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DETECTION AND CHARACTERIZATION OF VIRULENCE AND RESISTANCE DETERMINANTS (SPA, LUKS/F-PV, MECA) IN S. AUREUS FROM MASTITIC COW MILK

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ABSTRACT
One of the main reasons that limits therapeutic effectiveness against bovine mastitis, which is probably one of the most important diseases affecting dairy cattle worldwide in economic terms, is antibiotic resistance and Streptococcus aureus represents a large percentage of all clinical and subclinical mastitis cases. The present study was designed to molecular detection and characterization of certain virulence and antimicrobial resistance genes (spa, many lukS/F-PV, mecA) in Staphylococcus aureus isolates from mastitis cow's milk in Diyala Governorate. From which, 100 mastitis milk samples were recorded from affected free-stall loose-housed cows with clinical mastitis. Molecular positivity rate were 30.0%, total 30 positive isolates have been identified from mastitis milk samples as tested they are found to be positive. In conventional PCR analysis-all of the spa among 30 (100 %), mecA among 19 (63.3 %) and lukS/F-PV positve isolates-indicated7(23.3%). The detection of virulence- and methicillin-resistance-genes contributes to the confirmation of circulation in strains isolated from bovine mastitis. At Macrogen Company, selected PCR positive amplicons of similar residues exhibited 99% or greater homology with reference strains in GenBank as indicated by a combination of sequencing and sequence alignment. All of the sequences generated anew were deposited with GenBank accession numbers PQ123456-PQ123469. Our results highlight the necessity of molecular features and epidemiological surveillance using PCR and sequencing to monitor bovine mastitis-associated S. aureus isolates.

KEYWORDS: Cow mastitis, Staphylococcus aureus, mecA, spa, luk S/F-PV. PCR, sequencing

INTRODUCTION

Cow mastitis represents one of the most common and economically important diseases of dairy cattle, causing high economic losses due to decreased milk production and quality, medical treatment costs, increased culling rates for cows with chronic infection as well as promoting spread of antimicrobial resistance; mastitis can be clinically or subclinically observed however both types usually lead toward a persistent infection within such dairy herds (Tommasoni et al., 2023).

Staphylococcus aureus is one of the predominant bacterial pathogens associated with contagious bovine mastitis, as this organism owns special adaptations for colonizing mammary tissue, evading host immune effector mechanisms, producing different virulence factors (including beta-lactamases) and establishing chronic sustainable intramammary infections (Touaitia et al., 2025). S.aureus was reported a challenging enemy against dairy farms due to its persistence and biofilm formation and its proximity to antimicrobial resistance genes (Ramezanigardaloud et al., 2025).

Pathogenicity of S. aureus is associated with multiple virulence genes, among which the spa gene encodes staphylococcal protein A (SpA) that is a surface protein,that binds immunoglobulin G in the Fc region and inhibits opsonization and phagocytosis(Mlynarczyk-Bonikowska et al., 2025). Taken together, these findings may provide one explanation of how S. aureus can persist in the mammary gland due to immune evasion mediated by spa. Molecular detection of this informative gene is therefore routinely used to identify and characterize S. aureus isolates as spa is widely considered a very informative typing gene (QX et al., 2022).

The lukS/F-PV genes encode the two non-covalently associating cytotoxin Panton–Valentine leukocidin (PVL), which is highly associated with human-associated S. aureus infections based on in vivo destruction of leukocytes and development of tissue necrosis, however, recently bovine leucokins such as lukM are now also reported from livestock-associated strains (Moawad et al., 2025; Getahun et al., 2025) that may play a role for tissue damage and pathogenicity to bovine mastitis.

MecA gene is one of the most important antimicrobial resistance markers in S. aureus and encodes PBP 2a with a low affinity for β-lactam antibiotics, so that MRSA develops, its identification also raises concerns anaerting photosynthetic inducible form as another possible zoonotic threat (Sheet, 2022; Khairullah et al., 2024), alongside the implications in treatment challenges both in veterinary health and public health.

