# Conventional and molecular identification of methicillin-resistance Staphylococcus aureus (MRSA) associated with human skin lesions and bovine mastitis

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# Conventional and molecular identification of methicillin-resistance *Staphylococcus aureus* (MRSA) associated with human skin lesions and bovine mastitis

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## ABSTRACT

**Background.** The objective of this study was to identify the prevalence of *Staphylococcus aureus* (*S. aureus*) takes from human skin lesions and bovine mastitis samples as well as determine the rate of occurrence of methicillin-resistance *S. aureus* (MRSA) and analysis of the genetic connections between isolated bacteria with local and global strains.

**Methods.** A total of 350 specimens were collected, including 150 specimens from human with different skin lesions and 200 specimens from cattle with mastitis.

**Results.** Cultivation methods and biochemical evaluation demonstrated the presence of 86 (57.3%) and 98 (49%) *S. aureus* isolates in human and animal samples, respectively. The resistance pattern showed that each individual *S. aureus* isolate shown resistance to penicillin. (100%) followed by

erythromycin (86% and 95.5%), Clindamycin (69.7% and 90.8%), Oxacillin (63.9% and 82.6%), Levofloxacin (50% and 55.1%), Tetracyclin (19.7% and 12.2%), Vancomycin (15.1% and 9.1%), Teicoplanin (8.1% and 20.4%) and Gentamicin (9.3% and 5.1%) for human and animals, respectively. The *mecA* gene was detected in all human Oxacillin resistant isolates (100%), while it was detected only in 73 (82.6) phenotypically Oxacillin resistant isolates. PCR partial gene sequencing that targeted housekeeping gene loci; *arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqi* was done. The sequences were analyzed using multilocus sequence typing (MLST). These isolates carried the above-mentioned genes as follows; 2 (6.7%), 2 (6.7%), 2 (6.7%), 2 (6.7%), 6 (6.7%), 3 (10%), and 2 (6.7%) human isolates, and 10 (13.3%), 14 (18.7%), 8 (10.7%), 6 (8%), 10 (13.3%), 3 (4%), and 2 (2.7%) for milk isolates, respectively, and the MLST via a phylogenetic evaluation revealed close-nucleotide similarities between local and NCBI-based human and cow sequences.

**Conclusion.** Ultimately, the findings of the current investigation suggest that *S. aureus* is commonly identified in human skin lesions and milk samples and a lot of these isolates the bacteria included significant resistance genes, identifying a possible origin of the spread of resistance to human strains. Furthermore, the obtained information about the genetic similarities between these isolates highlights the animals as an important source for transporting the virulent pathogens to human which leads to health risks to public health.

**Keywords:** MRSA, mastitis, skin lesion, antibiotic resistance, housekeeping gene

## INTRODUCTION

*S. aureus* is a persistent gram-positive bacterium that is well recognized for its association with several types of clinical and sub-clinical infections [1]. The bacterium typically colonizes the skin and mucous membranes, and has the ability to infiltrate many organs so it recognized as a

prominent etiological agent responsible for various medical conditions, including endocarditis, bacteremia, osteomyelitis, as well as infections of soft tissue [2]. *S. aureus* regard the most common bacteria involved in skin infections worldwide. The pathogenesis of this pathogens attributed to variety of virulence factors; toxic shock syndrome toxin, exfoliative toxin, Panton-Valentine leucocidin are the main toxins which play an essential part in the clinical manifestation of staphylococcus's skin infections [3] . In cattle, *S. aureus* have a significant role as a causative agent for wide diversity of infections. It has an ability to adhere and colonize the mammary canal and recognized for its association with several types of clinical and sub-clinical mastitis [4]. This pathogen triggers a comparatively weak immune response in cows when compared to *E. coli*. Consequently, the pathogen infections consistently result in the development of chronic mastitis, which persists for a duration of several months [1]. The strains of this pathogenic organism that exhibit resistance to methicillin are often referred to as MRSA, which possess a *mecA* gene that is responsible for transmitting the resistance [5]. The colonization of MRSA has been found to elevate the likelihood of infection approximately 50-80% of cases. Despite continuous advancements in antibiotics, MRSA continues to be a potential highly fatal pathogen [6]. The introduction of antibiotics has significantly decreased the mortality rate associated with *S. aureus* bacteremia, reducing it from 80% to a range of 15-50%, which is still considered to be unacceptably high [16–18]. Due to the importance of MRSA as life threating pathogen, the current investigation has been conducted. intended to record the occurrence of S. aureus, specifically MRSA, in skin infection of human and cattle mastitis, as well as to find genetic relation between human and bovine global strains.

