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Study Biofilm Phenotyping and Genotyping of Staphylococcus Aureus from the Nasal Discharge of Sheep in Basrah Governorate

Khadeja Ch. Khalaf*, Hanaa K. Ibraheim, Mohammed H. Khudor

Department of Microbiology, College of Veterinary Medicine, University of Basrah, Iraq

*Email: khadeja.khalaf@uobasrah.edu.iq

ABSTRACT: The study was designed to determine and characterize the rate of nasal discharge of *S. aureus* in sheep and to characterize it genotypically by PCR targeting the gene *nuc* and culturing. The study was conducted in Basrah province, between September and October 2025. The study evaluated phenotyping of biofilm formation by microtiter plate assay and genetic to biofilm genes from the isolated *S. aureus* among the 50 tested nasal swabs, 42 (84%) isolates were positive for *S. aureus* isolation using culturing. Antibiotic susceptibility testing for *S. aureus* was conducted utilizing the standard Kirby-Bauer disk diffusion method. A total of 32 biochemical methods were employed, accounting for 64% of the approaches used. The findings showed that 28 isolates, representing 56%, tested positive for the *nuc* gene at a size of 423 bp, thus confirming the successful amplification of the *nuc* gene and the presence of *S. aureus*. The *S. aureus* isolates were categorized into three groups of strong, medium and weak biofilm producers by the biofilm formation assay. Results showed that the rate of detection of biofilm genes in *Staphylococcus aureus* was 50%, 39.28% and 10.71%, The PCR analysis indicated that all isolates included the *icaA* gene, achieving a detection rate of 100%. The *sigA* gene exhibited the highest frequency of detection at 92.85%, while the *katA* gene was identified in 75% of the cases. The *sodA* gene had the lowest detection rate among the genes examined, at 69.23%. The isolates exhibited varying levels of resistance to the tested antibiotics; the highest resistance rate was recorded against azithromycin (92.85%), whereas all isolates showed complete susceptibility (100%) to oxacillin.

Furthermore, two isolates showed resistance to cefoxitin and were identified as methicillin-resistant *Staphylococcus aureus* (MRSA). The presence of *S. aureus* and MRSA in sheep is alarming, as these animals could act as a source of infection for humans, especially for those working on farms.

INTRODUCTION

Staphylococcus aureus is both a commensal organism and a significant opportunistic pathogen responsible for various diseases in humans and animals alike. Its presence poses a considerable threat to public health and significantly affects the livestock section. This bacterium continues to be the subject of extensive research, a bacterial species affecting both humans and animals. include a highly adaptable and opportunistic pathogen capable of thriving and reproducing in diverse environments. This pathogen is responsible for a broad spectrum of illnesses, including severe conditions such as pneumonia, bacteremia, meningitis, sepsis, and pericarditis, as well as foodborne illnesses. Additionally, it frequently leads to infections of the skin and soft tissues. (3). In various animal species, *S. aureus* has been recognized as a significant opportunistic pathogen, capable of causing illnesses that range from mild to life-threatening (4). This bacterium belongs to the gram-positive genus