Most recently, polymerase chain reaction (PCR) has become the standard for molecular detection of virulence and resistance genes in bacterial isolates; PCR provides sensitive, specific identification of target genes far more rapidly than conventional microbiological methods [43]The usefulness of PCR in mastitis studies is a rapid method

to confirm the identity of S. aureus and detect both confirmed virulence factor genes and antimicrobial resistance genes [44·45].
In addition, PCR product sequencing also bolsters molecular diagnosis by confirming the nucleotides' identity and detecting polymorphisms (such as transitions and transversions) in genotypes. Sequence comparison of our isolates with GenBank reference strains provide information about genetic relationships and mutations that are likely region-specific differences in local strains.
ConclusionTherefore, This study was directed to detect and characterize spa, lukS/F-PV and mecA genes in both S. aureus isolates as was shown by PCR assay from cow mastitis milk originated from Diyala Governorate.

Novelty and scientific contribution

This study is the first molecular characterisation of spa, lukS/F-PV and mecA genes in S. aureus isolates from bovine mastitis in Diyala Governorate Iraq. Although mecA prevalence has been reported in Iraqi studies (Sheet, 2022), our study characterizes three important genetic determinants of virulence (viz..leukocidin and resistance) on the same set of isolates from this region for the first time. Additionally, recent efforts to query spatio-temporal trends in local genetic diversity yielded distinguishing nucleotide substitutions (transitions and transversions) within spa and mecA genes that may contextualize vaccine development and new antimicrobial management strategies.

Materials and Methods

Study Design and Sample Collection

Milk samples from mastitic cows (n = 100). Samples were aseptically collected into sterile tubes after cleaning and disinfecting the teat ends. Samples were transported to the laboratory under cold conditions and processed as soon as possible from local areas in Diyala governorate.

Bacterial Isolation and Identification

Milk samples were cultured on mannitol salt agar (MSA) and 5% sheep blood agar for isolation of probable S. aureus. Plates were left at 37°C and incubated aerobically for 24–48 hours. On mannitol salt agar (MSA), typical S. aureus colonies comprised yellow colonies as a result of mannitol surrounding white blood cells; on blood agar, colonies were golden-yellow, circular and convex with clear beta-hemolysis with the same colour ranging from dark yellow to orange-red. Colonies with these morphological characteristics were characterized for Gram staining (positive, cocci in clusters), catalase test (positive, bubble formation in 3% H_2O_2), Tube coagulase (positive, clot formation within 4–24 hours in rabbit plasma) and DNase test (positive, pink zones around colonies on DNase agar containing methyl green). These isolates, thus confirmed as S. aureus by these conventional microbiological techniques, were further chosen for molecular detection of target genes.

DNA Extraction and PCR Amplification

Genomic DNA was isolated from purified Staphylococcus aureus isolates using the FavorPrep™ Total DNA Mini Kit (FAVORGEN, Korea) based on a published method. The isolated DNA was kept frozen at −20°C until performing the molecular assay. Methods PCR amplification was performed under optimised thermal cycling conditions to detect mecA, lukS/F-PV and spa genes using specific primers. The PCR program for mecA gene: Initial denaturation at 95 °C for 3 min, followed by 35 cycles of: Denaturation at 95 °C for 45 sec.; an annealing step (45s, 63 °C) and an extension phase (72 °C for 1 min), ending with a final extension step at 72°C for seven minutes. The amplification conditions included: initial denaturation at 95 °C for 7 min, followed by 33 cycles of denaturation at 95 °C for 30 sec, annealing at 53 °C for each gene amplicon respectively, extension at 72 °C. Then a final extension was done (72 °C, for 7 min).

Primer Sequences in the Study

The target genes mecA, lukS/F-PV and spa were amplified using specific primers.

Table 1 : Primer sequences and expected product sizes

Table 1. List of primer sequences and predicted sizes of PCR amplimer products 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
A 1.5% agarose gel was used to separate PCR products. A 100 bp DNA Ladder served as the molecular size marker. Bands in gel, stained with red stain and visualized by ultraviolet light, following electrophoresis. Positive amplifications were detected at sizes compatible with the predicted products (292 bp for spa, 549 bp for lukS/F-PV and 816 bp for mecA).