## MATERIALS AND METHODS

### Sample collection

The investigation was carried out at a specific time period. December 2022 to May 2023. 200 cow Samples of milk were taken from five cow farms located in different area at Al-Qadisiyah Province, Iraq. The visual inspection was done for detection of any manifestations of mastitis in clinical signs such as pain, swelling and heat. As well as, the color and consistency of milk were checked. Aseptically, 10 ml milk samples were taken from cows with clinical and subclinical mastitis. (California mastitis test positive) in a sterile bottle then transported by aid of ice box to the University of Al-Qadisiyah, College of Veterinary Medicine, Microbiology Laboratory for examination according to Quinn *et al*. [7]. In addition, 150 different skin lesions samples were collected from human admitted to Al-Diwaniyah General Teaching Hospital, Al-Diwaniyah City, Iraq. The specimens were obtained from the lesions using aseptic swabs. in a sterile vial bearing the patient information like name, sex and age then transported to the laboratory. All samples were cultured immediately, if an immediate inspection was not feasible, the collected samples were stored at a temperature of 4 ºC until they were utilized.

**Ethical consideration**

The research procedures and sampling were approved by the Ethical Committee at the College of Veterinary Medicine, University of Al-Qadisiyah, Al-Diwaniyah City, Iraq.

**Bacteriological analysis**

All samples were subjected to traditional cultivation methods using peptone water (used as an enrichment media by adding 1ml milk to 5ml peptone water incubated at 37°C for 24hrs then inoculated on sheep blood agar (Oxoid, UK) and incubated aerobically for 24 h. The growing colonies were examined and primary diagnosed based on their hemolytic pattern, Gram's stain reaction, and morphological characterizations. The diagnosis was confirmed by subculturing on

mannitol salt agar (Oxoid, UK) and application of biochemical analysis (catalase, oxidase, coagulase and DNase tests) were done according to Quinn *et al.* (7). Later, analysis by VITEK2 Compact System was done on the isolated strains.

**Antibiotic Susceptibility Test**

The susceptibility of the isolated strains to antibiotics was performed by VITEK2 Compact System. The following antibiotics penicillin (PEN) (0.06, µg⁄ml) Erythromycin (ERY) (≥8 µg⁄ml), Clindamycin (CLI) (≥8 µg⁄ml), Oxacillin (OXA)( (≥4 µg⁄ml), Levofloxacin (LVX) (4 µg⁄ml), Tetracycline (TER) (≤ 1 µg⁄ml), Vancomycin (VAN)(≥32 µg⁄ml), Teicoplanin (TEC) (≤ 0.5 µg⁄ml), Gentamicin (GEN) (≤ 0.5 µg⁄ml), Trimethoprim sulfamethoxazole (TSX) (≤ 10 µg⁄ml),, Rifampin (RIF) (≤0.5 µg⁄ml), and Nitrofurantoin (NIT) (≤ 16 µg⁄ml), were used. The antibiotic resistance estimated according to the Clinical and Laboratory Standards Institute (8) .

**DNA extraction**

DNA extraction kit (Geneaid, USA) was used to perform the process of DNA extracting from fresh growth of *S. aureus*. The procedure was generated following the manufacturer's guidelines for gram-positive bacteria. The DNA was quantified using a NanoDrop spectrophotometer and subsequently stored at a temperature of -20°C until it was needed for further experiments.

