**Iraqi Journal of Veterinary Sciences**

www.vetmedmosul.com

# Molecular detection and phylogenetic diversity of *Staphylococcus aureus* isolated from goat subclinical mastitis in Nineveh governorate

**A.H. Taha, O.H. Sheet and D.M. Jwher**

Department of Veterinary Public Health, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

**Article information**

***Article history:***
Received 18 July, 2024
Accepted 03 November, 2024
Published online 01 January, 2025

***Keywords***:
Phylogenetic analysis tree
*S. aureus*
Goat milk
Virulence factors

***Correspondence:***
O.H. Sheet
omar.sheet@uomosul.edu.iq

**Abstract**

Mastitis in goats is thought to be caused mainly by *Staphylococcus* (*S.*) *aureus*. On the other hand, little information is known about methicillin-resistant *S. aureus* (MRSA) in goat milk. The current research aimed to determine the occurrence of *S. aureus* and MRSA in milk samples from Mosul City's goat farms. Sixty goat milk samples were collected from October 2023 to February 2024 from different governorate areas. This research used conventional techniques such as selective media and biochemical tests. A PCR assay was also used to detect the isolates' *nuc*, *mecA*, *clfA*, *clfB*, and *coa* genes. This study showed that the rate of *S. aureus* in goat subclinical mastitis was 5%, and the high occurrence of *S. aureus* in goat milk was 20% in the Ali Rash area. In addition, this research revealed that all *S. aureus* isolates possessed the *nuc*, *mecA*, *clfB*, and *coa* genes 100% (3/3), while the *clfA* gene was 66.7% (2/3). There are two types of gene profiles found in *S. aureus* isolates. Nine novel strains of *S. aureus* sequences are registered in the NCBI Genbank. The nine newly discovered *S. aureus* isolates had similarities to other strains of *S. aureus* found globally. Nine novel strains of *S. aureus* sequences are registered in the NCBI Genbank. The nine newly discovered *S. aureus* isolates had similarities to other strains of *S. aureus* found globally.

DOI: 10.33899/ijvs.2024.152066.3791, 

## Introduction

*Staphylococcus* (*S.*) *aureus* is one major pathogen identified in dairy goats responsible for both preclinical and symptomatic intramammary infections (1). *S. aureus* has been determined to cause 4.1% to 18.0% of all bacterial intramammary infections (2). Additionally, these microbes are commonly found in milk and its products (3). From mild-local, superficial skin lesions to significant invasive infections, *S. aureus* causes many clinical symptoms and may be fatal (4). There is a correlation between virulence factors and the severity and signs of *S. aureus* infections (5). The *nuc* gene encodes the virulence factor heat-resistant nuclease gene, strongly associated with enterotoxin production. At the same time, the pathogen attachment mechanism is carried out by fibrinogen-binding proteins termed clumping factors (*clf*) A and B, also a penicillin-binding protein (PBP 2a) with low affinity for β-lactam antibiotics that is encoded by the *mecA* gene, and staphylococcal enterotoxins (SEs) are the primary cause of foodborne poisoning among them. Food poisoning from *S. aureus* bearing SEs can occur quickly, develop quickly, and be extremely dangerous to human health (6). In addition, *S. aureus* possesses various types of toxics such as exfoliative toxins A and B (eta and etb), toxic shock syndrome toxin-1 (tst), staphylococcal enterotoxins (SEs), and toxic shock syndrome toxin-like (SEls) are expressed by certain *S. aureus* strains. It is commonly known that SEs, or SEls, are a primary reason for illness caused by food (7). Reports state that 95% of staphylococcal food poisoning (SFP) cases are caused by these five traditional enterotoxins, with newly identified SEs responsible for the remaining 5% of infections (8). When the mammary glands udder tissue becomes inflamed due to trauma or infectious bacteria, it results in

mastitis in ruminants (9). It is the most widespread illness that is economically harmful to dairy company operations because it reduces milk production by nearly 10% to 20% and has a negative impact on milk ingredients, making them less nutritious (low quality) and unfit to undergo processing and consumption (10,11). The direct and indirect mastitis costs result in a large financial loss (12). Bovine mastitis directly costs money for veterinary care and increases labour needs (13). The significant financial losses caused by mastitis-related losses in milk availability and quality are the cost of various forms of bovine mastitis (14). The mammary glands contain ruminants of more than 100 different species of bacteria, yet only a tiny percentage of these bacteria have been shown to cause mastitis (15). The cause of mastitis microorganisms may be categorized into two groups based on their reservoir, source, and transmission mechanism: the first category is an infectious bacterium. In contrast, the second category is an environmental bacterium (16). Mastitis risks public health because it can spread zoonoses and diseases associated with contaminated food (17). Because of the significant risk of harmful microorganisms from cattle, automated milking machines, and milk containers contaminating raw milk, it is not recommended to eat raw milk directly (18). Goat milk production is a burgeoning industry that produces a significant amount of mainstream dairy milk in hard-climate regions where large ruminants are either impossible to rear or very difficult to raise (19). Goat milk offers several advantages over human and cow milk, including superior digestion, alkalinity, buffer capacity, and therapeutic value (20). Goat milk plays a significant role in several countries' economies and dairy industries. Asia and Africa are home to more than 80% of the worldwide goats (21). Goat milk output is estimated to be 12 million tons worldwide (including 2 million tons in Europe), or 2% of all animal milk produced worldwide (22). Product consumption derived from goat milk has risen, which can be attributed to the health advantages and nutritional benefits associated with goat milk (23). The cleanliness of the milking system, the conditions during transportation, and storage all affect raw milk quality. On the other hand, it is also intimately linked to several animal illnesses, including mastitis, which could cause pathogenic bacteria in milk (24).

Because pathogenic *S. aureus* is important in goat subclinical mastitis, this research aims to determine the amount of damage caused by pathogenic *S. aureus* in milk samples collected during the governorate of Nineveh's goat subclinical mastitis, identify the methicillin-resistant strain of *S. aureus*, and identify the genes encoding virulence factors in *S. aureus* isolates.

## Materials and methods

### Ethical approval

With the approved ID of UM. Vet. 2024.003, all samples were obtained with the owners' consent and implemented in compliance with the ethical guidelines provided by the Institutional Animal Care and Use Committee at Mosul University's College of Veterinary Medicine.

