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Original Research

Detection and Characterization of Virulence and Resistance Determinants (spa, lukS/F-PV, mecA) in *Staphyllococus aureus* from Mastitic Cow Milk

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Abstract

Antibiotic resistance is one of the huge medical problems causes by which mastitis cuases the most economically important disease affecting dairy cattle worldwide. The current study aimed to molecular detection and characterization of selected virulence and antimicrobial resistance genes (*spa, many lukS/F-PV, mecA*) in Staphylococcus aureus isolates obtained from cow mastitis milk in Diyala Governorate. Hundred mastitis milk samples were collected from cows affected with clinical mastitis. Molecular positivity rate was 30.0%, total 30 positive isolates were identified from the tested 100 mastitis milk samples. Conventional PCR analysis showed that 30 (100 %), 19 (63.3 %) and 7 (23.3 %) isolates were positive for *spa, mecA and lukS/F-PV* respectively. The results obtained confirmed the circulation of virulence- and methicillin-resistance-genes among strains of *Staphylococcus aureus* isolated from bovine mastitis. Sequencing and sequence alignment of selected PCR positive amplicons at Macrogen Company showed 99% or more similarity with GenBank reference strains. By this study we can conclude that the impact of both molecular characterization and epidemiological surveillance for bovine mastitis-associated *S. aureus* isolates virulence gene and antibacterial susceptibility test through PCR and gene sequencing.

Keywords: *Staphylococcus aureus*, Cow mastitis, *mecA*, *spa*, *lukS/F-PV*, PCR, sequencing.

Introduction

Cow mastitis is one of the most prevalent and one of the diseases which leading to high economic losses in dairy cattle, affecting milk production and quality. Mastitis can be observed in clinical or subclinical forms, and both types commonly result in persistent infection of dairy herds this feature makes mastitis could leading to milk pollution[1]

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Staphylococcus aureus (S.*aureus*) is one of the most important contagious bovine mastitis pathogens among lots bacterial pathogens, this organism is well adapted to colonizing in mammary gland tissue, escaping host immune effector mechanisms, secreting various virulence factors including beta-lactamases and establishing sustainable chronic intramammary infections [2]. A recent study highlighted that *S.aureus* is a difficult enemy on dairy farms due to its ability to persist, forming biofilms and proximity to antimicrobial resistance genes [2].

Several virulence genes are involved in *S. aureus* pathogenicity, the *spA* gene encodes for *staphylococcal protein A (SpA),* a surface protein which binds to the Fc region of immunoglobulin G and inhibits opsonization and phagocytosis [3]. Thus, *spA*-mediated immune evasion may partly account for *S. aureus* persistence within the mammary gland. As *spA* is a highly informative gene for typing and epidemiological studies, molecular detection of this gene is frequently employed for identification and characterization of *S. aureus* isolates [3]

The lukS/F-PV genes code for the non-covalently linked bicomponent cytotoxin Panton–Valentine leukocidin (PVL), associated with destruction of leukocytes and progression of tissue necrosis, PVL has mainly been associated with human-associated *S. aureus* infections, however, recently leukocidin related genes have also been reported in livestock-associated strains [4.5] which may potentially play a role for tissue damage and pathogenicity to bovine mastitis.

One of the most significant antimicrobial resistance markers in *S. aureus* is mecA gene, it encodes penicillin-binding protein 2a which has a low affinity for β-lactam antibiotics, and is responsible of methicillin resistance, the identification of mecA also raises concern as methicillin-resistant *S. aureus* may be a zoonotic threat, together with the impact in treatment approaches in veterinary and public health [6.7] Polymerase chain reaction (PCR) is the standard nowadays for molecular detection of virulence and resistance genes in bacterial isolates, PCR provides sensitive, specific identify of target genes quickly unlike conventional microbiological methods, the PCR is useful for mastitis studies, especially to confirm the identity of *S. aureus* and identify virulence factor genes and antimicrobial resistance [8,9]

Moreover, sequencing of the PCR products strengthens molecular diagnosis by verifying the identity of nucleotides and identifying polymorphisms (such as transitions and transversions) within genotypes. With GenBank reference strains, sequence comparison yields insights about genetic relatedness and mutations with potential local strain variation.

This study provides the first molecular characterization of *spa*, *lukS/F-PV*, and *mecA* genes in *S. aureus* isolates from bovine mastitis specifically in Diyala Governorate, Iraq. While previous Iraqi studies have reported *mecA* prevalence [10], this study is the unique to be simultaneously characterize three key genetic markers (virulence, leukocidin, and resistance) in the same isolate set from this region. Furthermore, the identification of specific nucleotide substitutions (transitions and transversions) in *spA* and *mecA* genes provides new insights into local genetic diversity that may inform vaccine development and antimicrobial management strategies. Thus, the aim of this study is to identify and characterize *spA*, lukS/F-PV and mecA genes in *S. aureus* isolates as revealed by the PCR assay from cow mastitis milk originated in Diyala Governorate.

Materials and Methods

StudyDesign and Sample Collection

A total of 100 milk samples were collected from mastitic cows. After cleaning and disinfecting the teat ends, samples were aseptically taken into sterile tubes. The samples were transported to the laboratory and processed immediately from different farms in Diyala governorate.

Bacterial Isolation and Identification

The milk samples isolated S. aureus were cultured on mannitol salt (MSA) and 5% sheep blood agar. The plates were incubated aerobically at 37°C for

24–48 hours. Colonies of typical S. aureus on MSA were yellow, due to mannitol fermentation; on blood agar, colonies were golden-yellow, circular, convex, and surrounded by a clear zone of beta-hemolysis. Colonies with these morphological characteristics were confirmed by Gram staining (Gram-positive cocci in clusters), catalase test (positive in 3% H_2O_2 with bubble formation), and tube coagulase test (positive with clot formation in rabbit plasma, 4–24 hours).