**Detection of *mec* A gene by PCR**

The molecular confirmation of MRSA strains was conducted by PCR through amplification of *mec A* gene (gene responsible for methicillin resistance) in thermal cycler according to Stegger *et al* [9] by using specific primer (Table 1). The PCR results were examined

under ultraviolet (UV) light in the presence of agarose gel, which contains ethidium bromide at a concentration of 0.5 g/mL.

**TABLE 1.** Primer Used in This Study

| Primer | | Sequence (5'-3') | Product Size (bp) |
|---|---|---|---|
| *S. aurus mecA* | F | TCTTGGGGTGGTTACAACGT | 541 |
| | R | ACCACCCAATTTGTCTGCCA | |

**Identification of *S. aureus* STs/CCs**

The methodology utilized in this study involved the implementation of Multi-Locus Sequence Typing (MLST). This strategy encompassed the analysis of internal fragments derived from the user has provided a list of seven housekeeping genes: arcC (carbamate kinase), aroE (manganate dehydrogenase), glpF (glycerol kinase), gmk (guanylate kinase), pta (phosphate acetyltransferase), tpi (triphosphate isomerase), and yqiL (acetyl coenzyme A acetyltransferase). The process of PCR amplification was carried out for the seven housekeeping genes using the primers specified in Table (2) Sequences of the seven housekeeping gene fragments (strain from human skin lesion and from cow milk) the MLST scheme selected the seven gene segments that yielded the highest number of alleles.

The DNA was sequenced, and the data were analyzed using NCBI websites and MEGA 10 software for the phylogenetic analysis and building a phylogenetic tree. The partial gene sequencing targeted gene loci; *arcC, aroE, glpF, gmk, pta, tpi, and yqi*. The data representing the differentiation tree., along with auxiliary data consisting of 7 housekeeping the system was used to analyze the genes and clonal complexes that were inputted.

**TABLE 2.** Primers Used in This Study(10).

| Primer | | Sequence (5'-3') | Tm (C°) | Product Size (bp) |
|---|---|---|---|---|
| *arcC (Carbamate kinase)* | F | TTGATTCACAGCGCGTATTGTC | 55 | 570 |
| | R | AGGTATCTGCTTCAATCAGCG | | |
| *aroE* (Shikimate dehydrogenase) | F | ATCGGAAATCCTATTTCACATTC | 51.8 | 536 |
| | R | GGTGTTGTATTAATAACGATATC | | |
| *glpF(*Glycerol kinase) | F | CTAGGAACTGCAATCTTAATCC | 53.59 | 576 |
| | R | TGGTAAAATCGCATGTCCAATTC | | |
| *gmk* (Guanylate kinase) | F | ATCGTTTTATCGGGACCATC | 50.02 | 488 |
| | R | TCATTAACTACAACGTAATCGTA | | |
| *pta* (Phosphate acetyltransferase) | F | GTTAAAATCGTATTACCTGAAGG | 51.8 | 575 |
| | R | GACCCTTTTGTTGAAAAGCTTAA | | |
| *Tpi* (Triosephosphate isomerase) | F | TCGTTCATTCTGAACGTCGTGAA | 55.37 | 475 |
| | R | TTTGCACCTTCTAACAATTGTAC | | |
| yqi (Acetyle coenzyme A acetyltransferase) | F | CAGCATACAGGACACCTATTGGC | 57.15 | 598 |
| | R | CGTTGAGGAATCGATACTGGAAC | | |

**RESULTS**

The cultural and biochemical methods for detection of *S. aureus* in the samples revealed the presence of 49% (98 out of 200) in bovine samples and 57.3% (86 out of 150) in human samples. The *S. aureus* isolate exhibited characteristics of being gram positive cocci, positive for catalase and coagulase, and presented as yellow colonies on mannitol salt agar. (Figure 1)