### Samples collection

Sixty goat milk samples were obtained from various governorate areas from October 2023 to February 2024. These regions included Ali Rash, Brtala, Kukagly, and Hama Al Alil. The goat milk samples were collected in sanitary containers and sent straight to the laboratory of the Dept. Veterinary Public Health/College of Veterinary Medicine/Mosul University. All containers containing peptone water were placed in an incubator and underwent pre-enrichment for 18 to 24 hours at 37°C. After streaking milk samples across Blood and 7.5% Mannitol salt media plates, they were incubated at 37°C for 24 hr.

### *S. aureus* isolation and characterization

Morphological assessment, catalase and coagulase activity tests, and gram staining were used to study the characteristic *S. aureus* colonies (25).

### Extraction of DNA

To extract the *S. aureus* genomic DNA, the positive isolates of *S. aureus* were cultivated for 24 hours at 37°C on a mannitol salt medium. The DNeasy Blood and Tissue Kit (Qiagen, Germany) was used to extract DNA, following the Gram-positive bacteria-specific protocol provided by the producer. Following Nanodrop (Biodrop, UK) measurement, the separated DNA was maintained at -20°C.

### PCR Reaction

The PCR method detected the *nuc, mecA, clfA, clfB*, and *coa* genes of *S. aureus*. The molecular weight of the *nuc* gene is 166 bp (26), the *mecA* gene is 147 bp (27), the *clfA* gene is 288 bp (28), the *clfB* gene is 203 bp (28), and the *coa* gene is 674 bp (29) are shown in table 1. A total of 30 µl was used for the PCR reaction, and the mixture was prepared in a 200 µl tube (Biozym, Oldenhorf, Germany). The resultant amplicons were analyzed using gel electrophoresis on a 2% agarose gel (Peqlab, Erlangen, Germany) using a 100 bp ladder as a reference. 15 µl of Promega Corporation's (2×) GoTaq Green Mix Master, 1 µl of primer 1, 1 µl of primer 2, 9 µl of Promega Corporation's (USA) DNeasy-free water, and 4 µl of the *S. aureus* DNA template made up the reaction mixture.

### DNA sequencing

The samples were sent to Macrogen, a South Korean commercial sequencing company, to purify and sequence six PCR amplicons acquired from milk goat isolates previously found to be *S. aureus*-positive by classical PCR. The target genes for sequencing were the *clfA, clfB*, and *coa* genes. The obtained *clfA, clfB*, and *coa* genes sequences were then compared to previously published *S. aureus* sequences that

are available on GenBank using the NCBI BLASTn program, which is accessible at http://www.ncbi.nlm.nih.gov. The alignment and comparability of these sequences were further examined using the online multiple sequence alignment program CLUSTALW from GenomeNet (available at http://www.genome.jp/tools/clustalw/). The Neighbor-Joining (NJ) program and the same Genome Net tool, CLUSTALW, were used to generate phylogenetic trees. Five hundred duplicates of the *S. aureus clfA, clfB*, and *coa* gene sequences were utilized as an outgroup while creating the phylogenetic tree to increase robustness. This comprehensive approach aimed to shed light on the genetic relationships between the *S. aureus* isolates by using purification, sequencing, and subsequent bioinformatics analysis to better understand the phylogenetic context of the isolates from milk goats.

Table 1: PCR techniques and primers for identifying different *S. aureus* genes

| Gene | Primer | Sequence (5- 3) | Amplicon size [bp] | Programme* | Reference |
|---|---|---|---|---|---|
| *nuc* | nuc-1<br>nuc-2 | 5-CCTGAAGCAAGTGCATTTACGA-3<br>5-CTTTAGCCAA GCCTTGACGAACT-3 | 166 | I | (26) |
| *mecA* | MEC A-1<br>MEC A-2<br>SPA-2 | 5-GTGAAGATATACCAAGTGATT-3<br>5-ATGCGCTATAGATTGAAAGGAT-3<br>5-GCTTTTGCAATGTCATTTACTG-3 | 147 | II | (27) |
| *clfA* | clfA-1<br>clfA-2 | 5-ATTGGCGTGGCTTCAGTGCT-3<br>5-CGTTTCTTCCGTAGTTGCATTTG-3 | 288 | I | (28) |
| *clfB* | clfB-1<br>clfB-2 | 5-ACATCAGTAATAGTAGGGGCAAC-3<br>5-TTCGCACTGTTTGTGTTTGCAC-3 | 203 | III | (28) |
| *coa* | coa-1<br>coa-2 | 5-ATAGAGATGCTGGTACAGG-3<br>5-GCTTCCGATTGTTCGATGC-3 | 674 | I | (29) |

PCR program: I: 35 times (94°C - 30s, 55°C - 30s, 72°C - 30s), II: 35 times (94°C - 30s, 54°C - 30s, 72°C - 30s), III: 35 times (94°C - 30s, 60°C - 30s, 72°C - 30s).

**Results**

The colonies of *S. aureus* that yielded good results had a golden-yellowish tint based on how they appeared on Mannitol salt agar. In addition, positive results were obtained for particular biochemical evaluations, including coagulase and catalase, which verified the existence of *S. aureus* isolates. This study showed that the *S. aureus* isolates in this current research had a rate of occurrence of 5% (3/60). In the Ali Rash region, the highest percentage of *S. aureus* found in goat sub-clinical mastitis was 20% (3/15), whereas no *S. aureus* has been isolated from any other regions (Tables 2-4). According to Table 3, the results of the PCR assay agreed with those of the traditional methods, showing that all isolates of *S. aureus* had the *nuc* gene (Figure 1). According to Table 3, 100% (3/3) of the *S. aureus* possessed the *mecA* gene (Figure 2). Furthermore, our findings showed that just two *S. aureus* contained the *clfA* gene 66.7% (2/3) (Figure 3). Additionally, from this study, 100% (3/3) of *S. aureus* had the *clfB* gene (Figure 4). Furthermore, 100% (3/3) of *S. aureus* had the *coa* gene (Figure 5). The current study's findings additionally revealed that the *S. aureus* isolates were hooked on two separate gene profiles based on the presence of unique genes in each isolate (Table 4). Gene profile I (*nuc* + *mecA* + *clfA* + *clfB* + *coa*) was most frequently present in 2 (66.7%) *S. aureus* isolates, while gene profile II (*nuc* + *mecA* + *clfB* + *coa*) was reported in 1 (33.3%) isolate. Single genes were absent from all *S. aureus* isolates.