Staphylococcus and is characterized as a non-motile, non-spore-forming, facultative anaerobic coccus. Its importance lies in its ability to adapt to various environmental conditions and its numerous patterns of antibiotic resistance (5), including resistance to methicillin and other β -lactam antibiotics (6). Antimicrobials such as penicillin, ampicillin, amoxicillin, amoxicillin-clavulanic acid, third-generation cephalosporins, gentamicin, and ciprofloxacin are frequently utilized (7). The rising issue of antimicrobial resistance (AMR) in both obligate and opportunistic bacterial pathogens is largely driven by intense selective pressure and improper use of these medications (8). Biofilms, which consist of one or more microbial species attached to a surface, contrast with planktonic cells that exist independently. The formation of biofilms by bacteria not only allows them to escape detection by the immune system but also hinders the penetration of antibiotics into the biofilm matrix (9). There is a clear link between biofilm formation and antibiotic resistance in clinical isolates of *Staphylococcus aureus*, with biofilm-forming bacteria exhibiting a direct correlation can be observed between the formation of biofilms by bacteria and the resistance of clinical isolates of *Staphylococcus aureus* to antibiotics. It has been noted that bacteria capable of biofilm formation demonstrate antibiotic resistance levels that are approximately 100 to 1000 times higher than those found in their free-floating counterparts. (10). The capability of *Staphylococcus aureus* to induce infections is linked to its production of numerous virulence factors, including surface proteins, biofilms, exoenzymes, and exotoxins, as well as exfoliative toxins [11]. These factors enhance the bacteria's ability to attach to tissues, which can result in disease progression and allow them to overcome immune defenses, subsequently causing toxic effects [12]. The ability to form biofilms is an essential virulence characteristic found in pathogenic bacteria such as *S. aureus*. This capability greatly enhances their pathogenicity and resistance to multiple pharmaceuticals, serving as a protective strategy that allows these bacteria to persist and adapt to their surroundings. [13]. This biofilm composed of an extracellular polymeric matrix encapsulates clusters of microbial cells, enabling them to adhere to surfaces; it acts as a robust barrier against antibiotics and helps the bacteria evade the immune system. [14]. Infections caused by biofilm-forming bacteria pose a significant challenge in medical facilities, largely due to the extreme difficulty of eradicating them. It can evade host defense mechanisms while thriving within a protective biofilm that shields it from opsonophagocytosis. Furthermore, these bacteria have the ability to acquire resistance to standard antimicrobial agents used to treat planktonic (free-floating) forms, particularly those that inhibit cell wall synthesis, posing a significant health risk. Much research indicates that various genes contribute to the formation of biofilms. (15). Biofilms can lead to contamination or persistent infection, as the cells embedded within them exhibit significant resistance to cleaning methods, antimicrobial treatments, and immune system defense mechanisms. [16]. Regarding *Staphylococcus* species, biofilm formation is a complex process involving the activation of a variety of genes and proteins; these elements play a fundamental role in facilitating bacterial adhesion and the formation of the extracellular matrix [17]. The *icaADBC* gene operon is a key factor in biofilm formation, as it enables *Staphylococcus aureus* bacteria to synthesize the polysaccharide intercellular adhesin (PIA), a substance that plays a fundamental role in biofilm formation [18]. The PIA complex is primarily composed of the polymer poly-N-acetylglucosamine (PNAG), which is produced through the coordinated action of four genes located within the gene cluster (operon) known as *icaADBC*. [19, 20]. The process of biofilm formation begins with the attachment of bacterial cells to surfaces, facilitated by surface-associated adhesion proteins. Following this initial attachment, the bacteria proliferate and mature, forming a complex, multilayered structure. The *ica* gene cluster (the *ica* operon) plays a crucial role in this developmental process, facilitating the production of poly-N-acetylglucosamine (PIA) and other adhesive polysaccharide molecules. These components are essential for cell aggregation and contribute to the formation and stability of staphylococcal biofilms. In cases where the *ica* gene is absent, studies show that the *sigB* gene plays a crucial role in biofilm formation in *Staphylococcus aureus* bacteria. (21) *Staphylococcus aureus* bacteria are characterized by a set of adhesion proteins (adhesins) that play a crucial role in their ability to cause disease by facilitating attachment to host tissues; these factors are a fundamental determinant of their virulence. [22]. These bacteria demonstrate a remarkable ability to rapidly adapt to antibacterial treatments. The aim of this research is to isolate biofilm-producing *Staphylococcus aureus* from sheep nasal secretions and to evaluate their antibiotic resistance in vitro.

MATERIAL AND METHODS

Sample collection

A veterinarian oversaw the collection of 50 nasal discharge samples from sheep at various farms in Basra between September and October 2025. Sterile, moistened cotton swabs were gently inserted into each nostril to a depth of approximately 1 cm and rotated five times. Subsequently, the swabs were placed in Stuart transport medium and immediately transported to the laboratory for analysis. The nasal swab samples were initially placed in Brain-Heart Infusion (BHI) broth and incubated at

37°C for 24 hours. Following this incubation period, the samples were transferred to Mannitol Salt Agar (HiMedia, India) and CHROMagar™ *Staph. aureus* media for further analysis. (CHROMagar, France). The air-inoculated agar plates were incubated at 37°C for 24 hours. Presumptive *Staphylococcus aureus* colonies appearing on the CHROMagar™ medium were confirmed using Gram staining, followed by biochemical tests to detect catalase and oxidase enzymes.

Isolation and identification of *S. aureus*

Nasal secretion samples were collected and subjected to bacterial analysis to isolate and identify *Staphylococcus aureus*; the samples were first cultured on mannitol salt agar and then incubated under aerobic conditions at 37°C for 24 hours. The resulting colonies were examined for specific morphological characteristics, particularly the appearance of golden-yellow colonies. Gram staining revealed them to be Gram-positive cocci arranged in clusters. Colonies exhibiting the expected characteristics were cultured on mannitol salt agar to verify their purity. Final identification was confirmed through standard biochemical tests, including catalase, oxidase, and coagulase tests [23].

Molecular study

Genomic DNA Extraction: In accordance with the manufacturer's instructions, genomic DNA was extracted from isolates suspected to be *S. aureus* using a DNA extraction kit from the US-based company Geneaid.

Detection of *Staphylococcus aureus* by PCR

All collected DNA samples were examined to molecularly identify *Staphylococcus aureus* using the polymerase chain reaction (PCR) technique, with a particular focus on the *nuc* gene. The amplification process utilized specific pairs of primers: forward primer (F: 5'- GCTTGCTATGATTGTGGTAGCC-3') and reverse primer (R: 5'- TCTCTAGCAAGTCCCTTTTCCA -3'). A polymerase chain reaction (PCR) designed to amplify the *nuc* gene (423 bp in length) was performed in a total reaction volume of 25 µL. The reaction mixture consisted of 12.5 µL of Green Master Mix, 1 µL each of the forward and reverse primers, 5 µL of DNA template, and 5.5 µL of nuclease-free water. To initiate the amplification process, the protocol began with a denaturation step at 95°C for five minutes. This was followed by 35 cycles comprising denaturation, annealing, and extension phases, and the process concluded with a final extension step at 72°C for ten minutes. The PCR products obtained were analyzed by agarose gel electrophoresis (1.5%) and visualized under ultraviolet light.