**Antibiotic Resistance in human and cow milk isolates**

The antibiotics sensitivity test was performed on all samples of *S.aureus* acquired from human and bovine milk in order to assess its susceptibility to antimicrobial agents. The results indicated that the highest antibiotic resistance was to Penicillin (100%), Erythromycin (86%), Clindamycin (69.7%), Oxacillin (63.9%), Levofloxacin (50%), Tetracyclin (19.7%), Vancomycin (15.1%), Teicoplanin (8.1% ) and Gentamicin (9.3%) in human isolates. While resistance pattern in bovine isolates were (100, 95.9, 90.8, 82.6, 55.1, 9.1, 20.4 and 5.1) % for the same antibiotics, respectively Table (3).

**TABLE 3.** Antimicrobial sensetivity test of *S. aureus* isolated from human skin lesions and cow milk samples.

| Antibiotics | PEN | ERY | CLI | OXA | LVX | TER | VAN | TEC | GEN | MUN | FOS | TSX | RIF | NIT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Human Samples N0.( %) | 86 (100) | 74 (86) | 60 (69.7) | 55 (63.9) | 43 (50) | 17 (19.7) | 13 (15.1) | 7 (8.1) | 8 (9.3) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Cow milk Samples | 98 (100) | 94 (95.9) | 89 (90.8) | 81 (82.6) | 54 (55.1) | 12 (12.2) | 9 (9.1) | 20 (20.4) | 5 (5.1) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N0 (%) | | | | | | | | | | | | | | |

The amplification of *mecA* gene in suspected MRSA isolates (phenotypically resistant to oxacillin) demonstrated that all human isolates (100% ), and 73 out of 81 (82.6 %) bovine milk isolates were harboring *mecA* gene, which considered as genotypically confirmed MRSA (Figure 2).

**Accession numbers for nucleotide sequences**

The DNA sequences of each allele at the seven loci used in the current study have been documented in GenBank, together with their corresponding accession codes, Table (4).

**TABLE 4.** Nucleotide sequence accession numbers of *S.aureus* isolated from human and bovine milk

| **Genes** | **Cattle** | **Human** |
|---|---|---|
| arcC | OR723529 | OR723522 |
| ***aroE*** | OR723530 | OR723523 |
| ***glpF*** | OR723531 | OR723524 |
| ***gmK*** | OR723532 | OR723525 |
| ***pta*** | OR723533 | OR723526 |
| ***tpi*** | OR723534 | OR723527 |
| ***yqi*** | OR723535 | OR723528 |

**MLST Typing results**

The isolates have allele mutation *arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqi* as follows; 13 (13.2%), 18 (18.3%), 10 (10.2%), 8 (8.1%), 13 (13.2%), 4 (4.1%), and 3 (3.1%) milk isolates, respectively, and 6 (6.9%), 6 (6.9%), 6 (6.9%), 6 (6.9%), 18 (20.9%), 9 (10.4%), and 6 (6.9%) for human isolates, the major clone identified in this study (ST30) in human and (ST45) in animal isolate (Table 5).

**TABLE 5.** Pub-MLST typing analysis in local *S. aureus* isolates

| **Local *S.aureus*** | ***Staphylococcus aureus* MLST genes**<br>**Pub-MLST Typing allele mutation analysis** | | | | | | | **ST typing** | **Clonal complex** |
|---|---|---|---|---|---|---|---|---|---|
| | ***arcC*** | ***aroE*** | ***glpF*** | ***gmk*** | ***pta*** | ***tpi*** | ***yqiL*** | | |
| **Human isolate** | 6 | 6 | 6 | 6 | 18 | 9 | 6 | 30 | CC30 |
| **Animal isolate** | 13 | 18 | 10 | 8 | 13 | 4 | 3 | 45 | CC45 |

The seven housekeeping genes (*Arc, Aro, Glp, Gmk, Pta, Tpi*, and *Yqi*) showed positive PCR at (570bp, 536bp, 576bp, 488bp, 575bp, 475bp, and 598bp), respectively (Figures 3, 4).