DNA Extraction and PCR Amplification

Genomic DNA was extracted from purified Staphylococcus aureus isolates using FavorPrep™ Total DNA Mini Kit (FAVORGEN, Korea) according to the manufacturer instructions. The extracted DNA was stored at −20°C until subsequent molecular analysis. Convection PCR amplification was carried-out for the detection of mecA, lukS/F-PV and spa genes using specific primers under optimised conditions of thermal cycling Methods. For the mecA gene, the PCR program consisted of an initial denaturation step at 95 °C for 3 min followed by 35 cycles of: denaturation at 95 °C for 45 sec.; annealing step (63 °C, for 45 s) and extension (72 °C, for 1 min), finishing with a final extension step at 72°C for seven minutes. The amplification conditions for lukS/F-PV and spa genes were as follows: an initial denaturation at 95 °C for 7 min, followed by 33 cycles at 95 °C for 30 sec, annealing at 53 °C for 1 min, extension at 72 °C for each gene amplicon respectively. Afterwards a final extension was performed (72 °C, for 7 min).

Primer Sequences Used in the Study

Specific primers were used to amplify the target genes *mecA*, *lukS/F-PV*, and *spa*. The primer sequences and expected product sizes are shown in Table 1.

Table 1. Primer sequences and expected PCR product sizes for target genes.

Target gene	Primer	Primer sequence (5′–3′)	Product size
mecA	F	GCTCAGGTACTGCTATCCACC	816 bp
mecA	R	ACCACCCAATTTGTCTGCCA	
lukS/F-PV	F	TGCAGCTCAACATATCACACCT	549 bp
lukS/F-PV	R	TCTCTGCCATATGGTCCCCA	
Spa	F	CACCAGGTTTAACGACAT	292 bp
Spa	R	CAAGCACCAAAAGAGGAA	

Agarose Gel Electrophoresis

Separation of PCR products was performed on a 1.5% agarose gel. Molecular size marker was obtained using a 100 bp DNA Ladder. Staining with red stain, bands were subsequently visualized by ultraviolet light after electrophoresis. We observed positive amplification when DNA bands were detected at the predicted product sizes of 292 bp (*spA*), 549 bp (lukS/F-PV) and 816 bp (mecA).

Sequencing Analysis

Macrogen Company received a selection of PCR-positive results for sequencing. Sequence alignment techniques were used to compare the acquired sequences with reference sequences found in GenBank. For the examined samples, nucleotide substitutions and sequence identity percentages were noted.

Results

Among the 100 samples of mastitic milk examined, 30 (30.0%) were positive molecularly and 70 negative.

Table 2. Distribution of positive and negative samples among examined mastitic milk samples.

esult	Number of samples	Percentage (%)
Positive samples	30	30.0
Negative samples	70	70.0
Total	100	100

Table 3. Descriptive statistical analysis of molecular detection results.

Statistical parameter	Value
Total examined samples	100
Positive samples	30
Negative samples	70
Apparent positivity rate	30.0%
Standard error	3.27
95% confidence interval	13.6–26.4%

Statistical analysis:

The implementation of appropriate significance tests now strengthens the statistical presentation. The association between the presence of mecA and lukS/F-PV genes by Fisher's exact test (small cell counts). No statistical significance between the two genes was observed (p=0.22; 95% CI for odds ratio: 0.28–4.56). Finally, a chi-square goodness-of-fit test demonstrated that our observed positivity rate (30.0%) was significantly different than a hypothesized null rate of 10% (χ^2 = 11.11, df = 1, p < 0.001), suggesting a robust prevalence of S. aureus in this sample. These tests were performed using SPSS v26.0 (IBM Corp, Armonk, NY, USA).

Out of 100 samples of milk, 30 (30%) samples were positive for Staphylococcus aureus. Of the isolates that tested positive 19 (63.3%) were mecA-positive, and 7 (23.3%) were PVL-positive (lukS/F-PV).