Table 2: The number of samples and percentage of positive *S. aureus* isolates

| Areas | Samples (No.) | Positive *S. aureus* (No.) | Percentages (%) |
|---|---|---|---|
| Ali Rash | 15 | 3 | 20% |
| Brtala | 15 | 0 | 0 |
| Kukagly | 15 | 0 | 0 |
| Hama Al Alil | 15 | 0 | 0 |
| Total | 60 | 3 | 5% |

Table 3: The number and percentage of the genes found in *S. aureus* isolates

| Gene | *S. aureus* |
|---|---|
| | Number (%) |
| 1. *nuc* | 3 (100) |
| 2. *mecA* | 3 (100) |
| 3. *clfA* | 2 (66.7) |
| 4. *clfB* | 3 (100) |
| 5. *coa* | 3 (100) |

Table 4: Genetic characterization of three *S. aureus* isolates obtained from milk samples of sheep with sub-clinical mastitis

| | Staphylococcus genes | Isolates n (%) |
|---|---|---|
| I | *nuc* + *mecA* + *clfA* + *clfB* + *coa* | 2 (66.7) |
| II | *nuc* + *mecA* + *clfB* + *coa* | 1 (33.3) |

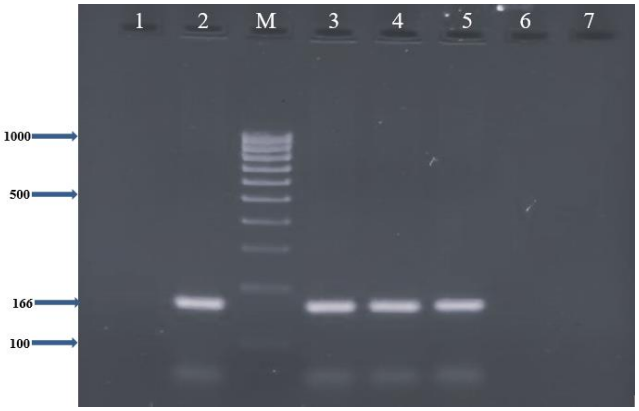


Figure 1: Electrophoretic analysis of PCR products for *S. aureus nuc* gene detection. The DNA amplification appears as a ladder-like pattern. Lane 1 contains a negative control, Lane 2 shows the positive control, and Lanes marked as "M" are DNA markers using a 100 bp ladder from Biozym Diagnostic. Lanes 3 to 5 display positive isolates, while Lanes 6 and 7 show negative isolates.

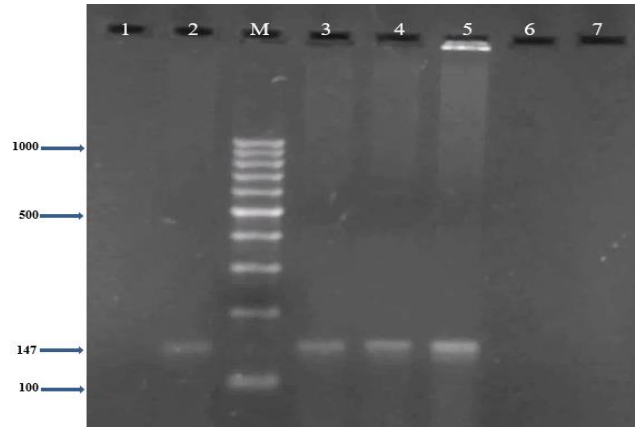


Figure 2: Electrophoretic analysis of PCR products for *S. aureus mecA* gene detection. The DNA amplification appears as a ladder-like pattern. Lane 1 contains a non-template control, Lane 2 shows the positive control, Lanes marked as "M" are DNA markers using a 100 bp ladder from Biozym Diagnostic, and Lanes 3 to 5 display positive isolates, while Lanes 6 and 7 show negative isolates.

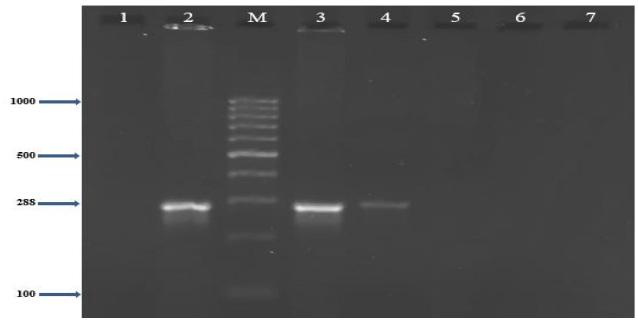


Figure 3: Electrophoretic analysis of PCR products for *S. aureus clfA* gene detection. The amplification of DNA appears as a ladder-like pattern. Lane 1 contains a non-template control, Lane 2 shows the positive control, Lanes marked as "M" are DNA markers using a 100 bp ladder from Biozym Diagnostic, and Lanes 3 and 4 display positive isolates, while Lanes 5 to7 show negative isolates.

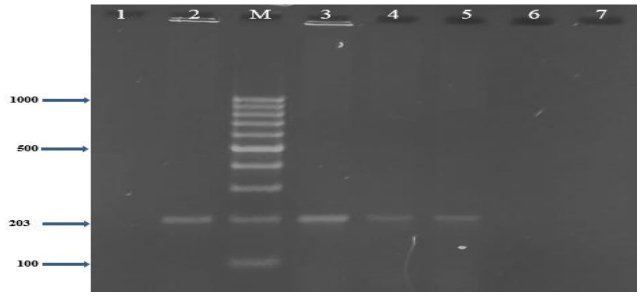


Figure 4: Electrophoretic analysis of PCR products for *S. aureus clfB* gene detection. The DNA amplification appears as a ladder-like pattern. Lane 1 contains a non-template control, Lane 2 shows the positive control, Lanes marked as "M" are DNA markers using a 100 bp ladder from Biozym Diagnostic, and Lanes 3 to 5 display positive isolates, while Lanes 6 and 7 show negative isolates.

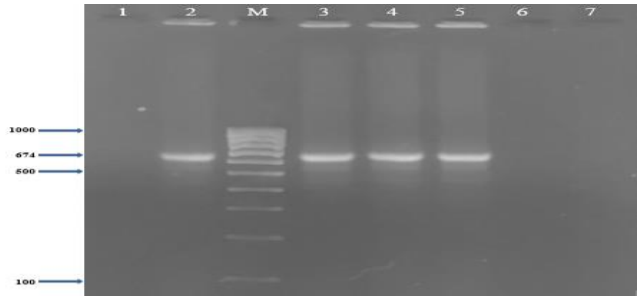


Figure 5: Electrophoretic analysis of PCR products for *S. aureus coa* gene detection. The DNA amplification appears as a ladder-like pattern. Lane 1 contains a non-template control, Lane 2 shows the positive control, Lanes marked as "M" are DNA markers using a 100 bp ladder from Biozym Diagnostic, and Lanes 3 to 5 display positive isolates, while Lanes 6 and 7 show negative isolates.

