fine-scale genetic diversity and potential transmission dynamics of *S. aureus* associated with subclinical mastitis in goats. Despite these constraints, the present phylogenetic analysis provides a robust molecular baseline and contributes foundational information on the genetic positioning of *S. aureus* circulating in dairy goats in North Sumatra.

In conclusion, the findings of this investigation integrate phenotypic characterization, antimicrobial susceptibility profiling, and molecular analysis to establish *S. aureus* was the predominant bacterial isolate among CMT-positive goats examined in this study. The pronounced resistance to β-lactam antibiotics observed in both Deli Serdang and Serdang Bedagai, with higher numerical levels in Deli Serdang, suggests diminished reliability of commonly applied first-line treatments and highlights an immediate need for strengthened veterinary extension initiatives that emphasize judicious antimicrobial use at the farm scale. Although resistance frequencies varied numerically between regencies, no statistically significant differences were observed. Phylogenetic evaluation using partial 16S rRNA sequences positioned the isolates within widely disseminated *S. aureus* groups, consistent with species-level affiliation. Further investigations incorporating higher resolution molecular typing alongside detailed farm-level antimicrobial usage data are required to clarify local epidemiological patterns and to support targeted mastitis control and antimicrobial stewardship in small ruminant production systems.

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