The MLST via a phylogenetic evaluation revealed close-nucleotide similarities between local bovine with local and NCBI-based human sequences (Figure 5).

## DISCUSSION

*Staphylococcus aureus* is a prominent and widespread bacterial illness that causes numerous skin infections and potentially hundreds of thousands to millions of more severe, invasive infections worldwide each year [11]. The infection with *S. aureus* may last for several months, this result from the weak immune response elicited by this pathogen which leads to

chronic cases [12]. The degradative enzymes of *S. aureus* cause various degree of damaging effect on the infected tissues [13]. The current study revealed the presence of the *S.aureus* in high incidences in both human and bovine milk samples. *S. aureus* infection can be transmitted via direct exposure to infected patients, individuals carrying the bacteria, or a contaminated environment. Particularly, those who contain Staphylococcus spp. experience higher levels of resistance when they have had direct exposure to animals that have received prior antibiotic treatment, as well as among those employed in hospitals or veterinary clinics [14].

The study showed an elevated occurrence of *S. aureus* (49%), which aligns with the findings of Belay *et al* [15] additionally, they observed a high prevalence of *S. aureus* in cases of bovine mastitis. while it higher than the result obtained in previous study in Iraq [15] and lower than 82.81% prevalence rate obtained by Al-Debbag and Hamid [16].

The differences in the occurrence of *S. aureus* observed in this study, in comparison to previous studies, can be attributed to various factors including sample size, isolation procedures, husbandry practices, farm owners' understanding and skills, animal health delivery systems, and geographic regions of the sampled area. The dominance of this bacterial species may be due to continual colonization of teats, as they are commensals to the skin. As a result, the bacterium can readily penetrate the teat canal during milking or suckling, and it can propagate from one quarter to another and from cow to cow throughout the milking process.

On the other hand, *S.aureus* was isolated from 57.3 % of human samples. Mohanty *et.al.*(16) reported a much lower 20.9% skin colonization in India. Previous study reported colonization of *S. aureus* in 75% of dairy farmer and 40% of Dangke makers [17]. Because farmworkers and dairy cattle are in close proximity to one another, cross-transmission between humans and animals has been documented frequently [17].

*S. aureus* isolates in the present study were totally resistant to penicillin (100%), this data is compatible with Al-Dahbi and Al-Mathkhury [18]. Similarly, Methicillin-resistant *S. aureus* (MRSA) is widespread among cattle and is a major cause of mastitis, the most prevalent illness in cattle. This infection results in significant economic losses. Therefore, it is imperative to utilize the appropriate antibiotic for the treatment and management of bovine mastitis caused by *S. aureus* [19]. Hence, the obtained the isolates underwent both phenotypic and genotypic identification of MRSA using the Oxacillin disk diffusion technique and PCR to detect the *mec*A gene. The result indicated that Oxacillin disk diffusion method had lower sensitivity and accuracy than molecular method. In addition, Phenotypic false-positive scan occurs due to many factors, such as the generation of hyper beta-lactamase, and the outcomes highlight how crucial it is to validate phenotypic testing for methicillin resistance using more sensitive phenotypic techniques or molecular techniques. The high incidence of MRSA is in agreement with the previous studies in Iraq [15,20]. This information can aid in assessing the potential for spread of MRSA among animals and humans, as well as between farms and other susceptible populations, particularly consumers of dairy products [21]. A new meta-analysis examining the relationship between MRSA and bovine mastitis revealed a notable rise in prevalence over time. The study further proposed that this increase may be attributed to improvements in the employed detection techniques. Furthermore, It is important to mention that there is a higher presence of Methicillin-resistant Staphylococcus aureus (MRSA) in Asia, in comparison to other geographical areas, may be attributed to the widespread utilization of antimicrobial agents in animals [22] and could potentially be linked to the larger populations of animals in these areas, as well as the availability and utilization of distinct antimicrobial agents that exert varying selective pressures on MRSA [23,24]. Notoriously, nine isolates from human and animals showed resistance to Vancomycin