Biofilm Formation Assay

The biofilm-forming ability of various *Staphylococcus aureus* isolates was evaluated using the microtiter plate method. Briefly, overnight bacterial cultures of the isolates were diluted in Tryptic Soy Broth (TSB) supplemented with 1% glucose, and the bacterial concentration was adjusted to a 0.5 McFarland standard. Subsequently, 200 µL of each suspension was added in triplicate to sterile 96-well polystyrene microtiter plates; wells designated as negative controls were filled exclusively with 200 µL of TSB broth. The plates were then incubated at 37°C for 24 hours. Following the incubation period, the wells were washed three times with phosphate-buffered saline (PBS, pH 7.2) and then fixed in methanol for 20 minutes. Subsequently, they were allowed to air-dry and were stained with 0.1% crystal violet. Excess dye was removed by washing, and the bound dye was solubilized by adding 200 µL of 33% glacial acetic acid to each well. Absorbance was measured at a wavelength of 570 nm using a BioTek 800TS microplate reader. (BioTek, UK, 25). The optical density cut-off value (OD_c) was calculated as the average OD of negative controls plus three standard deviations. Based on these optical density measurements, the isolates were categorized as non-biofilm producers (OD ≤ OD_c), weak producers (OD_c < OD ≤ 2×OD_c), moderate producers (2×OD_c < OD ≤ 4×OD_c), or strong producers (4×OD_c < OD).

Molecular detection biofilm genes

Table 1 lists all the oligonucleotide primers utilized in this study. We processed a total of 28 DNA samples through PCR amplification, carried out in a final volume of 50 µL. The components and concentrations of the reaction were tailored to each specific primer as outlined in Table 2. Following amplification, the resulting PCR products were separated and visualized using electrophoresis on a 1.5% agarose gel. (26).

Table 1: polymerase chain reaction primers for amplification of target genes

Primer name	Primer sequence 5'-3'	Product size (bp)
icaA	F CTGGCGCAGTCAATACTATTTCGGGTGTCT R GACCTCCCAATGTTTCTGGAACCAACATCC	195
sigB	F CGTCTCGGAACATGTACACTCCAAG R GTCCTTTGAACGGAAGTTTGAAGCC	102
katA	F AAAGGTTCTGGTGCATTTGG R AACGCAAATCCTCGAATGTC	173
sodA	F TGCACGCTTTGGTTTCAGGTGGG R GCGCCAATGTAGTCAGGGCGTTTG	177

Table 2 :Primer Names, Target Gene Amplicon Sizes, and Polymerase Chain Reaction (PCR) Cycling Conditions

Primer name	primary denaturation	Amplification (35 cycles)			Extension
		Secondary Denaturation	Annealing	Extension	
icaA	95 5m	95 30s	58 C ⁰ 45s	72 30s	72 8m
sigB	95 5m	95 30s	50 C ⁰	72 30s	72 8m
katA	95 5m	95 30s	55 C ⁰	72 30s	72 8m
sodA	95 5m	95 30s	58 C ⁰	72 30s	72 8m

Antibiotics susceptibility test

Staphylococcus aureus isolates, verified using molecular techniques, were subjected to antimicrobial susceptibility testing using the standard disk diffusion method on Mueller-Hinton agar. To prepare the bacterial inoculum, colonies grown on mannitol salt agar for 18–24 hours were suspended in sterile saline, and the turbidity of the suspension was adjusted to match the 0.5 McFarland standard. A sterile cotton swab was used to evenly distribute the standard suspension onto the surface of Mueller-Hinton agar plates. Subsequently, antibiotic discs—representing five different antimicrobial agents—were carefully placed onto the inoculated plates under aseptic conditions. Subsequently, the plates were incubated at temperatures ranging from 35 to 37°C for 18 to 24 hours. Following this incubation period, the diameters of the zones of inhibition were measured in millimeters, and the results were classified as susceptible, intermediate, or resistant, in accordance with standard criteria. (28).

RESULTS

The process of identifying the target bacteria began with an analysis of their cultural and microscopic characteristics; on Mannitol Salt Agar (MSA), colonies suspected to be *Staphylococcus aureus* appeared as yellow colonies surrounded by yellow halos, indicating successful mannitol fermentation. After a 24-hour incubation period at 37°C on ChromAgar medium, the colonies exhibited a pale pink color (see Figure 1 A and B), suggesting the potential identification of *S. aureus* bacteria. Microscopic examination—following Gram staining—revealed the presence of Gram-positive cocci arranged in irregular clusters resembling bunches of grapes, a characteristic feature of *Staphylococcus aureus*. These findings, combined with observations from bacterial cultures, indicate the specific identity of the bacteria; 42 isolates representing 84% were Initially classified as *Staphylococcus aureus*. To confirm this classification, biochemical tests were performed on all suspected isolates. Sample test results showed the presence of catalase and coagulase, while oxidase test results were negative. Biochemical analyses indicated that 32 out of 45 nasal swab samples, representing 64%, were identified as *Staphylococcus aureus* bacteria, as presented in Table 1.