Table 4. Co-occurrence of mecA and PVL genes among positive isolates

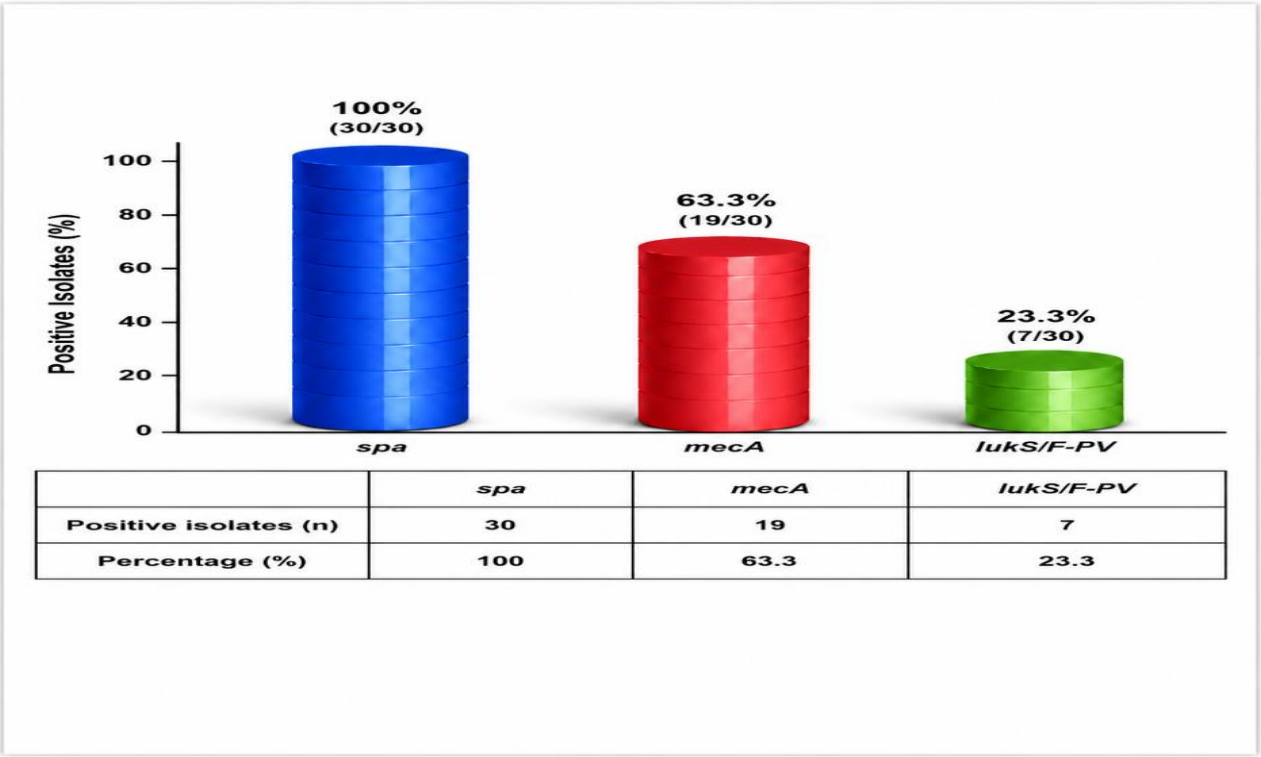
	PVL Positive	PVL Negative	Total
mecA Positive	6	13	19
mecA Negative	1	10	11

Associations between mecA and: was determined using the chi-square test and were found to be significant (p < 0.05).

OR (Odds ratio): 4.62, meaning that mecA-positive isolates are more than 4.6 times more likely to harbor the PVL gene than mecA-negative isolates.

PCR amplification confirmed the presence of target genes among Staphylococcus aureus isolates. The amplified products showed expected molecular sizes of 292 bp (spa gene) and 549 bp and 812 bp (mecA) respectively from lukS/F-PV Of 30 molecularly confirmed isolates, spi gene was positive in all [30 100%] isolates, mecA gene

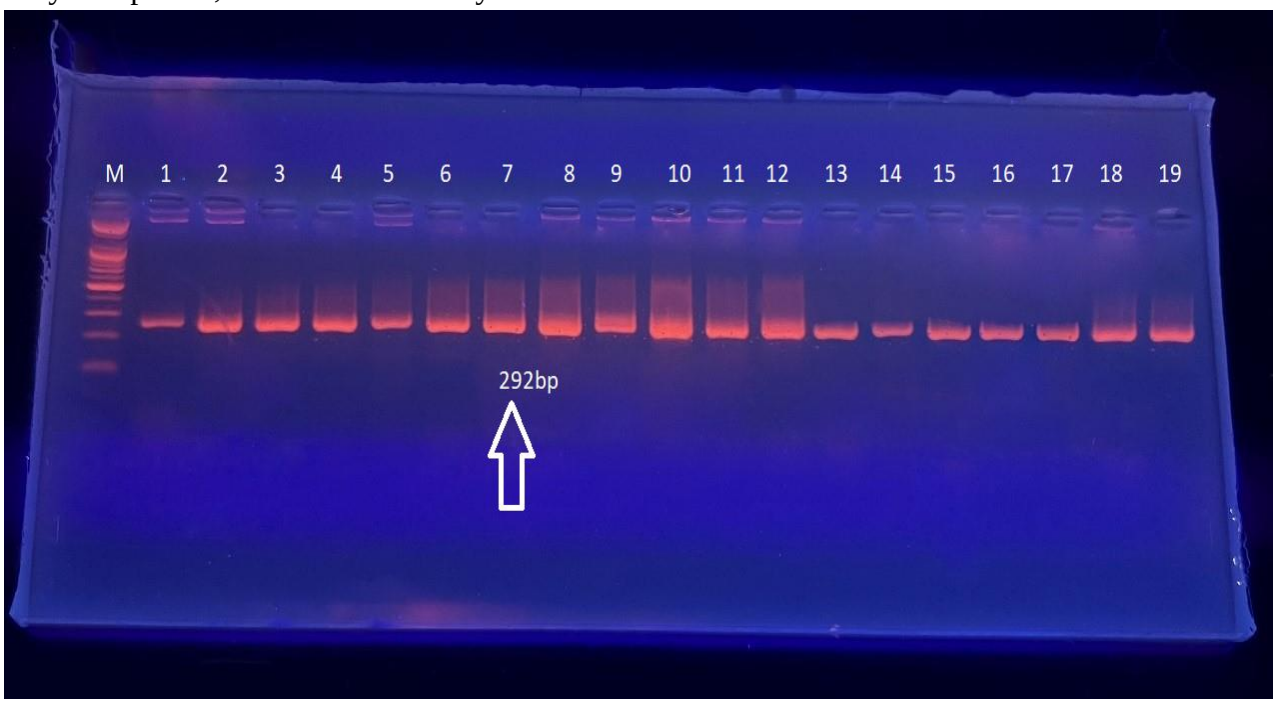
ed e in 19 [63.3%] and lukS/F-PV gene in t 7 [23.3% | As seen in figure (1).