(VRSA) which represent important risk factor for dispersion and infection with VRSA in human and animals. Hence, the judicious selection of vancomycin for clinical application is imperative to decrease the emergence of VRSA. The high rate of antibiotic resistance may attributed to incorrect prescription, misuse and overuse of antibiotics, as well as, usage of commercialized antibiotics(25) In order to ascertain the source and relatedness of the *S. aureus* isolates found in human and bovine milk, we employed the MLST molecular typing technique to investigate the dissemination of clonal *S. aureus* strains across different healthcare facilities. The ST45 strains exhibited a higher rate of population growth compared to strains belonging to alternative sequence types (STs) [24]. The ST45 strains were found to possess the selm/selo genes, which encode the superantigens SElM and SElO, along with the adhesin gene *cna*. Previous studies have established a correlation between these genes and cow milk [26]. Hence, it can be inferred that unidentified factors in ST45 play a crucial role in facilitating the expansion of this particular strain in the udder of cows, outcompeting other resident bacteria. Additionally, The ST30 strains exhibited the highest prevalence of virulence genes, particularly the superantigen genes sea and *tst*, as well as the adhesin genes *bsp* and *cna*. These findings align with previous research on the subject [5].

The present study showed that each one of the seven human isolates of *S. aureus* was perfectly aligned with an animal isolate. This indicates that they could belong to the same strain due to high fragment similarity and the isolates belonged to the same city of sample collection. Enright *et al* [10] detected assembling of different strains of their isolates in the same cluster, which they suggested a same strain identity. Zhou *et al.* found the MLST on 81 isolates of *S. aureus* revealed the presence of 18 distinct strains with 42% of the isolates were typical strains [26]. Our study results agree with Ullah *et a*l [27] who performed MLST profiling and found that their strains carried specific locus determinants for arrangement according to the clades of isolates.

## CONCLUSION

The present study findings provide important information about *S. aureus* as a significant pathogen that poses a major risk to the human and animals. The frequency of antibiotic resistance in *S.aureus* isolates, namely MRSA, is worrisome and highlights the necessity to reassess the utilization of antibiotics in veterinary medicine. Furthermore, the presence of genetic similarities between these isolates is a critical signal about zoonosis.

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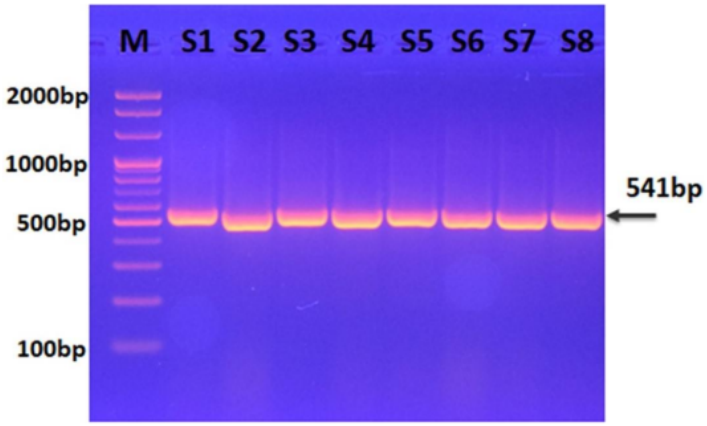