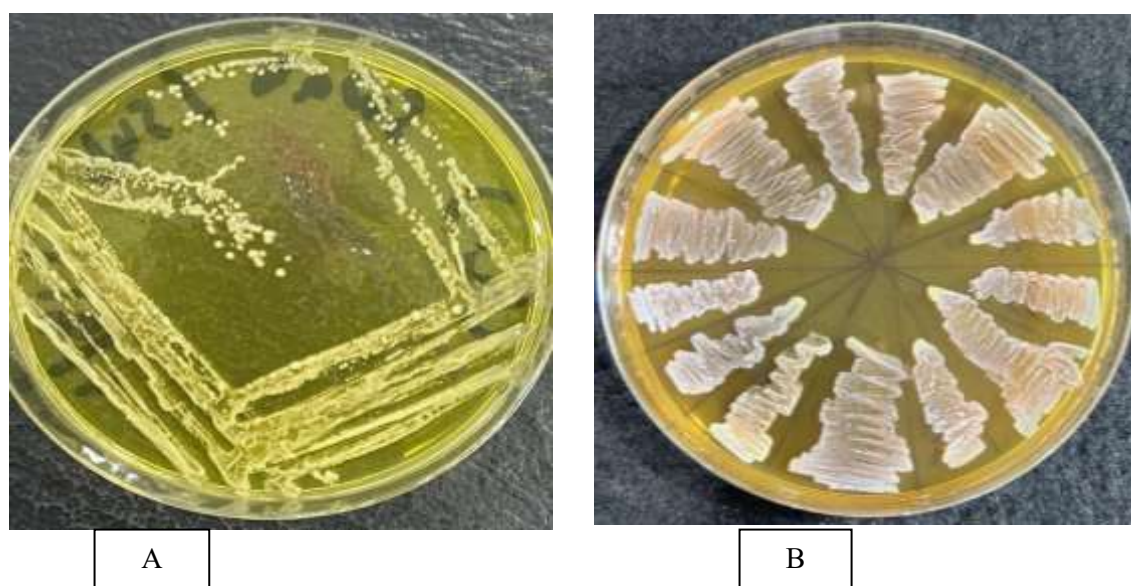


Figure 1: A *Staphylococcus aureus* on MSA, B: *Staphylococcus aureus* on chrom agar

Table 1: Isolation of *S. aureus* using Culture media, biochemical test

Source of sample	Examined sample no=	Culturing no+ (%)	Bio. Test no+ (%)
	50		
Nasal discharge from sheep	50	42(84%)	32 (64%)

Molecular techniques

DNA extraction

All bacterial genomes were extracted from positive cultures and subsequently analyzed using 1% agarose gel electrophoresis.

Molecular Confirmation of *S. aureus* by PCR

S. aureus bacteria were first identified using biochemical tests, which confirmed the classification of 32 samples as belonging to this specific bacterial species. Subsequently, polymerase chain reaction (PCR) analysis was performed to detect the nuc gene at the molecular level. The results showed that 28 isolates opposite to 56% tested positive for the target gene.

Furthermore, gel electrophoresis (using agarose gel) revealed distinct bands at the expected size of 423 base pairs, confirming the successful amplification of the nuc gene and thereby demonstrating the presence of *Staphylococcus aureus*. (Figure 2).