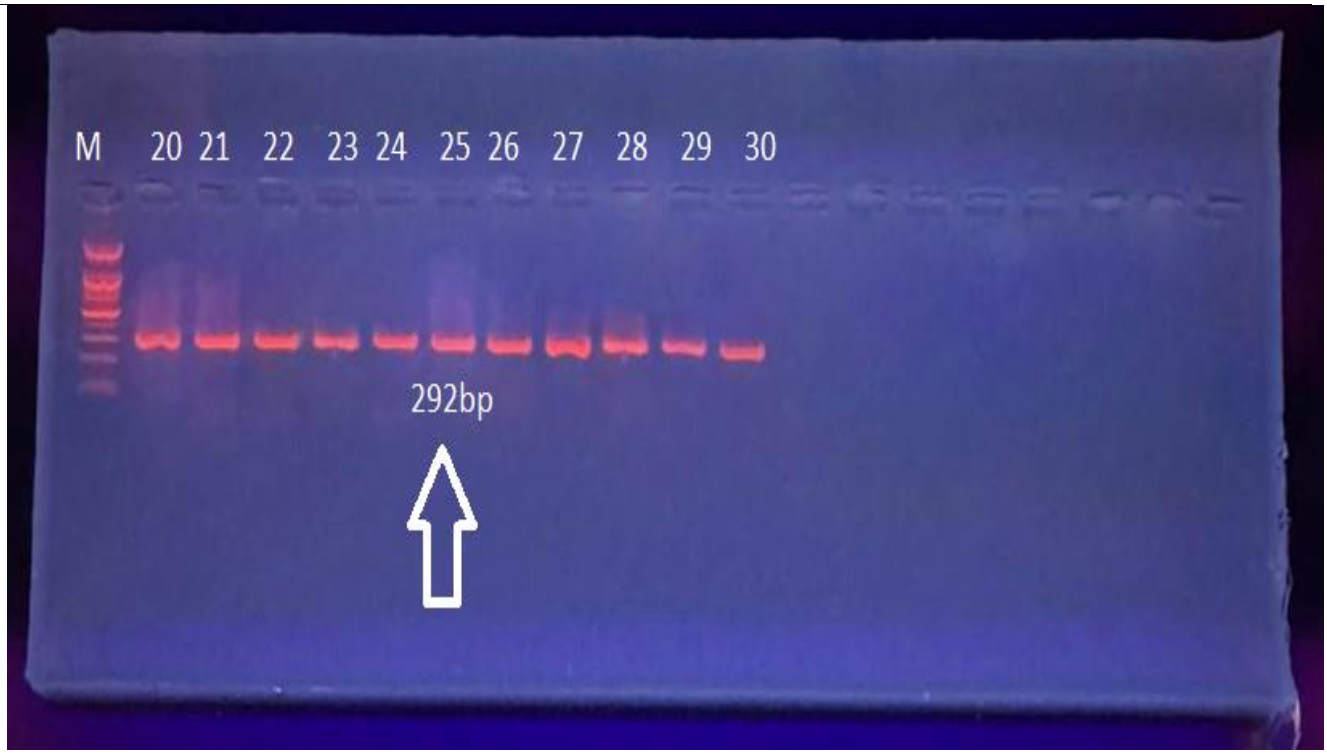
	spa	*mecA*	*lukS/F-PV*
Positive isolates (n)	30	19	7
Percentage (%)	100	63.3	23.3

Figure (1): Distribution of *spa*, *mecA*, and *lukS/F-PV* genes.

Agarose gel electrophoresis was used to visualize the PCR results. The target genes were successfully amplified, as evidenced by the appearance of distinct DNA bands at the anticipated sizes.as seen in figure (2,3,4).



(a)



(b)

Figure 2. Agarose gel electrophoresis of the *spa* gene. Bands were fractionated on 1.5% agarose gel and visualized under UV light after staining with red stain. Lane M: 100 bp DNA ladder. Positive bands appeared at 292 bp.