The sequencing results of this research showed that the individual sequencing analysis (BLASTn) was carried out on nine novel gene sequences (three novel *clfA* gene sequences, three novel *clfB* gene sequences, and three novel *coa* gene sequences) obtained from the subclinical mastitis milk of goats. As indicated in table 5, the *S. aureus* sequences that are provided in the NCBI Genbank are indexed under the following accession numbers:( PP958240, PP958241, PP958242, PP973841, PP973842, PP973843, PP951509, PP951510, and PP951511). A phylogenetic tree analysis in MegAlign software applying the maximum likelihood technique showed that local difference gene sequences displayed a distinct relationship from those previously described and available in the GenBank database. Furthermore, the *S. aureus* sequence types based on the *clfA* gene (PP958240, PP958241, and PP958242) and the United Kingdom's CP001996.1 and France CP025395 sequence types have a tight link was 100%. Whereas the sequence types (PP958240, PP958241, and PP958242) of this research have 99.6% and 98.3% similarity with LT615218.1 Australia and FR821779.1 United Kingdom, respectively. Furthermore, the *S. aureus* sequence types based on the *clfB* gene (PP973841, PP973842, and PP973843) and HQ424333.1 Germany and HQ424333.1 USA appeared to have the closest relationship of 100%. Meanwhile, the sequence types (PP973841, PP973842, and PP973843) have a 99.4% similarity with CP134882.1 South Korea. The *S. aureus* sequence types based on the *clfA* and *clfB* did not compare with those of *S. aureus* isolated from Iraq. Moreover, a strong correlation was seen between the *S. aureus* sequence types depending on the *coa* gene (PP951509, PP951510, and PP951511) and the sequence types of CP042650.1 China and CP085320.1 South Korea achieved 100%. In addition, the sequence types (PP951509, PP951510, and PP951511) were similar to CP041634.1 China, CP127671.1 Australia, CP075511.1 Thailand, and CP020354.1 Republic of Korea were 97.2%, 95.3%, 95%, and 94.2%, respectively. Moreover, the sequence types (PP951509, PP951510, and PP951511) appeared similar with LC425081.1 Al Basra, Iraq and ON631029.1 Al Diyala, Iraq was 95% and 94.5%, respectively (Figures 6 and 7).

Table 5: The NCBI GenBank accession numbers for the *clfA, clfB*, and *coa* genes of *S. aureus* sequences in goat's milk

| Accession numbers | Bacteria | Gene | Types of samples |
|---|---|---|---|
| PP958240 | *S. aureus* | *clfA* | Goat's milk |
| PP958241 | *S. aureus* | *clfA* | Goat's milk |
| PP958242 | *S. aureus* | *clfA* | Goat's milk |
| PP973841 | *S. aureus* | *clfB* | Goat's milk |
| PP973842 | *S. aureus* | *clfB* | Goat's milk |
| PP973843 | *S. aureus* | *clfB* | Goat's milk |
| PP951509 | *S. aureus* | *coa* | Goat's milk |
| PP951510 | *S. aureus* | *coa* | Goat's milk |
| PP951511 | *S. aureus* | *coa* | Goat's milk |

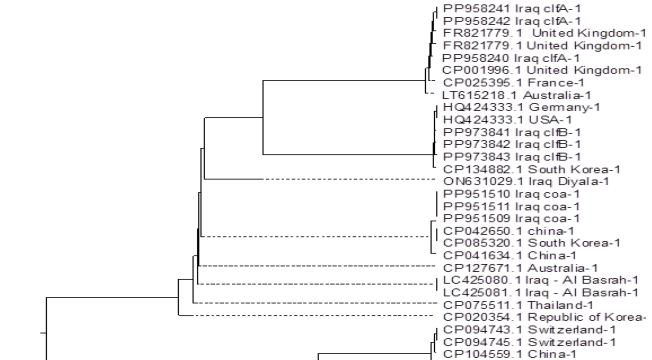


Figure 6: Clustering analysis of the *clfA, clfB,* and *coa* gene sequences of *S. aureus* and other *various* gene sequences of *S. aureus* isolates retrieved from NCBI GenBank. The designation in the parentheses indicates the NCBI accession number.

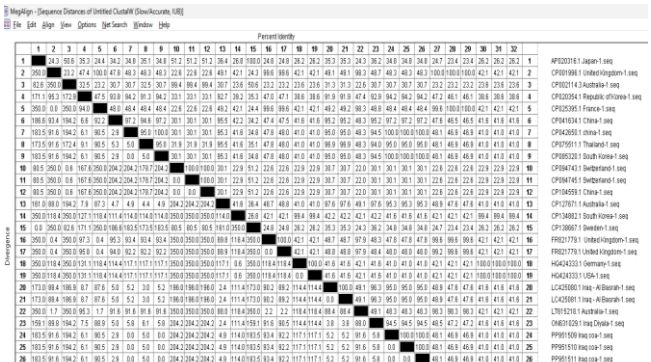


Figure 7: *clfa, clfB*, and *coa* gene sequences similarity and divergence of each pair for *S. aureus* calculated by DNASTAR.

## Discussion

A microorganism infection is typically the cause of mastitis, a significant mammary gland illness that can be either subclinical (mild) or clinical (severe). To increase our knowledge of the spread of *S. aureus* in Nineveh goat herds, we characterized *S. aureus* detected in cases of goat subclinical mastitis collected in several herds and various areas of Nineveh governorate/Iraq. Several reports of *S. aureus* isolated from bovine subclinical mastitis (30) and camel mastitis (31) in Nineveh governorate/Iraq. The present investigation revealed that 5% (3/60) of goat milk samples with subclinical mastitis had *S. aureus* isolated from them. Several studies showed that clinical and subclinical goat mastitis caused by *S. aureus* varies from 5.6% to 37% in different counties (32-36). The rate of occurrence of *S. aureus* in goat's milk has been reported in many previous studies to be greater than the findings of our research; it was 46% (47/104) in the United States (37), 66% in Spain (38), and 76.9% (60/78) in Italy (39). The rate of occurrence of *S. aureus* in this research was near to the findings of other investigations, at 6.3% in Iran (40) and 6.2% in Norway (41). While several additional studies have revealed a lower rate of occurrence of *S. aureus* in goat's milk than the current research results, it was 1.4% in the USA (42) and 1.5% in China (43). The variations in *S. aureus* occurrence rates are based on the varying geographic distributions and sanitary conditions in milk production facilities and farms. Numerous earlier investigations revealed that *S. aureus* was found in udder skin, bedding, labourers' hands, insects, and dust, significant in transmitting *S. aureus* among mammary glands of animals and tainted milk (44).