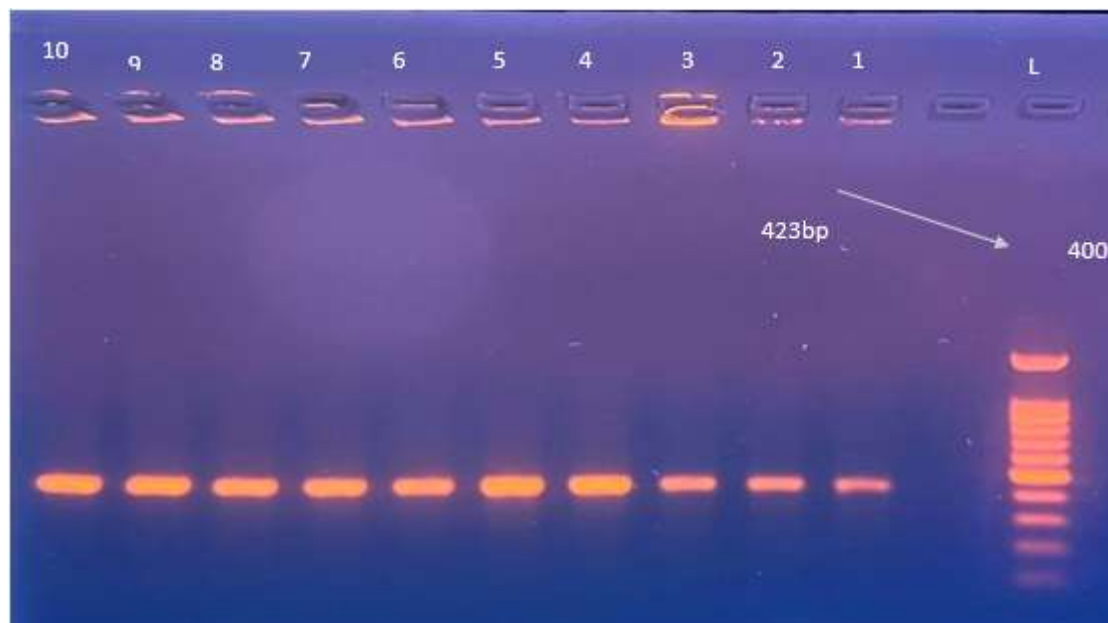


Figure 2: Agarose gel electrophoresis (2%) of PCR-amplified nuc gene from nasal discharge *S. aureus* isolates, stained with ethidium bromide. Lane (L): DNA ladder (100–1500 bp); Lanes (1–10): positive PCR products at 423 bp (run at 100 V, 80 mA for 1h)

Biofilm Formation Assay

Biofilm formation in *S. aureus* isolates was evaluated using a microtiter plate assay, and the results were classified into three distinct categories: strong, moderate, and weak biofilm producers. This is illustrated in the figure. 5. The results indicated that half of the isolates—specifically 14 isolates—exhibited high levels of biofilm production. Additionally, 11 isolates (39.28%) showed moderate biofilm production, while 3 isolates (10.71%) were classified as weak biofilm producers, as shown in Table 2.

Table 2: Results of standard conditions for *S. aureus* biofilm formation.

Source of sample	Total no. of isolation	Biofilm production		
		Strong (%)	Moderate (%)	Weak (%)
Nasal discharge from sheep	28	14(50%)	11 (39.28%)	3(10.71%)

detection biofilm genes in *S. aureus*

Using polymerase chain reaction (PCR) analysis, researchers identified genes associated with biofilm formation in 28 isolates of *Staphylococcus aureus* known for their ability to form biofilms. As shown in figure (2 and 3), the icaA gene was found to be the most prevalent, appearing in all isolates and resulting in a prevalence rate of 100%. The sigA gene showed the highest detection rate at 92.85%, followed by the katA gene at 75%, while the sodA gene recorded the lowest detection rate at 69.23% among the studied isolates.

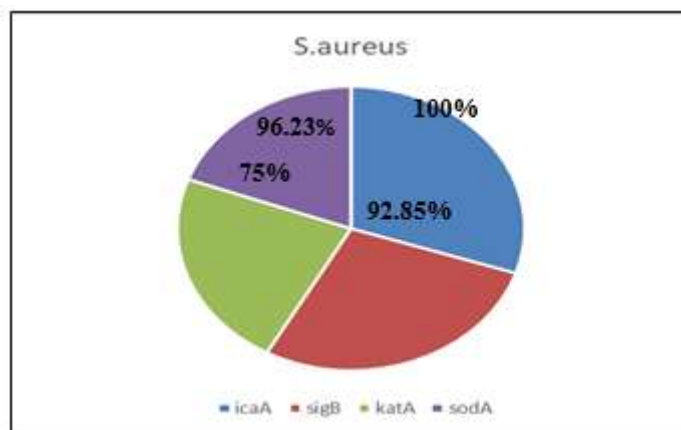


Figure (3): Percentage of biofilm-associated genes in *S. aureus* isolates

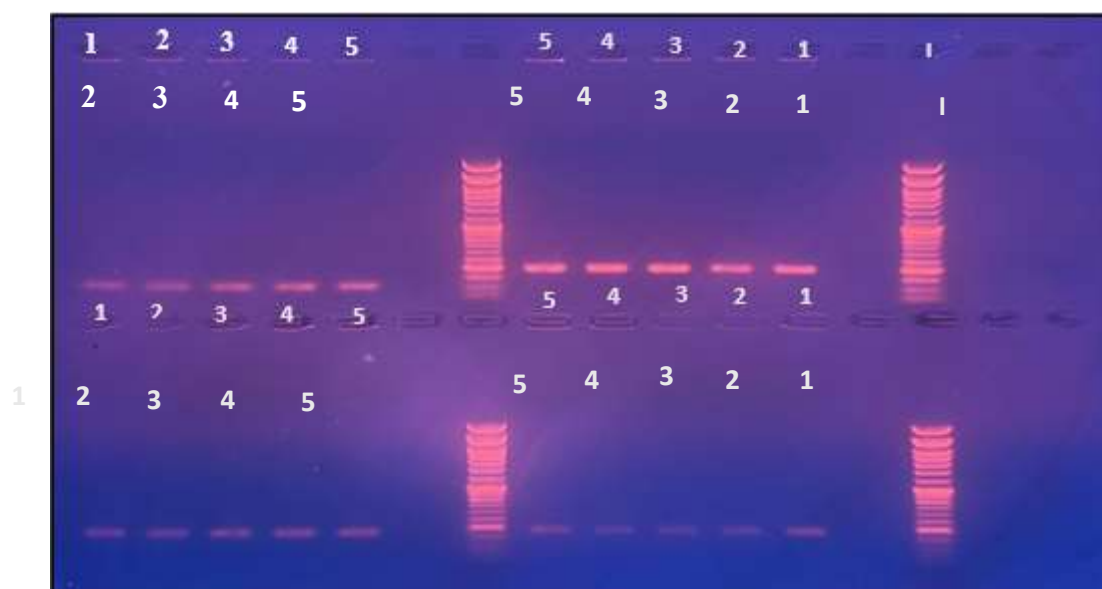


Figure 3. Agarose gel electrophoresis (2%) of PCR-amplified genes in *Staphylococcus aureus* isolates. Lane (L): DNA ladder (50–1500 bp); lanes (1–5): positive PCR products with the expected amplicon sizes. Electrophoresis was performed at 100 V and 80 mA for 1 h.