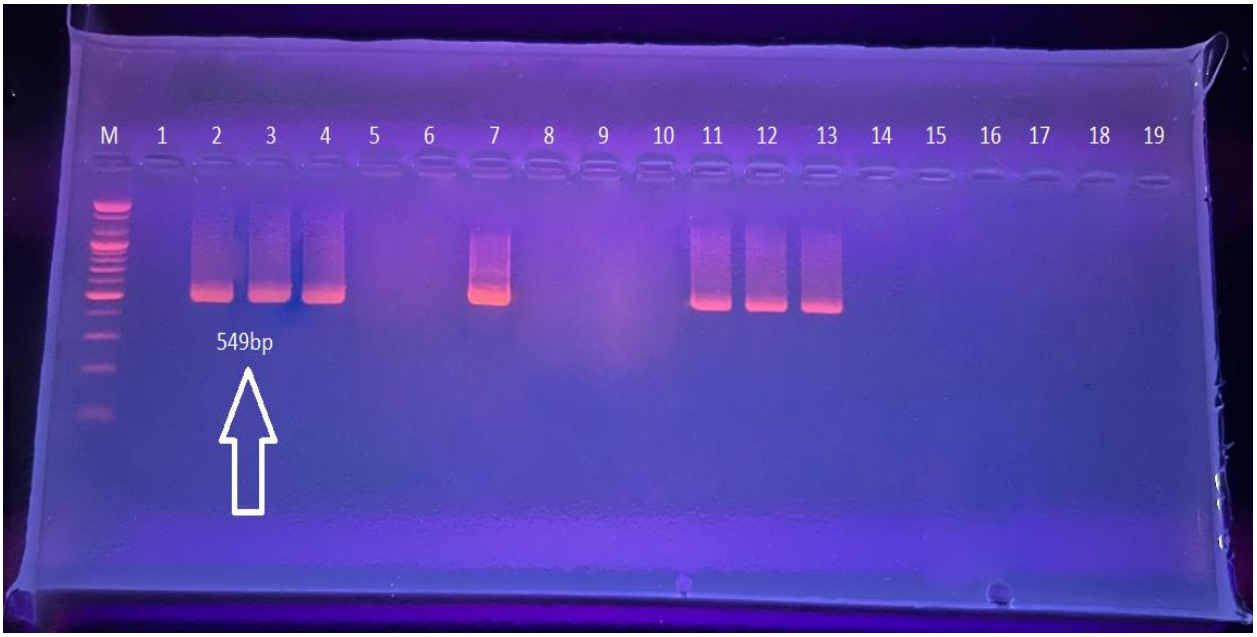
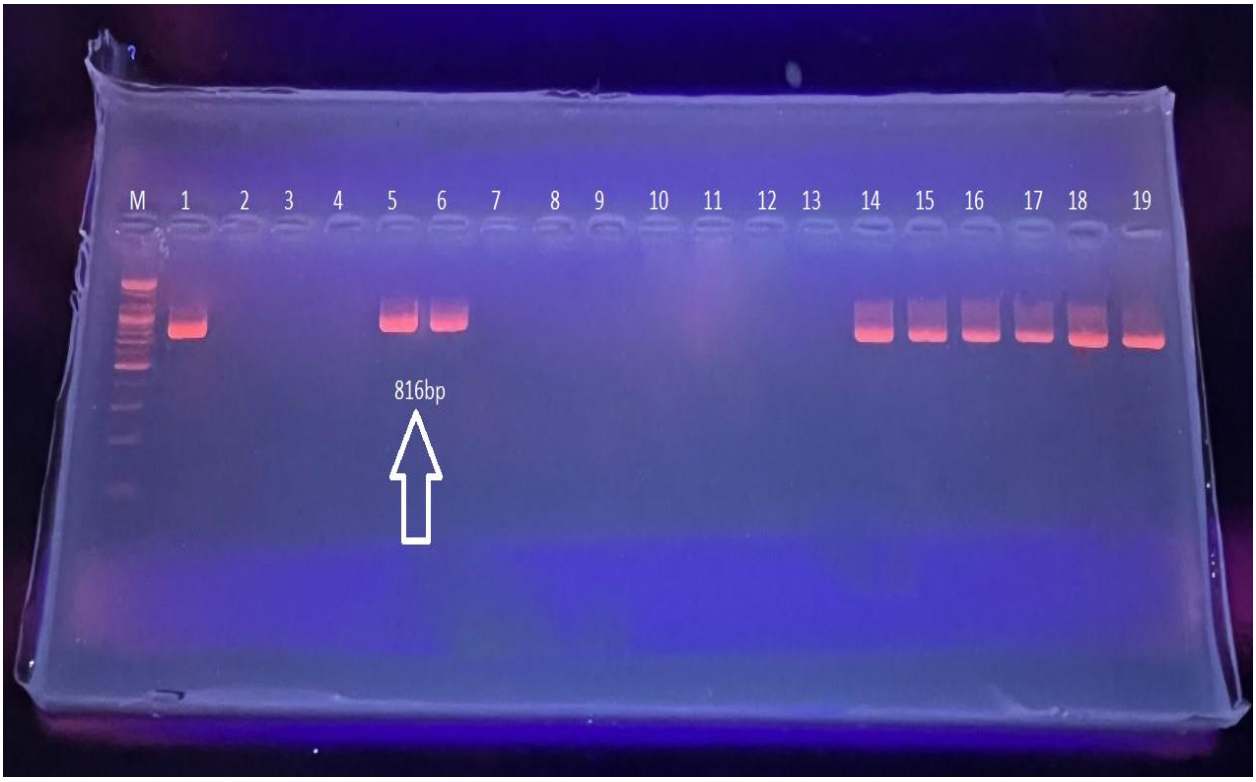
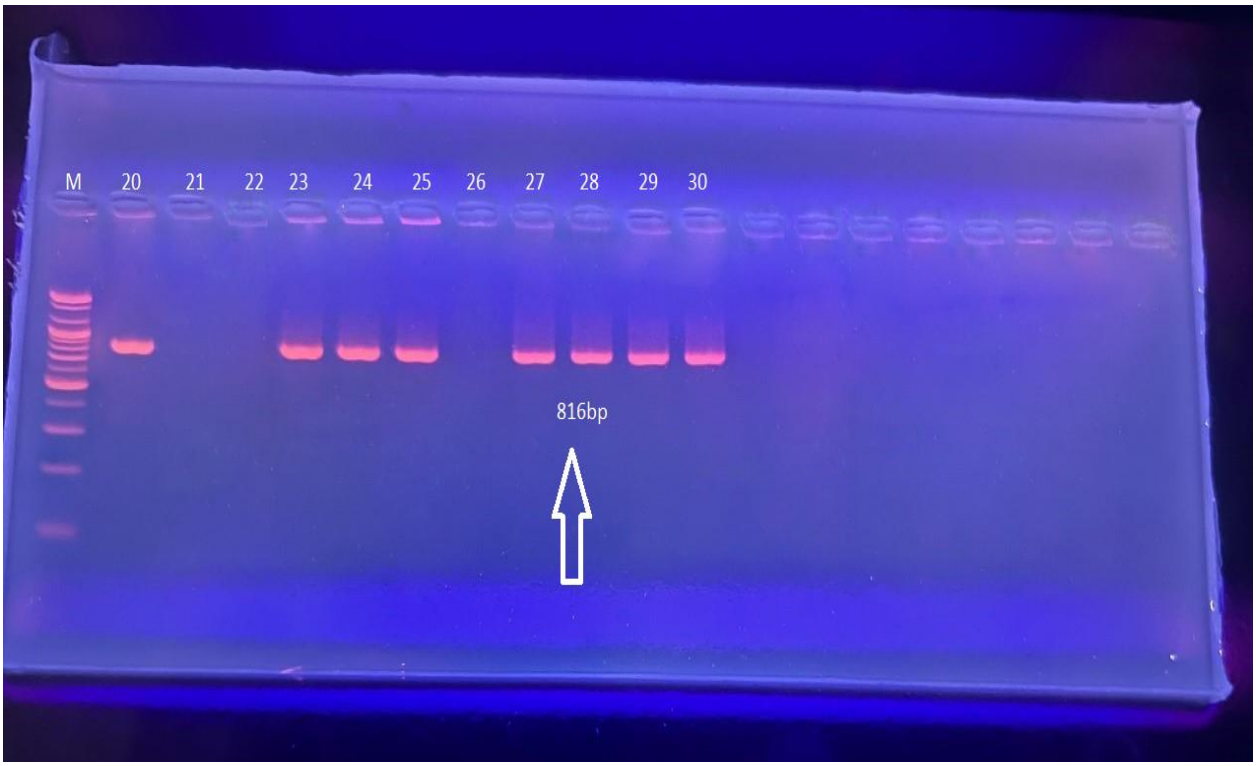