Furthermore, the isolation of *S. aureus* from various animals' organs, including the vagina, muzzle, and skin wounds, causes the spread of *S. aureus* from body sites to environments on the one hand and from dairy herds to their calves on the other, via ventilation or by providing infants milk from animals that has *S. aureus* (45). In addition, often pointed-out issues included dirty milking utensils, insufficient cleanliness of milking staff, inadequate udder preparation, unhygienic farms, carrying milk without cold chains, and not realizing enough about foodborne illnesses (46). All of the investigated farms had refrigerated milk storage facilities in place. Therefore, seasonal management methods (i.e., grazing in the summer and confinement in the winter) are more likely to be to blame for this effect than seasonal variations in the temperature (47).

Additionally, all *S. aureus* isolates had 100% (3/3) of the *nuc, mecA, clfB*, and *coa* genes; only two isolates had 66.7% (2/3) of the *clfA* gene. Many previous investigations discovered that the *nuc* gene was identified in all coagulase-positive *S. aureus* (48,49). Moreover, the *mecA* gene was present for each isolate of *S. aureus*, indicating that the organism was methicillin-resistant *S. aureus* (MRSA). According to earlier investigations, the *S. aureus* isolated from goat milk in Spain lacked the *mecA* gene (47). People in close proximity to animals are exposed to colonization and subsequent infection in areas where the occurrence of livestock-associated clones is significant. The MRSA outbreak may be prevented by systematically surveilling MRSA in animals produced for food (50). Numerous studies concluded that there was no MRSA isolated from goat's milk (51-53). While the occurrence of MRSA in Greece was 5.5% (9/162) (54), in China it was 51.6% (55), and in Uganda it was 56.1% (56). Encoding adhesion factors are the *clfA* and *clfB* genes. Numerous studies have reported revealing these genes in *S. aureus* in goat milk throughout the globe (57-59). The results of our research concurred with those of prior investigations, which showed that all strains of *S. aureus* possessed the *clfA* and *clfB* genes (60). In Iran, 84 and 65.3% of *S. aureus* in bovine milk carried the *clfA* and *clfB* genes (61). Numerous investigations found *clfA* in 19%-100% of isolates with bovine mastitis and *clfB* in 91.8%-92.9% (62-65). Although every isolate displayed a coagulase-positive reaction on mannitol salt agar, all *S. aureus* isolates carried the *coa* gene when the primers mentioned above were used. After agarose gel investigation of the amplified products, PCR amplification of the *coa* gene revealed a single amplicon for the 674 bp. Our results are consistent with previous research that the *coa* gene existed in all *S. aureus*, and the *coa* gene produced five distinct PCR products with a range of around 500, 580, 660, 740, and 820 bp (66,49). A different study revealed that all *S. aureus* isolates had the *coa* gene, which produced 580 bp amplicons (67,68).

## Conclusion

The presence of *S. aureus*, and mainly MRSA in subclinical mastitis goat milk, raises serious concerns for the health of the animals because *S. aureus* is capable of resisting β-lactams, one of the most effective antimicrobials for treating mastitis. Raw milk has *S. aureus* and MRSA, which can spread throughout the community via the dairy supply chain. Therefore, from their existence in raw milk, there is a significant health danger to people of all ages. In low-input goat farms, the high incidence rate of *S. aureus* is justified by bad husbandry techniques, a disregard for basic hygiene precautions, and the haphazard use of antibiotics. Numerous genes encode various virulence factors in *S. aureus* isolated from goat milk at various geographical locations worldwide.

## Acknowledgment

We thank the College of Veterinary Medicine / Mosul University for furnishing the essential resources needed to complete this study.

## Conflict of interest

The writer of the manuscript has affirmed that neither the writing nor the information analysis phases included any conflicts of interest.