Antibiotics susceptibility test

Figure 3 clarifies the susceptibility of 28 *Staphylococcus aureus* isolates confirmed by PCR to ten commonly used antibiotics, a summary of which is provided in Table 2. The results indicated significant antibiotic resistance; the drug azithromycin topped the list with a resistance rate of 92.85%, followed immediately by tetracycline at 89.28%, while ciprofloxacin recorded a resistance rate of 85.71% and erythromycin showed a rate of 78.57%. All isolates demonstrated complete susceptibility to oxacillin, resulting in a 100% susceptibility rate. Furthermore, two isolates exhibited resistance to cefoxitin and tested positive in the screening test for this drug, confirming their classification as methicillin-resistant *Staphylococcus aureus* (MRSA) strains.

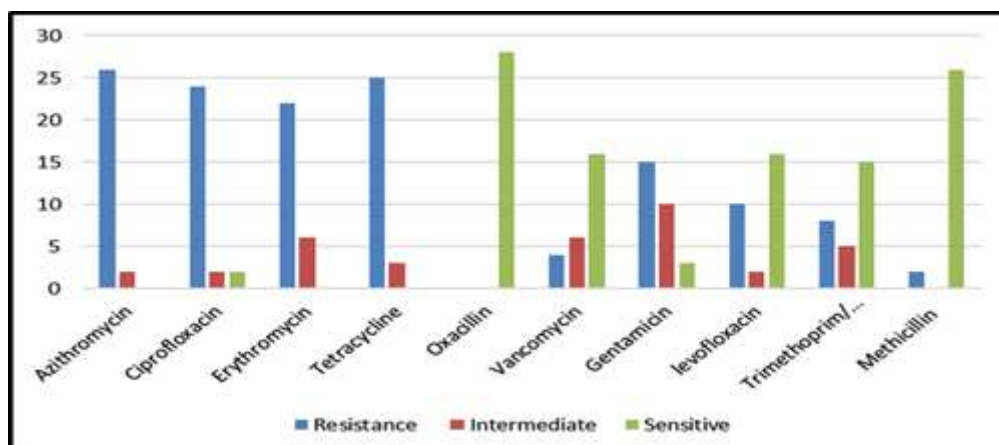


Figure 2: Antibiotic susceptibility of *Staphylococcus aureus*.

Table 2: Antibiotic susceptibility patterns of PCR-confirmed *S. aureus* isolates (n = 28)

Antibiotics	Resistance	Intermediate	Sensitive
Azithromycin	26(92.85%)	2(7.14%)	0(0%)
Ciprofloxacin	24(85.71)	2(7.14%)	2(7.14%)
Erythromycin	22(78.57%)	6(21.42%)	0(0%)
Tetracycline	25(89.28%)	3(10.171%)	0(0%)
Oxacillin	0(0%)	0(0%)	28(100%)
Vancomycin	4(4.28%)	6(21.42%)	16(57.14%)
Gentamicin	15(53.57%)	10(35.71%)	3(10.71%)
levofloxacin	10(35.71%)	2(7.14%)	16(57.14%)
Trimethoprim/ Sulfamethoxazole	8(28.57%)	4(14.28%)	12(42.85%)
Cefoxitin	2(7.14%)	0(0%)	26(92.85%)

DISCUSSION

Isolation and identification of *S. aureus*

It is likely that *Staphylococcus aureus* bacteria found in the nasal passages of ruminants are a major contributing factor to staphylococcal infections. (29). A study conducted on 50 nasal swabs collected from sheep revealed that 28 samples representing 56% tested positive for *Staphylococcus aureus* using standard bacteriological methods and specific polymerase chain reaction (PCR) testing. This prevalent rate is consistent with findings from other studies on dairy sheep, underscoring the concept that the nasal cavity serves as an important reservoir for *S. aureus* in small ruminants. This emphasizes its significant role in the continued presence and transmission of bacteria among animal populations. (30).