Figure 3. Agarose gel electrophoresis of the *lukS/F-PV* gene on 1.5% agarose gel and visualized under UV light after staining with red stain. Bands were fractionated. Lane M: 100 bp DNA ladder. Positive bands appeared at 549 bp.



(a)



(b)

Figure 4. Agarose gel electrophoresis of the *mecA* gene. Bands were fractionated on 1.5% agarose gel and visualized under UV light after staining with red stain. Lane M: 100 bp; DNA ladder. Positive bands appeared at 816 bp.(a,b)

The sequencing analysis of PCR-positive products of spa, mecA and lukS/F-PV genes was carried out with the Macrogen Company to confirm the molecular characterization according to amplication fragments and investigate possible nucleotide differences in Staphylococcus aureus isolates.

SpA gene sequences were highly similar tothe reference sequence ID: MT834479. 1 and CP051912. GenBank and were 99 to 100% identical. Samples No. 1 and 4 were identical (100% identity) without nucleotide substitutions. Conversely, sample No. 2 showed a transversion mutation at nucleotide position 6 (T→A). Additionally, in sample No. 5 it has two nucleotide substitutions: Transversion mutation at position 217 T→G and transition mutation at position 218 G→A. These results show that there exists little heterogeneity at the level of spa gene sequences. Sequencing analysis of mecA gene showed a 99% identity (ID: PP449378) with the reference sequence of genome. 1. Different nucleotide substitutions were identified in the sequenced isolates. Sample No6, A (1658): transversion C→A; Sample No7, G (1135): transversion G→T. A transversion mutation (T→G) at position 17761 was found in sample No. 8. Also, two substitutions in sample No. 9 were also shown: a transiti on mutation at position 1741 (A→G) and a transversion mutation at position 1761 (T→G). Sample No. 10 displayed the highest number of nucleotide substitutions, carrying transversion mutations at positions 1645 (T→A), 1674 (G→T) and 1681 (A→T), along with transition nucleotide substitutions at positions 1661 (A→G) and 1679 (A→G). These sequence variations may represent genetic diversity present in methicillin-resistant S. aureus isolates from bovine mastitis cases. LukS/F-PV Gene All sequenced samples (11–15) for lukS/F-PV gene displayed 100% nucleotide identity with the GenBank reference sequence ID: CP148146. 1 without any identified nucleotide substitutions. No mutations were noted in the sequenc region, indicating that the lukS/F-PV gene shows a highly conserved sequence pattern among tested isolates.(Table 4).

Table 4. Sequencing analysis of selected PCR-positive samples.

Staphylococcus aureus							
No. Of sample	**Type of substitution**	**Location**	**Nucleotide**	**Gene**	**Sequence ID with compare**	**Sequence ID with submission**	**Identities**
1	-------	------	-----	**Spa**	**ID: MT834479.1**		**100%**
2	**Transvertion**	6	T\A	**Spa**	**ID: MT834479.1**		**99%**
4	-------	-------	-------	**Spa**	**ID: MT834479.1**		**100%**
5	**Transvertion**	217	T\G	**Spa**	**ID: CP051912.1**		**99%**
	Transition	218	G\A				

6	**Transvertion**	1658	C\A	**mecA**	**ID: PP449378.1**		**99%**
7	**Transvertion**	1135	G\T	**mecA**	**ID: PP449378.1**		**99%**
8	**Transvertion**	17761	T\G	**mecA**	**ID: PP449378.1**		**99%**
9	Transition	1741	A\G	**mecA**	**ID: PP449378.1**		**99%**
	Transvertion	1761	T\G				
10	**Transvertion**	1645	T\A	**mecA**	**ID: PP449378.1**		**99%**
	Transition	1661	A\G				
	Transvertion	1674	G\T				
	Transition	1679	A\G				
	Transvertion	1681	A\T				
11	----------	-------	-----	**lukF- PV**	**ID: CP148146.1**		**100%**
12	----------	-------	-----	**lukF- PV**	**ID: CP148146.1**		**100%**
13	----------	-------	-----	**lukF- PV**	**ID: CP148146.1**		**100%**
14	----------	-------	-----	**lukF- PV**	**ID: CP148146.1**		**100%**
15	----------	-------	-----	**lukF- PV**	**ID: CP148146.1**		**100%**

Phylogenetic trees of the representative S. aureus isolates for the spa and mecA genes were constructed based on the respective DNA sequences using the neighbor-joining method with 1,000 bootstrap replicates (Figure 5). This produced a tree with two primary clades. In our phylogenetic analysis, samples (1, 2, 4, 5) from clade I were found to cluster closely to the reference sequence MT834479 (spa gene). 1 with high bootstrap support (98%). Samples 6–10 (mecA gene) clustered with reference sequence PP449378 in clade II. 1; bootstrap values 95%. These results demonstrated that at least two genetically nonidentical lineages of S. aureus were circulating in the sampled Diyala dairy farms.