## References


1. Doğruer G, Saribay MK, Ergün Y, Aslantaş Ö, Demir C, Ateş CT. Treatment of subclinical mastitis in Damascus goats during lactation. Small Rumin Res. 2010;90(1-3):153-5. DOI: 10.1016/j.smallrumres.2010.01.003
2. Virdis S, Scarano C, Cossu F, Spanu V, Spanu C, De Santis EP. Antibiotic resistance in *Staphylococcus aureus* and coagulase negative staphylococci isolated from goats with subclinical mastitis. Vet Med Int. 2010;2010. DOI: 10.4061/2010/517060
3. Vyletělová M, Hanuš O, Karpíšková R, Šťástková Z. Occurrence and antimicrobial sensitivity in staphylococci isolated from goat, sheep and cow's milk. Acta Univ Agric Silvic Mendel Brun. 2011;59:209-14. [available at]
4. Turner NA, Sharma-Kuinkel BK, Maskarinec SA, Eichenberger EM, Shah PP, Carugati M, Holland TL, Fowler Jr VG. Methicillin-resistant *Staphylococcus aureus*: An overview of basic and clinical research. Nat Rev Microbiol. 2019;17(4):203-18. DOI: 10.1038/s41579-018-0147-4
5. Xing X, Zhang Y, Wu Q, Wang X, Ge W, Wu C. Prevalence and characterization of *Staphylococcus aureus* isolated from goat milk powder processing plants. Food Control. 2016;59:644-50. DOI: 10.1016/j.foodcont.2015.06.042
6. Basanisi MG, La Bella G, Nobili G, Franconieri I, La Salandra G. Genotyping of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from milk and dairy products in South Italy. Food Microbiol. 2017;62:141-6. DOI: 10.1016/j.fm.2016.10.020
7. Argudín MÁ, Mendoza MC, Rodicio MR. Food poisoning and *Staphylococcus aureus* enterotoxins. Toxins. 2010;2(7):1751-73. DOI: 10.3390/toxins2071751
8. Papadopoulos P, Angelidis AS, Papadopoulos T, Kotzamanidis C, Zdragas A, Papa A, Filioussis G, Sergelidis D. *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) in bulk tank milk, livestock and dairy-farm personnel in north-central and north-eastern Greece: Prevalence, characterization and genetic relatedness. Food Microbiol. 2019;84:103249. DOI: 10.1016/j.fm.2019.103249
9. Gomes F, Henriques M. Control of bovine mastitis: Old and recent therapeutic approaches. Curr Microbiol. 2016;72:377-382. DOI: 10.1007/s00284-015-0958-8
10. Rathod P, Shivamurty V, Desai AR. Economic losses due to subclinical mastitis in dairy animals: A study in Bidar district of Karnataka. Indian J Vet Sci Biotech. 2017;13(1):37-41. DOI: 10.21887/ijvsbt.v13i01.87325
11. Romero J, Benavides E, Meza C. Assessing financial impacts of subclinical mastitis on Colombian dairy farms. Front Vet Sci. 2018;5:273. DOI: 10.3389/fvets.2018.00273
12. Kossaibati MA, Esslemont RJ. The costs of production diseases in dairy herds in England. Vet J. 1997;154(1):41-51. DOI: 10.1016/s1090-0233(05)80004-8
13. Lescourret F, Coulon JB. Modeling the impact of mastitis on milk production by dairy cows. J Dairy Sci. 1994;77(8):2289-301. DOI: 10.3168/jds.S0022-0302(94)77172-1
14. Seegers H, Fourichon C, Beaudeau F. Production effects related to mastitis and mastitis economics in dairy cattle herds. Vet Res. 2003;34(5):475-91. DOI: 10.1051/vetres:2003027
15. Owens WE, Watts JL. Antimicrobial susceptibility and beta-lactamase testing of staphylococci isolated from dairy herds. J Dairy Sci. 1988;71(7):1934-9. DOI: 10.3168/jds.S0022-0302(88)79763-5
16. Hogeveen H, Huijps K, Lam TJ. Economic aspects of mastitis: New developments. N Z Vet J. 2011;59(1):16-23. DOI: 10.1080/00480169.2011.547165
17. Blum S, Heller ED, Krifucks O, Sela S, Hammer-Muntz O, Leitner G. Identification of a bovine mastitis Escherichia coli subset. Vet Microbiol. 2008;132:135–148. DOI: 10.1016/j.vetmic.2008.05.012
18. Zouharova M, Rysanek D. Multiplex PCR and RPLA identification of *Staphylococcus aureus* enterotoxigenic strains from bulk tank milk. Zoonoses Public Health. 2008;55:313–319. DOI: 10.1111/j.1863-2378.2008.01134.x
19. Silanikove N, Leitner G, Merin U, Prosser CG. Recent advances in exploiting goat's milk: quality, safety and production aspects. Small Rumin Res. 2010;89(2-3):110-24. DOI: 10.1016/j.smallrumres.2009.12.033
20. Park YW. Hypo-allergenic and therapeutic significance of goat milk. Fd Sci Indust. 2001;34(4):6-13. [available at]
21. Oliveira CJ, Hisrich ER, Moura JF, Givisiez PE, Costa RG, Gebreyes WA. On-farm risk factors associated with goat milk quality in Northeast Brazil. Small Rumin Res. 2011;98(1-3):64-9. DOI: 10.1016/j.smallrumres.2011.03.020
22. Danków R, Pikul J. Technological suitability of sheep milk for processing. Nauka Przyr Technol. 2011;5(2):1-5. [available at]
23. Silanikove N, Leitner G, Merin U, Prosser CG. Recent advances in exploiting goat's milk: Quality, safety and production aspects. Small Rumin Res. 2010;89(2-3):110-24. DOI: 10.1016/j.smallrumres.2009.12.033
24. Quinn PJ, Markey BK, Carter ME, Donnelly WJC, Leonard FC, Maguire D. Veterinary Microbiology and Microbial Diseases. 1st ed. UK: Blackwell Science Ltd; 2002. [available at]
25. Graber HU, Casey MG, Naskova J, Steiner A, Schaeren W. Development of a highly sensitive and specific assay to detect *Staphylococcus aureus* in bovine mastitic milk. J Dairy Sci. 2007;90(10):4661-9. DOI: 10.3168/jds.2006-902
26. Zhang K, McClure JA, Elsayed S, Louie T, Conly JM. Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome mec types I to V in methicillin-resistant Staphylococcus aureus. J Clin Microbiol. 2005;43:5026–5033. DOI: 10.1128/JCM.43.10.5026-5033.2005
27. Tristan A, Ying L, Bes M, Etienne J, Vandenesch F, Lina G. Use of multiplex PCR to identify *Staphylococcus aureus* adhesins involved in human hematogenous infections. J Clin Microbiol. 2003;41(9):4465–4467. DOI: 10.1128/jcm.41.9.4465-4467.2003
28. Javid F, Taku A, Bhat MA, Badroo GA, Mudasir M, Sofi TA. Molecular typing of *Staphylococcus aureus* based on coagulase gene. Vet World. 2018;11(4):423–430. DOI: 10.14202/vetworld.2018.423-430
29. Sheet OH. Molecular detection of mecA gene in methicillin-resistant Staphylococcus aureus isolated from dairy mastitis in Nineveh governorate, Iraq. Iraqi J Vet Sci. 2022;36(4):939-43. DOI: 10.33899/ijvs.2022.132643.2115
30. Sheet OH, Jwher DM, Al-Sanjary RA, Alajami AD. Direct detection of *Staphylococcus aureus* in camel milk in the Nineveh governorate by using the PCR technique. Iraqi J Vet Sci. 2021; 35(4):669-672. DOI: 10.33899/ijvs.2020.127725.1524
31. Deinhofer M, Pernthaner A. *Staphylococcus spp.* as mastitis-related pathogens in goat milk. Vet Microbiol. 1995;43(2-3):161-6. DOI: 10.1016/0378-1135(95)92532-G
32. White EC, Hinckley LS. Prevalence of mastitis pathogens in goat milk. Small Rumin Res. 1999;33(2):117-21. DOI: 10.1016/S0921-4488(99)00013-9
33. da Silva ER, Siqueira AP, Martins JC, Ferreira WP, da Silva N. Identification and in vitro antimicrobial susceptibility of Staphylococcus species isolated from goat mastitis in the northeast of Brazil. Small Rumin Res. 2004;55(1-3):45-9. DOI: 10.1016/j.smallrumres.2004.01.001
34. Moroni P, Pisoni G, Vimercati C, Rinaldi M, Castiglioni B, Cremonesi P, Boettcher P. Characterization of *Staphylococcus aureus* isolated from chronically infected dairy goats. J Dairy Sci. 2005;88(10):3500-9. DOI: 10.3168/jds.S0022-0302(05)73035-6
35. Aydin I, Kav K, Celİk HA. Identification and antimicrobial susceptibility of subclinical mastitis pathogens isolated from hair goats' milk. J Adv Vet Anim. 2009;8(6):1086-90. [available at]
36. Merz A, Stephan R, Johler S. *Staphylococcus aureus* isolates from goat and sheep milk seem to be closely related and differ from isolates detected from bovine milk. Front Microbiol. 2016;7:184387. DOI: 10.3389/fmicb.2016.00319
37. Alvarez-Suarez ME, García-López ML, Otero A, Santos A. Microbiological examination of bulk tank goat's milk in the Castilla y