During the initial isolation phase, all 42 presumptive isolates developed yellow colonies accompanied by yellow zones on mannitol salt agar medium. This indicates mannitol fermentation, a characteristic feature of *Staphylococcus aureus*. In addition, all tested isolates yielded positive results for the catalase and coagulase tests, while testing negative for the oxidase test. This is consistent with the biochemical characteristics expected for this species. Previous research utilizing selective media also reported similar results regarding phenotype and biochemical behavior. (31). Taking these characteristics into

account, the 42 isolates were Initially classified as *S. aureus* and subsequently subjected to further confirmation by biochemical analysis. [32]. Molecular identification via polymerase chain reaction targeting the species-specific *nuc* gene revealed that only 28 isolates (56%) were correctly identified, as evidenced by the expected amplification products observed upon agarose gel electrophoresis. (Figure 2). The lower number of confirmed isolates detected via PCR analysis highlights the improved accuracy of molecular methods, suggesting that relying exclusively on culture and biochemical characteristics may lead to an overestimation of the prevalence of *Staphylococcus aureus*. Consequently, the results highlight the importance of combining traditional phenotypic methods with molecular techniques to accurately identify *S. aureus* isolates. (33).

Biofilm Formation Assay

The recent study showed that every isolate examined was capable of producing biofilm; however, production levels varied significantly, falling into three distinct categories: strong, moderate, and weak. (see Table 5). In particular, half of the isolates exhibited significant biofilm formation, while approximately 39.28% showed moderate production, and 10.71% were classified as weak producers. This is consistent with a local study on *S. aureus* that found all analyzed isolates had the ability to form biofilms. In contrast, a separate regional study revealed that 15% of *S. aureus* bacteria exhibited a weak capacity for biofilm formation, while a similar proportion showed moderate levels; meanwhile, a significant 70% demonstrated a strong capacity for biofilm formation [25].

The results of this study are consistent with those reported in reference (34), which also concluded that all analyzed isolates were capable of forming biofilms. Specifically, 37.93% of them were classified as strong producers, while 48.28% fell into the average category, and 13.79% were considered weak producers. Nevertheless, the findings only partially align with those of other studies. (35) A significant finding was that 42% of *S. aureus* isolates demonstrated a high capacity for producing strong biofilms on non-living surfaces. Additionally, approximately 20% and 38% of the isolates were classified as moderate and weak biofilm producers, respectively. The presence of biofilms complicates the treatment of *S. aureus* infections, as these bacteria can form biofilms within the tissues of the host. These infections present a significant challenge due to antibiotic resistance, often require the surgical excision of infected tissues (36). The resistance to antimicrobials linked with biofilms can be partially explained by the presence of dormant *S. aureus* cells, commonly known as persister cells, which are sheltered within the biofilm. These cells stay inactive during antimicrobial therapies but can reactivate once treatment ceases, resulting in recurrent infections. (37). Creative treatment methods will be essential for addressing the infections linked to *S. aureus* biofilms. Therefore, it is essential to conduct overall research on *S. aureus* biofilms to gain a deep understanding, employing appropriate microbiological techniques alongside other relevant approaches. (38).

detection biofilm genes in *S. aureus*

The likelihood of *Staphylococcus aureus* infection is linked to the bacterium's ability to produce various virulence factors that facilitate bacterial invasion. [39]. Furthermore, biofilm formation enhances the virulence of *S. aureus*; certain strains possess the ability to form biofilms that exhibit greater resistance to antibiotics, disinfectants, and harsh environmental conditions. [40]. The *ica* genes are essential for slime production in *Staphylococcus aureus*, as they regulate the formation of polysaccharide intercellular adhesin (PIA); this regulation directly influences the organism's ability to form biofilms. A study conducted by Namvar and his colleagues highlights this fundamental relationship. [41] It has been established that biofilm formation in *S. aureus* bacteria depends on the presence of the *icaD* gene. [42]. A comprehensive analysis of all *S. aureus* isolates (Table 2) revealed the presence of the *icaA* gene a result that aligns closely with previous studies that found *ica* genes in all isolates. [43].

These results are consistent with the study conducted by Ahmed and Abdullah (44), which revealed that a remarkable 99.2% of *Staphylococcus aureus* isolates carried the *icaA* gene. Infections caused by *Staphylococcus aureus* particularly strains that produce an extracellular, sticky substance and promote biofilm growth pose significant challenges for clinical treatment. Biofilm formation relies on cell adhesion mediated by polysaccharides, which are synthesized by enzymes encoded by the intercellular adhesion (*ica*) gene cluster. Adhesion molecules are crucial for the initiation of infection, and the detection of "ica" genes may shed light on the various adhesion mechanisms contributing to the development of infections within healthcare settings (45). Infections caused by *Staphylococcus aureus* strains carrying the *ica* genetic locus can lead to complex clinical scenarios; Accordingly, identifying this specific site in clinical bacterial samples would significantly improve treatment methods and preventive measures. Furthermore, this understanding could contribute to the development of strategies to

counter the bacteria's ability to colonize and penetrate medical devices. Accordingly, the use of polymerase chain reaction (PCR) technology to detect the "ica" gene cluster (ica operon) serves as a valuable method for distinguishing between pathogenic and non-pathogenic strains (46).