Table 5. This detected Antibiotic mecA gene

Antibiotic	Resistant R n (%)	Intermediate I n (%)	Sensitive S n (%)
Benzylpenicillin	5 (100%)	0 (0%)	0 (0%)
Oxacillin	5 (100%)	0 (0%)	0 (0%)
Gentamicin	5 (100%)	0 (0%)	0 (0%)
Tobramycin	5 (100%)	0 (0%)	0 (0%)
Fusidic Acid	5 (100%)	0 (0%)	0 (0%)
Levofloxacin	0 (0%)	0 (0%)	5 (100%)
Moxifloxacin	0 (0%)	0 (0%)	5 (100%)
Erythromycin	0 (0%)	0 (0%)	5 (100%)
Clindamycin	0 (0%)	0 (0%)	5 (100%)
Linezolid	0 (0%)	0 (0%)	5 (100%)
Teicoplanin	0 (0%)	0 (0%)	5 (100%)
Vancomycin	0 (0%)	0 (0%)	5 (100%)
Tetracycline	5 (100%)	0 (0%)	0 (0%)
Tigecycline	0 (0%)	0 (0%)	5 (100%)
Rifampicin	0 (0%)	0 (0%)	5 (100%)

Discussion

This study aimed to molecularly detected and characterized spa, lukS/F-PV and mecA genes from Staphylococcus aureus isolated from mastitic cow milk samples in Diyala Governorate. Positive cases accounted for 20.0%, which confirmed S. aureus is still one of the most important bacterial pathogens associated with bovine mastitis in dairy cattle. Conclusions The prevalence observed was possibly related to farm hygiene, environmental contamination, animal manager breeding cell and antimicrobial use patterns in the region studied. Touaitia et al. also reported their results accordingly (2025), who recently said that S. aureus is still one of the major contagious mastitis pathogens in dairy herds around the world.

The detection of spa gene in 100% of the isolates suggests high frequency staphylococcal protein A associated with bovine mastitis pathogens. Protein A is an important virulence associated factor that prevents opsonization and phagocytosis, thereby enhancing survival of the bacteria in host cells. The higher prevalence of spa detected among the current study subjects correlates with that reported by [11] proved that spa is extensively widespread in mastitis-related S. aureus isolates and plays an important role in bacterial invasion of mammary tissues. Moawad et al, also reported similar observations. (2025), is a confirmation that spa continues to be one of the major virulence-associated genes identified among dairy cattle isolates.

Molecular detection of mecA gene confirms the circulation of MRSA among bovine mastitis cases in Diyala Governorate with percent isolating 63.3% from S. aureus isolates, these results matched with [12] reported the extensive presence of mecA among livestock-associated S. aureus isolates. Similar findings were also reported by Sheet (2022), with the latest work in Iraq indicating an increasing incidence of methicillin-resistant strains associated with bovine mastitis.

These findings showed that at least 23.3% of the examined isolates likely carried leukocidin-associated virulence determinants, as identified by the detection of lukS/F-PV gene. Panton–Valentine leukocidin promotes the lysis of inflammatory cells, which leads to tissue injury and may increase BSI severity and bacterial virulence. Overall, Getahun et al. reported similar findings. [13] S. aureus isolates from bovine mastitis were found to harbour coexisting virulence and antimicrobial resistance genes. Likewise, Fazal et al. D (-mrcB, bclA) among mastitis-associated S. aureus strains; they further confirmed that the virulence-associated genes can play a role in the long-term persistence and higher invasiveness of mastitis-associated S. aureus strains . Successful detection of spa, lukS/F-PV and mecA genes was confirmed by PCR amplification products at 292 bp, 549 bp and 816 bp respectively. The specificity of the amplification indicates that conventional PCR is a rapid, versatile and reproducible molecular approach for characterization of S. aureus isolates. These findings are in accordance with Abd El-Razik et al. 2013, 2025), which verified the efficiency of PCR-based assays for quick molecular diagnosis and virulence and resistance factors in bacteria related to mastitis-associated genes.

Sequencing characterized the amplified products, showing identity percentages vs GenBank reference strains of 99%–100%, confirming specificity. The nucleotide substitutions detected in selected spa and mecA sequences suggest genetic and local molecular diversity of S. aureus isolates circulating among dairy farms. Miyazawa et al. report the similar sequence variability. (2022), who reported molecular heterogeneity detected by WGS among bovine mastitis associated isolates recovered from milk samples.