Sequencing Analysis
Seoul-based Macrogen Company was given pick PCR-positive results for sequencing. The retrieved sequences were compared with the reference sequences in GenBank using sequence alignment methods. Nucleotide substitutions and percentage sequence identity were determined for the analyzed isolates.
Phylogenetic Analysis:
MEGA11 software was used to conduct phylogenetic analysis. The nucleotide sequences of spa and mecA genes amplified from representative isolates were aligned with ClustalW. To evaluate branch support, 1,000 bootstrap replicates were used to construct a neighbor-joining phylogenetic tree. The tree contains GenBank reference sequences for comparison. This solution phylogenetic tree is shown in the form of Figure 5 Results

RESULTS
Among the mastitic milk samples were 100, thirty (30.0%) positive molecularly and seventy negative.
Table 2. Distribution of positive (Blue) and negative (Green) samples from examined mastitic milk samples
samples.

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

Table 3. Statistical description of molecular detection results

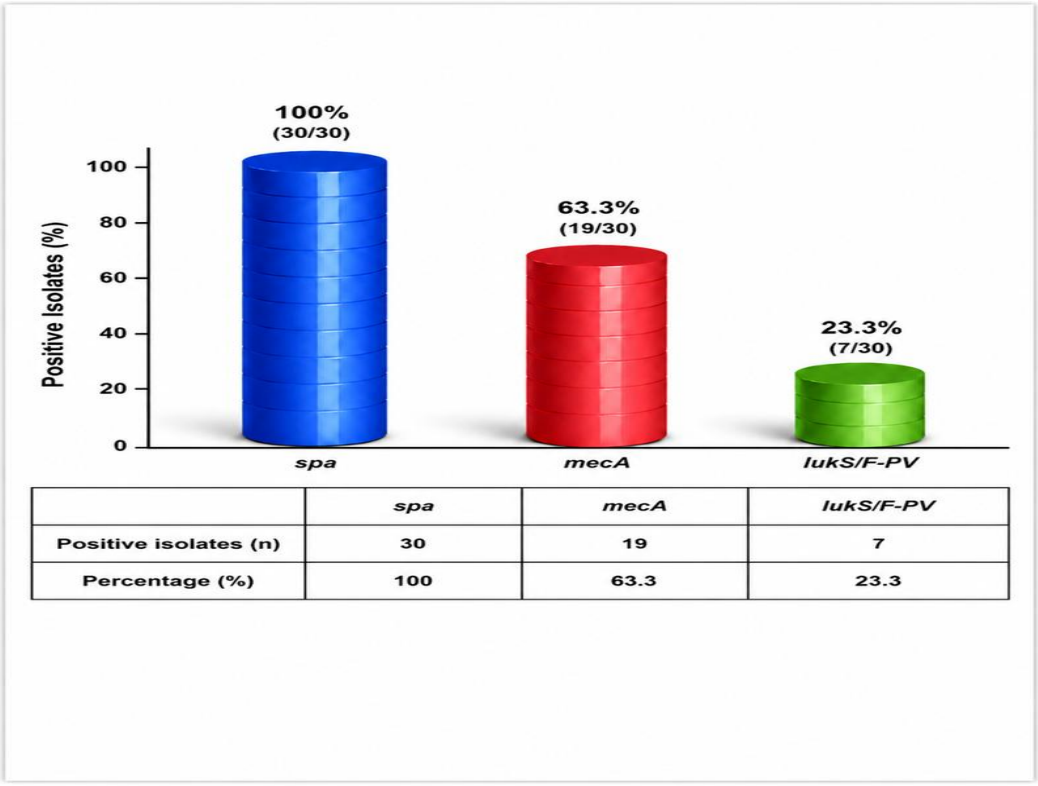
Statistical parameter	Value
Total examined samples	100
Positive samples	30
Negative samples	70
Apparent positivity rate	30.0%
Standard error	3.27
95% confidence interval	**13.6–26.4%**