The sigB gene plays a vital role in promoting biofilm formation in *Staphylococcus aureus* [47]. Our research found that 92.85% of the analyzed isolates harbored the sigB gene. (see Table X). The detection of this factor in the samples indicates its role as a global regulator that influences biofilm formation and supports bacterial adaptation. The Sigma B (SigB) factor regulates the expression of multiple genes that provide resistance to various environmental stresses, including heat, oxidative damage, and antimicrobial agents. Consequently, SigB is a crucial component of the bacteria's ability to survive and thrive under challenging conditions. [48] The current study identified the katA and sodA genes in *S. aureus* isolates, highlighting fundamental mechanisms for coping with oxidative stress. These genes are likely to contribute to the bacteria's ability to thrive and adapt under harsh conditions. Specifically, the katA gene encodes catalase, an enzyme that breaks down hydrogen peroxide (H_2O_2). The SodA gene produces the enzyme superoxide dismutase, which protects bacterial cells from reactive oxygen species (ROS). During aerobic metabolism and in response to infection, neutrophils produce reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2), as a vital component of their defense mechanisms against pathogens, highlighting the importance of oxidative stress resistance for bacterial survival [49].

Consequently, the identification of these genes in the current study may shed light on how *S. aureus* strains withstand oxidative stress, thrive within the host environment, and maintain their pathogenicity.

Antibiotics susceptibility test

An assessment of antibiotic resistance in PCR-verified *Staphylococcus aureus* isolates revealed notable resistance to various antibiotics commonly used in veterinary practice. It is worth noting that the drug Azithromycin showed the highest level of resistance at 92.85%, followed immediately by Tetracycline with a resistance rate of 89.28%, then Ciprofloxacin at 85.71%, and Erythromycin at 78.57%. The results reveal the widespread emergence of antimicrobial-resistant strains of *Staphylococcus aureus* (*S. aureus*) among animals. The observed resistance to tetracycline aligns with previous research identifying it as one of the antibiotics to which *S. aureus* exhibits the highest levels of resistance. Previous studies have also indicated similar trends; notable levels of tetracycline resistance have been documented in animal-associated isolates. The observed increase in resistance is likely due to the extensive use of tetracycline in livestock for both therapeutic and preventive purposes, which fosters the emergence of resistant strains. Furthermore, resistance rates to the macrolide group were alarmingly high; 92.85% of the isolates exhibited resistance to azithromycin, while 78.57% showed resistance to erythromycin. It is worth noting that the level of erythromycin resistance observed in this study exceeds the rates recorded in previous reports. (52,53), A study conducted by the World Health Organization showed that 65% of *Staphylococcus aureus* (*S. aureus*) isolates exhibited resistance to erythromycin. Differences in resistance levels may arise from a range of factors, including variations in antimicrobial usage patterns, animal species, geographical influences, and inconsistencies in management practices. The resistance rate to ciprofloxacin reached an alarming 85.71%, a figure that far exceeds previous findings; in contrast, an earlier study indicated a much lower resistance rate of only 12.7%. (54). Variations in antibiotic resistance patterns are often linked to differing rates of antibiotic consumption, associated selective pressures, and the presence of resistant strains in diverse environments. The increasing use of fluoroquinolones in veterinary medicine may contribute to the emergence and spread of ciprofloxacin-resistant bacteria. On a positive note, all tested isolates demonstrated complete susceptibility to oxacillin (100%), indicating a significant reduction in the incidence of methicillin resistance within this group. Two specific isolates demonstrated resistance to ceftiofur, confirming their identification as methicillin-resistant *Staphylococcus aureus* (MRSA). Ceftiofur serves as a reliable indicator for detecting MRSA, as it triggers methicillin-associated resistance mechanisms more effectively than oxacillin. (55). The detection of methicillin-resistant *Staphylococcus aureus* (MRSA), even in small numbers, raises significant public health concerns because these strains can act as carriers of antibiotic resistance genes within animal populations. The resistance observed in this study can be attributed to the improper and excessive use of antibiotics in agriculture, as well as the transmission of resistant strains among animals and their exposure to antibiotic residues in the environment. Previous studies on antibiotic resistance in animal-associated *Staphylococcus aureus* isolates have indicated similar effects.

Antibacterial drugs face significant obstacles due to their limited ability to penetrate the biofilm matrix, allowing *Staphylococcus aureus* (*S. aureus*) cells to survive even in the presence of low levels of antibiotics. Bacterial biofilms play a

vital role in the development of drug resistance. However, there are additional factors that contribute significantly to the rise of antimicrobial resistance; these include the ability of bacteria to evade the host's innate immune defense mechanisms and to undergo genetic mutations that facilitate adaptation, as well as the transfer of genetic material by horizontal gene transfer. (56).

CONCLUSION

This study reveals a notable presence of *Staphylococcus aureus* in sheep, highlighting a significant level of antimicrobial resistance among the isolated strains. The discovery of genes associated with biofilm formation highlights the capability of polymerase chain reaction (PCR) technology to detect these genetic markers. The ubiquitous presence of the *icaA* gene across all isolates indicates an enhanced capacity to produce the polysaccharide intercellular adhesin (PIA). This compound is a key component in the formation of robust biofilms and may contribute to the development of persistent, chronic infections. In addition, two isolates were identified as methicillin-resistant *Staphylococcus aureus* (MRSA), given that they exhibited resistance to cefoxitin. This poses a potential health threat, as these strains could be transmitted to humans, particularly those in close contact with animals. Further investigations are necessary to understand the transmission routes of these strains and to identify their sources, whether they originate from animals or humans.

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