León region in Northern Spain. J Food Prot. 2015;78(12):2227-32. DOI: 10.4315/0362-028X.JFP-15-133
38. Spanu V, Scarano C, Virdis S, Melito S, Spanu C, De Santis EP. Population structure of *Staphylococcus aureus* isolated from bulk tank goat's milk. Foodborne Pathog Dis. 2013;10(4):310-5. DOI: 10.1089/fpd.2012.1356
39. Alian F, Rahimi E, Shakerian A, Momtaz H, Riahi M, Momeni M. Antimicrobial resistance of *Staphylococcus aureus* isolated from bovine, sheep and goat raw milk. Glob Vet. 2012;8(2):111-4. [available at]
40. Mørk T, Kvitle B, Mathisen T, Jørgensen HJ. Bacteriological and molecular investigations of *Staphylococcus aureus* in dairy goats. Vet Microbiol. 2010;141(1-2):134-41. DOI: 10.1016/j.vetmic.2009.08.019
41. Anderson KL, Kearns R, Lyman R, Correa MT. Staphylococci in dairy goats and human milkers, and the relationship with herd management practices. Small Rumin Res. 2019;171:13-22. DOI: 10.1016/j.smallrumres.2018.11.021
42. Xing X, Zhang Y, Wu Q, Wang X, Ge W, Wu C. Prevalence and characterization of *Staphylococcus aureus* isolated from goat milk powder processing plants. Food Control. 2016;59:644-50. DOI: 10.1016/j.foodcont.2015.06.042
43. Piccinini R, Tassi R, Dapra V, Pilla R, Fenner J, Carter B. Study of *Staphylococcus aureus* collected at slaughter from dairy cows with chronic mastitis. J Dairy Res. 2012;79(2):249-55. DOI: 10.1017/S002202991200009X
44. Mork T, Kvitle B, Jorgensen HJ. Reservoirs of *Staphylococcus aureus* in meat sheep and dairy cattle. Vet Microbiol. 2012;155(1):81-7. DOI: 10.1016/j.vetmic.2011.08.010
45. Regasa S, Mengistu S, Abraha A. Milk safety assessment, isolation, and antimicrobial susceptibility profile of *Staphylococcus aureus* in selected dairy farms of Mukaturi and Sululta town, Oromia region, Ethiopia. Vet Med Int. 2019;2019(1):3063185. DOI: 10.1155/2019/3063185
46. Xing X, Zhang Y, Wu Q, Wang X, Ge W, Wu C. Prevalence and characterization of *Staphylococcus aureus* isolated from goat milk powder processing plants. Food Control. 2016;59:644-50. DOI: 10.1016/j.foodcont.2015.06.042
47. Akineden Ö, Hassan AA, Schneider E, Usleber E. Enterotoxigenic properties of *Staphylococcus aureus* isolated from goats' milk cheese. Int J Food Microbiol. 2008;124(2):211-6. DOI: 10.1016/j.ijfoodmicro.2008.03.027
48. European Food Safety Authority. Scientific opinion of the Panel on Biological Hazards on a request from the European Commission on assessment of the public health significance of meticillin-resistant *Staphylococcus aureus* (MRSA) in animals and foods. EFSA J. 2009;993:1–73. [available at]
49. Porrero MC, Hasman H, Vela AI, Fernández-Garayzábal JF, Domínguez L, Aarestrup FM. Clonal diversity of *Staphylococcus aureus* originating from the small ruminants goats and sheep. Vet Microbiol. 2012;156(1-2):157-61. DOI: 10.1016/j.vetmic.2011.10.015
50. Rola JG, Sosnowski M, Ostrowska M, Osek J. Prevalence and antimicrobial resistance of coagulase-positive staphylococci isolated from raw goat milk. Small Rumin Res. 2015;123(1):124-8. DOI: 10.1016/j.smallrumres.2014.11.010
51. García-Álvarez L, Holden MT, Lindsay H, Webb CR, Brown DF, Curran MD, Walpole E, Brooks K, Pickard DJ, Teale C, Parkhill J. Meticillin-resistant *Staphylococcus aureus* with a novel mecA homologue in human and bovine populations in the UK and Denmark: A descriptive study. Lancet Infect Dis. 2011;11(8):595-603. DOI: 10.1016/S1473-3099(11)70126-8
52. Angelidis AS, Komodromos D, Giannakou R, Arsenos G, Gelasakis AI, Kyritsi M, Filioussis G, Hadjichristodoulou C, Torounidou P, Papa A, Sergelidis D. Isolation and characterization of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) from milk of dairy goats under low-input farm management in Greece. Vet Microbiol. 2020;247:108749. DOI: 10.1016/j.vetmic.2020.108749
53. Kou X, Cai H, Huang S, Ni Y, Luo B, Qian H, Ji H, Wang X. Prevalence and characteristics of *Staphylococcus aureus* isolated from retail raw milk in Northern Xinjiang, China. Front Microbiol. 2021;12:705947. DOI: 10.3389/fmicb.2021.705947
54. Asiimwe BB, Baldan R, Trovato A, Cirillo DM. Prevalence and molecular characteristics of *Staphylococcus aureus*, including methicillin-resistant strains, isolated from bulk can milk and raw milk products in pastoral communities of South-West Uganda. BMC Infect Dis. 2017;17:1-8. DOI: 10.1186/s12879-017-2524-4
55. Wolf C, Kusch H, Monecke S, Albrecht D, Holtfreter S, von Eiff C, Petzl W, Rainard P, Bröker BM, Engelmann S. Genomic and proteomic characterization of *Staphylococcus aureus* mastitis isolates of bovine origin. Proteomics. 2011;11(12):2491-502. DOI: 10.1002/pmic.201000698
56. Sheet OH, Grabowski NT, Klein G, Reich F, Abdulmawjood A. Characterisation of mecA gene negative *Staphylococcus aureus* isolated from bovine mastitis milk from Northern Germany. Folia Microbiol. 2019;64:845-55. DOI: 10.1007/s12223-019-00698-z
57. Ote I, Taminiau B, Duprez JN, Dizier I, Mainil JG. Genotypic characterization by polymerase chain reaction of *Staphylococcus aureus* isolates associated with bovine mastitis. Vet Microbiol. 2011;153(3-4):285-92. DOI: 10.1016/j.vetmic.2011.05.042
58. Sheet OH. Identification and characterization of *Staphylococcus aureus* isolated from bovine mastitis milk in Northern Germany [Ph.D. dissertation]. Hannover: University of Veterinary Medicine, Hannover/Germany; 2016. 74 p.
59. Ahangari Z, Ghorbanpoor M, Shapouri MR, Gharibi D, Ghazvini K. Methicillin resistance and selective genetic determinants of *Staphylococcus aureus* isolates with bovine mastitis milk origin. Iran J Microbiol. 2017;9(3):152. [available at]
60. Momtaz H, Rahimi E, Tajbakhsh E. Detection of some virulence factors in *Staphylococcus aureus* isolated from clinical and subclinical bovine mastitis in Iran. Afr J Biotechnol. 2010;9(25):3753-8. [available at]
61. Klein RC, Fabres-Klein MH, Brito MA, Fietto LG, Ribon AD. *Staphylococcus aureus* of bovine origin: Genetic diversity, prevalence and the expression of adhesin-encoding genes. Vet Microbiol. 2012;160(1-2):183-8. DOI: 10.1016/j.vetmic.2012.05.025
62. Gogoi-Tiwari J, Waryah CB, Eto KY, Tau M, Wells K, Costantino P, Tiwari HK, Isloor S, Hegde N, Mukkur T. Relative distribution of virulence-associated factors among Australian bovine *Staphylococcus aureus* isolates: potential relevance to development of an effective bovine mastitis vaccine. Virulence. 2015;6(5):419-23. DOI: 10.1080/21505594.2015.1043508
63. Ikawaty R, Brouwer EC, Duijkeren EV, Mevius D, Verhoef J, Fluit AC. Virulence factors of genotyped bovine mastitis *Staphylococcus aureus* isolates in The Netherlands. Int. J Dairy Sci. 2010;5(2):60-70. DOI: 10.3923/ijds.2010.60.70
64. Scherrer D, Corti S, Muehlherr JE, Zweifel C, Stephan R. Phenotypic and genotypic characteristics of *Staphylococcus aureus* isolates from raw bulk-tank milk samples of goats and sheep. Vet Microbiol. 2004;101(2):101-7. DOI: 10.1016/j.vetmic.2004.03.016
65. Stephan R, Annemüller C, Hassan AA, Lämmler C. Characterization of enterotoxigenic *Staphylococcus aureus* strains isolated from bovine mastitis in north-east Switzerland. Vet Microb. 2001;78(4):373-82. DOI: 10.1016/S0378-1135(00)00341-2
66. Hadi HM, ALmallah OD, AL-Hassan FH. Effect of genotypes for growth hormone gene in Awassi ewes on milk production and components. Mesopotamia J Agric. 2023;51(4):50-8. DOI: 10.33899/mja.2023.142496.1264
67. Rayan NM, Thamer AK. Effect of ohmic heating treatment on different properties of whole cow milk. Mesopotamia J Agric. 2022;50(2):68-76. DOI: 10.33899/magrj.2022.133744.1171
68. Alkass JE, Mustafa KN, Baker IA. Performance of Karadi sheep in Kurdistan region/Iraq: A review. Mesopotamia J Agric. 2022;50(4). DOI: 10.33899/mja.2023.142252.1259