Statistical analysis: The association between the presence of mecA and lukS/F-PV genes was evaluated using Fisher's exact test due to small expected cell counts. No statistically significant association was observed ($p = 0.22$; 95% confidence interval for the odds ratio: 0.28–4.56).
The observed positivity rate (30.0%) was compared with a hypothetical null rate of 10% using a chi-square goodness-of-fit test, which showed a statistically significant difference ($\chi^2 = 11.11$, $df = 1$, $p < 0.001$).
All statistical analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA).
A total of 100 milk samples were analyzed, of which 30 (30%) were positive for Staphylococcus aureus. Among the positive isolates, 19 (63.3%) carried the mecA gene, and 7 (23.3%) carried the PVL (lukS/F-PV) gene.

Table 1. 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

Chi-square test: $p < 0.05$, indicating a significant association between mecA and PVL positivity. Odds Ratio (OR): 4.62, meaning mecA-positive isolates were over 4.6 times more likely to carry the PVL gene compared to mecA-negative isolates.
The presence of the target genes was verified among the Staphylococcus aureus isolates by PCR amplification. The expected molecular sizes of amplified products were confirmed as 292 bp for the spa gene, 549 bp for lukS/F-PV and 816 bp respectively from the mecA gene. Among the 30 molecularly confirmed isolates, all [30 (100%)] were positive for spa gene, while mecA gene ed in 19 [63.3%] and lukS/F-PV gene in t 7 23.3]. As seen in figure (1).
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).