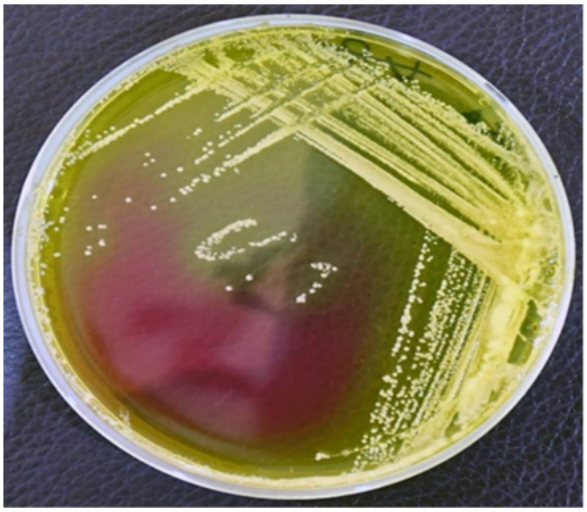

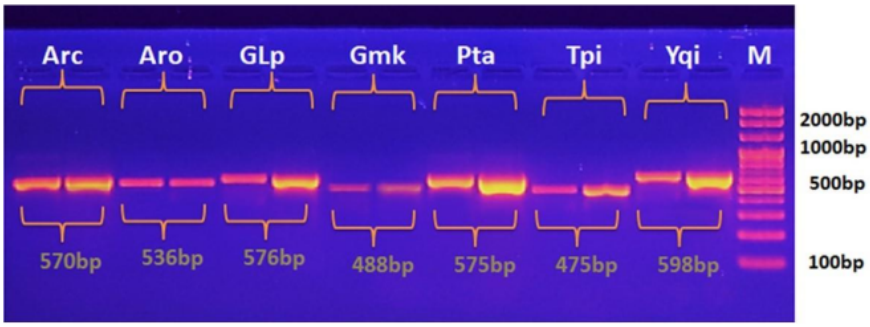
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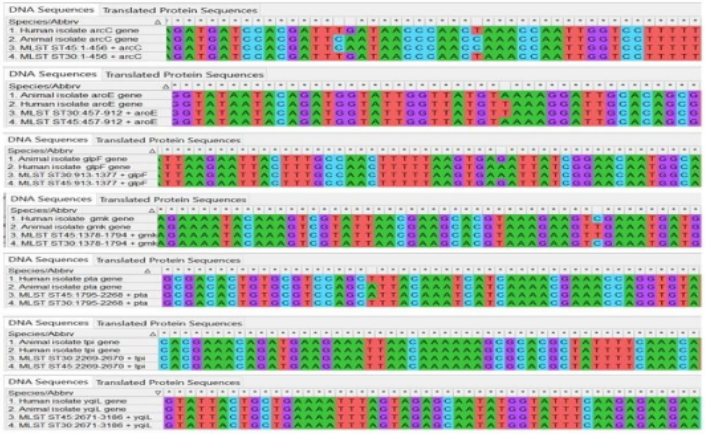

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**Figure legends**

**FIGURE 1.** Yellow colonies of *S. aureus* on Mannitol salt agar.

**FIGURE 2.** PCR-agarose gel electrophoresis of mecA gene in Staphylococcus aurous (human and animal isolates). M: DNA ladder (2000-100bp), lanes (S1-S4): Positive PCR (human samples), and lanes (S5-S8): Positive PCR (animal samples). PCR product: 541bp.

**FIGURE 3.** PCR-agarose gel electrophoresis of MLST housekeeping genes in *S. aureus* (human and animal isolates). M: DNA ladder (2000-100bp). The seven housekeeping genes (*Arc, Aro, Glp, Gmk, Pta, Tpi*, and *Yqi*) showed positive PCR at (570bp, 536bp, 576bp, 488bp, 575bp, 475bp, and 598bp, respectively).

**FIGURE 4.** Multiple sequence alignment analysis of MLST housekeeping genes in local *S. aureus* (Human and animal isolates) constructed using Crustal alignment tool (Online version). The analysis reveals similarity between local and global isolates

**FIGURE 5.** Phylogenetic tree according to the MLST housekeeping genes for methicillin resistance *S. aureus* from and human skin lesion and bovine mastitis samples.