Characterization of *Staphylococcus aureus* isolated from subclinical mastitis of Peranakan Ettawa goat in Pekanbaru

D C Widianingrum¹ and S I O Salasia²*

¹Department of Animal Science, Faculty of Agriculture, University of Jember, 68121 Jember, Indonesia
²Department of Bioresources Technology and Veterinary, Vocational College, Universitas Gadjah Mada, 55281 Yogyakarta, Indonesia
*E-mail: isrinasalasia@ugm.ac.id

**Abstract.** *Staphylococcus aureus* (*S. aureus*) causing mastitis needs to be identified as the basis of treatment. This study aimed to characterize *S. aureus* isolated from Peranakan Ettawa (PE) goat's milk in Pekanbaru. A total of 50 milk samples were collected from the Farm in Pekanbaru. Based on the cultural and biochemical properties and the amplification of the 23S rRNA, *coa*, and *nuc* genes, eight isolates (53.33%) could be identified as *S. aureus*. The characterization of *S. aureus* based on the fermenting of Mannitol Salt Agar media, positive for Voges-Proskauer, catalase, and coagulase tests. The abilities of *S. aureus* isolated from goats to form β-hemolysis on blood agar plates, agglutinate rabbit blood, compact formed colonies in the Soft Serum Agar (SSA) test indicates the pathogen of the isolates to host.

1. **Introduction**

During the current pandemic, we need to strengthen the body's immune system. Consumption of milk accompanied by regular exercise can boost immunity [1]. Based on Indonesia's data statistics, the national milk production in 2019 is around 996,442.44 tons, which only can supply the consumption of about 16.23 kg/capita/year [2]. The problems because of low productivity and the total dairy population in Indonesia. Several simulation models to raise the dairy cow business in this country have been carried out [3]. Another obstacle is the presence of diseases like mastitis [4,5]. Mastitis is inflammation of the udder, with physical signs (clinical mastitis) or asymptomatic inflammatory (subclinical mastitis) [6]. Subclinical mastitis is very dangerous because it potentially contains harmful bacteria [7], such as *Staphylococcus aureus*. [4] These bacteria can become zoonotic [8]; therefore, it is very important to identify their character as a database for treatment and prevention.

2. **Material and Methods**

2.1. **Collecting Subclinical Mastitis Sample**

A total of 50 samples of Ettawa goat milk were collected from Umban Sari Farm, Pekan Baru, Indonesia. Subclinical mastitis samples were known based on the California Mastitis Test (CMT) results [9] as described previously study [4]. Samples with +++ results further tested on the Clinical Pathology Laboratory, Faculty of Veterinary Medicine, University of Gajah Mada.
2.2. Identification and characterization of Staphylococcus aureus

2.2.1 Phenotypic Identification.
The strains were identified as S. aureus by Gram staining, mannitol salt agar (MSA), catalase, and Methyl Red Voges-Proskauer (MR-VP) tests as described in previous research [4].

2.2.2 Genotypic Identification and characterization.
The isolates of S. aureus were confirmed by polymerase chain reaction (PCR), amplification of the 23S rRNA and nuc genes as the gold standard [10]. To determine the coagulase character of the isolates, the coa gene test was carried out (Table 1).

Table 1. Oligonucleotide primers and PCR programs

| Gene | Oligonucleotide primers | Program* |
|------|-------------------------|----------|
| 23S rRNA | 5'ACG GAG TTA CAA AGG ACG AC 3' | 1 |
|       | 5' AGC TCA GCC TGA ACG AGT AC 3' |          |
| Nuc   | 5' AGC AAC GGC TTG ACG AAC TAA AGC 3' | 2 |
| Coa   | 5' ATA GAG ATG CTG GTA CAG G 3' | 3 |
|       | 5' GCT TCC GAT TGT TCG ATG C 3' |          |

*1. 30x 94°C-120 s, 64°C-40 s, 72°C-75 s; 2. 37x 94°C-60 s, 55°C-30 s, 72°C-30 s; 3. 30x 94°C-60 s, 58°C-60 s, 72°C-60 s

2.2.3 Phenotypic Characterization. The characters of S. aureus observed in this study including hemolysin, growth in liquid media, coagulase test, and hydrophobicity properties. The zone of hemolysis showed on blood agar (BA) media [11], observation bacteria growth on the liquid media using Todd Hewitt broth (THB) medium. The coagulase test was carried out by cultivating 1 ml of bacteria on THB and mix with 1 ml of rabbit plasma, then incubated for 24 hours at 37 °C. Coagulase positive signed by clot solution after overnight incubation. The hydrophobicity character was known by mixing 50 µl serum on agar base 0.15% (serum soft agar/ SSA media), then punctured this media with S. aureus and incubation overnight [12]. All data in this study were reported descriptively.