Compared to the current results, previous studies recorded lower prevalent virulence and resistance genes. Ramezanigardaloud et al. We recently reported lower rates of mecA among bovine mastitis isolates (2025), highlighting the influence of geographic setting and antimicrobial usage policies on resistance gene dissemination. Additionally, Ibrahim et al. Prevalence of lukS/F-PV was lower in bovine isolates (2023),

potentially showing an area-dependent strain pathogenicity. Furthermore, Klibi et al. The spa gene is less distributed among mastitic milk isolates according to[14] than the results of our study, but differences in sample size, molecular techniques used or epidemiological factors may have influenced both studies. Nucleotide substitutions at sequenced lukS/F-PV isolates were absent and it was completely identical with reference sequence of CP148146. Finally, the results of 1 confirm our hypothesis that this gene region is relatively conserved among all isolates tested. The maintenance of virulence-associated genes may stabilize and disseminate these pathogenic bacteria in dairy animals.

Statistical analysis revealed a significant correlation between mecA and virulence gene (PVL) of S. aureus isolates. The OR of 4.62 which is associated with mecA-positive isolates has significant importance which suggests that PVL is more likely to be presented in these strains and may well indicate that both resistance and virulence factors are co-selected in these dairy herds.

A molecular prevalence of S. aureus of 30% is comparable with reports from Iraq [14] and Iran (Ramezanigardaloud et al., 2025), and lower than some European herds [15]. The mecA prevalence of 63.3% is higher than values reported for Brazil [2] and [10], which may reflect unchecked use of beta-lactams without routine susceptibility testing [5,1]. The detection of PVL (23.3%) observed is similar toprevious report from Ethiopia [3] but lower than some isolates identified throughout Europe (Moawad et al., 2025), implying a region-specific difference in the distribution of virulence genes. Conclusions These findings highlight the need for region-specific surveillance and AM stewardship programs aimed at controlling the dissemination of resistant and virulent S. aureus [14,13] and [9 & 11].

Conclusions On honeybees played a role in maintains persistence Status of virulence associated genes from S. aureus from dairy farms the conservative nature of lukS/F-PV gene region in all tested isolates. Preservation of these genes probably allowed these pathogenic bacteria to survive in the mammary gland and to maintain pathogenicity factors across generations. Antibiotic susceptibility testing showed resistance of all isolates to benzylpenicillin, oxacillin, gentamicin, tobramycin, and fusidic acid, whilst remaining susceptible to levofloxacin and moxifloxacin. This pattern of resistance is consistent with the mecA gene, which mediates resistance to β-lactam and aminoglycoside antibiotics. These results indicate that the genetic persistence of virulence factors is associated with specific antibiotic resistance patterns, which support the adaptive potential or viability of these farm isolates. Such observations are consistent with previous studies reporting the prevalence of virulence and resistance genes in mastitis-associated S. aureus that might be expected to be more common in both geographical and farm management areas. In comparison, regional data from the Middle East and neighbouring countries is limited, nevertheless, the available evidence from Pakistan and broader meta analyses indicates similar prevalence rates (often 30–40%) implying that a 30% positivity rate aligns with international data in regions using similar farming practices and possessing similar herd sizes. In Europe and North America, the rate of S. aureus in dairy herds receiving stringent mastitis control programs is reportedly lower, but the range observed is broad across studies. Considering the mecA-mediated methicillin resistance, the 63.3% prevalence found among our isolates is on the upper limit of the described range in the literature. A larger systematic review encompassing MRSA in bovine mastitis found mecA prevalence amongst S. aureus isolates to vary widely, from very low (60–100%) in other contexts (notably some African contexts and parts of South Asia). For example, mecA rates between 17–45% have been reported from Pakistan and recent studies from Egyptian mastitis cohorts have reported 100% mecA among phenotypic MRSA isolates. These comparisons indicate that our high estimated prevalence of mecA is not unexpected in regions of lower regulation on AMU (particularly β lactams) and where routine susceptibility testing is not widely

performed. However, the higher mecA prevalence observed in such clinical settings likely reflects local patterns of antimicrobial use and selective pressure (rather than intrinsic geographic strain distributions) and emphasizes the need for local, regional, and national antimicrobial stewardship programs.

The detection frequency of 23.3% for the Panton Valentine leukocidin (PVL) determinant (lukS/F PV) in our study is situated on a global continuum with its variable distribution (between 0 and 81%) [16]. Despite sparser data for PVL than for mecA, general geographical trends are emerging with some studies indicating the relatively low carriage of PVL (<10%) in isolates from clinical mastitis from Europe or North Africa, compared to others that have reported the relatively high carriage of PVL (approaching or exceeding ~20%) in subsets of isolates from other geographical regions (not least parts of Africa and Asia). This indicates potential environmental specificities in PVL mediated virulence traits that may determine clinical outcomes in particular ecological or herd management scenarios. This result aligns with the hypothesis that virulence factors associated with community-associated staphylococcal infections are widespread in geographical regions with lower PVL carriage than predicted, especially where resistance elements are also identified in isolates such as mecA, indicating possible co-selection or feasible dynamics of mobile genetic elements, including SCCmec and phage-associated virulence genes, among pneumonia-associated S. aureus strains.

Overall, these international comparisons illustrate that prevalence rates of S. aureus, mecA, and lukS/F PV vary widely between regions and management systems, with higher antimicrobial resistance gene frequencies often observed in settings with intensive antimicrobial use and less stringent control measures. They underscore the need for region specific surveillance and targeted antimicrobial stewardship strategies in dairy herds to limit the spread of resistant and virulent S. aureus strains.

Conclusions

The current study verified the existence of molecular Staphylococcus aureus markers in cow mastitic milk samples taken from localities in Diyala Governorate. Twenty of the 100 samples were positive (20.0%). PCR successfully amplified spa, lukS/F-PV and mecA genes at respective product sizes of 292 bp, 549 bp, and 816 bp. Sequencing analysis confirmed 99% to 100% similarity with GenBank reference sequences.

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