# الكشف الجزيئي والتنوع التطوري لجراثيم المكورات العنقودية الذهبية المعزولة من حليب الماعز المصابة بالتهاب الضرع تحت السريري في محافظة نينوى

**ايمن هاني طه، عمر هاشم شيت و ضياء محمد طاهر جوهر**

فرع الصحة العامة البيطرية، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

**الخلاصة**

يُعتقد أن أحد أسباب التهاب الضرع في الماعز في اغلب الأحيان هي جراثيم المكورات العنقودية الذهبية. من ناحية أخرى، لا توجد هناك الكثير من المعلومات عن المكورات العنقودية الذهبية المقاومة للميثيسيلين الموجودة في حليب الماعز. اهم أهداف الدراسة هو الكشف عن معدل انتشار جرثومة المكورات العنقودية الذهبية والمكورات العنقودية الذهبية المقاومة للميثيسيلين في عينات الحليب من مزارع تربية الماعز في مدينة الموصل. تم جمع ستين عينة من حليب الماعز في الفترة من تشرين الأول ٢٠٢٣ إلى شباط ٢٠٢٤ من مناطق مختلفة من المحافظة. لغرض عزل وتحديد المكورات العنقودية الذهبية من حليب الماعز، تم استخدام في هذه الدراسة التقنيات التقليدية مثل الوسائط الانتقائية والاختبارات البيوكيميائية. بالإضافة إلى ذلك، تم استخدام اختبار تفاعل البلمرة المتسلسل للكشف عن الجينات *nuc* و*mecA* و*clfA* و*clfB* و *coa* في العزلات. أظهرت نتائج هذه الدراسة أن معدل انتشار المكورات العنقودية الذهبية في التهاب الضرع تحت السريري في الماعز كان ٥%، وكان معدل انتشار المكورات العنقودية الذهبية في حليب الماعز في منطقة على راش هو ٢٠%، ولم يتم عزل أي المكورات العنقودية الذهبية في مناطق اخرى من مدينة الموصل في هذه الدراسة. بالإضافة إلى ذلك، كشفت هذه الدراسة أن جميع عزلات جراثيم المكورات العنقودية الذهبية تمتلك جينات *nuc* و *mecA* و *clfB* و *coa* بنسبة ١٠٠% (٣/٣)، بينما كانت جين *clfB* ٦٦,٧%(٣/٢). هناك نوعان من الملف الجيني الموجود في عزلات المكورات العنقودية الذهبية. تم تسجيل تسع سلالات جديدة من تسلسلات المكورات العنقودية الذهبية في بنك الجينات. أظهرت الدراسة الى وجود تتشابه العزلات التسع المكتشفة حديثًا من المكورات العنقودية الذهبية مع سلالات أخرى من نفس الجرثومة التي تم العثور عليها عالميًا.