3. Results and Discussion

3.1 Subclinical mastitis case.
Data of subclinical mastitis in this study was 15 (30%) from a total of 50 samples. Only goat milk Data of subclinical mastitis in this study was 15 (30%) from a total of 50 samples. Only goat milk with CMT +++ results that we classified as subclinical mastitis in our study. CMT tests work based on the arylsulfonate reagent that breaks down the cell nucleus. Different from cows, subclinical mastitis in goat milk was classified from CMT test ≥ +++ based on the cell somatic count data [9, 13]. The number of somatic cells in goats with CMT test + results has SCC 143.8 x 10³, and the CMT test ++ has SCC 417.9 x 10³ that they still on standard International Dairy Federation that SCC on the milk should not exceed 500,000 cells/ml. Whereas in the CMT test +++ the SCC is more than 542.4 x 10³ [9, 13].

3.2 Identification of Staphylococcus aureus.
Based on phenotypic identification (Figure 1) and genotypic confirmation (Table 2), there were eight samples (53.33%) S. aureus in this study. This bacteria is known as the main cause of subclinical mastitis in dairy cows in Indonesia [10, 11, 12. 13]. Staphylococci also were reported causing 38.5% of subclinical mastitis cases in goats [4].

S. aureus bacteria are Gram-positive that can ferment mannitol. The fermentation of carbohydrates produces organic acids such as lactic, formic, and acetic acids—the formation of these acids changing the color of MSA media. The catalase test was used to identify bacteria’s ability to use oxygen and break down hydrogen peroxide (H2O2). Hydrogen peroxide can destroy bacteria cells, but the strain
produced by enzyme catalase can convert hydrogen peroxide into air and oxygen. Acetyl methyl carbinol (acetoline) in the MR-VP test results from \textit{S. aureus} fermentation signing with the red ring layer. Biochemical tests are a conventional screening method to determine \textit{S. aureus}. However, as the gold standard, it must be confirmed by genotypic methods with amplification of species-specific genes [10].

![Figure 1](image1.png)

**Figure 1.** Phenotypic identification of \textit{S. aureus}. a. California Mastitis Test (CMT), signed by clot formation after mixing; b. Gram staining; c. Mannitol Salt Agar (MSA) test, turning the red media to the yellow; d. catalase test, form bubbles (indicated by arrows); e. MR-VP test, form red ring layer.

| Isolat Code | 23SrRNA | nuc | coa | Polymorphisms |
|-------------|---------|-----|-----|---------------|
| 1           | +       | +   |     | 600           |
| 2           | +       | +   |     | 2500          |
| 3           | +       | +   |     | 430           |
| 4           | +       | +   |     | 840           |
| 5           | +       | +   |     | 840           |
| 6           | +       | +   |     | 600           |
| 7           | +       | +   |     | 840           |
| 8           | +       | +   |     | 840           |

**Table 2.** Genotypic identification by 23SrRNA, nuc, and coa genes

3.3 Characterization of \textit{Staphylococcus aureus}.

Based on our data, the eight \textit{S. aureus} approves with their ability to clot rabbit plasma, forming β hemolysis in the blood agar media, settling on liquid media, and forming compact colonies (hydrophobic) in SSA media (Figure 2).

![Figure 2](image2.png)

**Figure 2.** Characterization of \textit{S. aureus}. a. coagulase +, b. form β hemolysa, c. settles on liquid media, d. form colonies compact on hydrophobicity test.

Hemolysin is an exoprotein and one of the factors virulent from \textit{S. aureus} because, from this ability, they can lyse red blood cells. There were 4 types of hemolytic, among α (forming a light zone around the colony), β (forming a slightly dark zone), γ (no zone), and a combination of α and β by forming a dark and light area around the colony [14]. Subclinical mastitis in goats can occur by Coagulase...
Positive Staphylococci (CPS) and Coagulase Negative Staphylococci (CNS) strain. One of the characters of S. aureus was they have a coa gene. The most CNS strain causing subclinical mastitis is S. pasteuri, S. xylosus, and S. haemolyticus [4] Hydrophobicity determines bacteria's ability to adhere to the host cell. Hydrophobic bacteria have strong adhesion properties [15].

4. Conclusion
PE goat used in this study 30% has subclinical mastitis, which is caused by S. aureus infection. The character of S. aureus can be used as an essential reference in drug development and the classification of this bacteria in the future study.

5. References
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