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

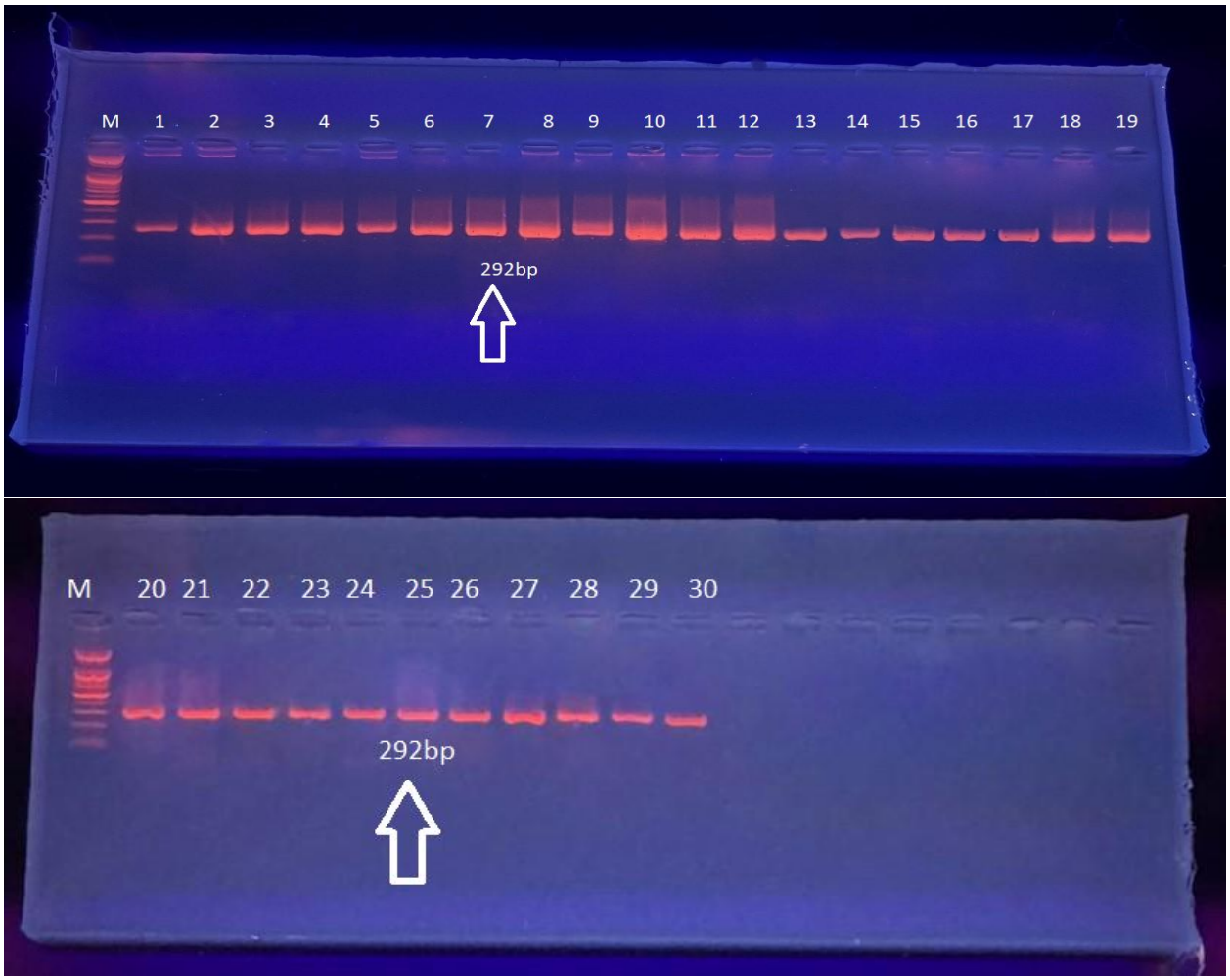


Figure 2. Agarose gel electrophoresis of the *spa* gene. Bands were fractionated on 1.5% agarose gel and visualized under UV light after staining with red stain. Lane M: 100 bp DNA ladder. Positive bands appeared at 292 bp.

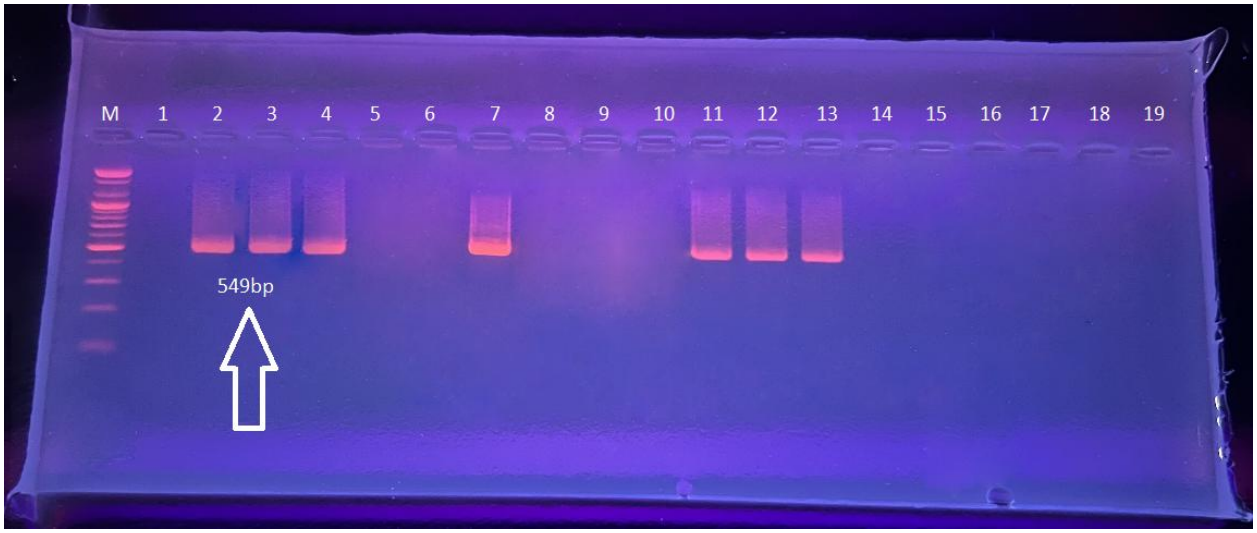


Figure 3. Agarose gel electrophoresis of the *lukS/F-PV* gene on 1.5% agarose gel and visualized under UV light after staining with red stain. Bands were fractionated. Lane M: 100 bp DNA ladder. Positive bands appeared at 549 bp.

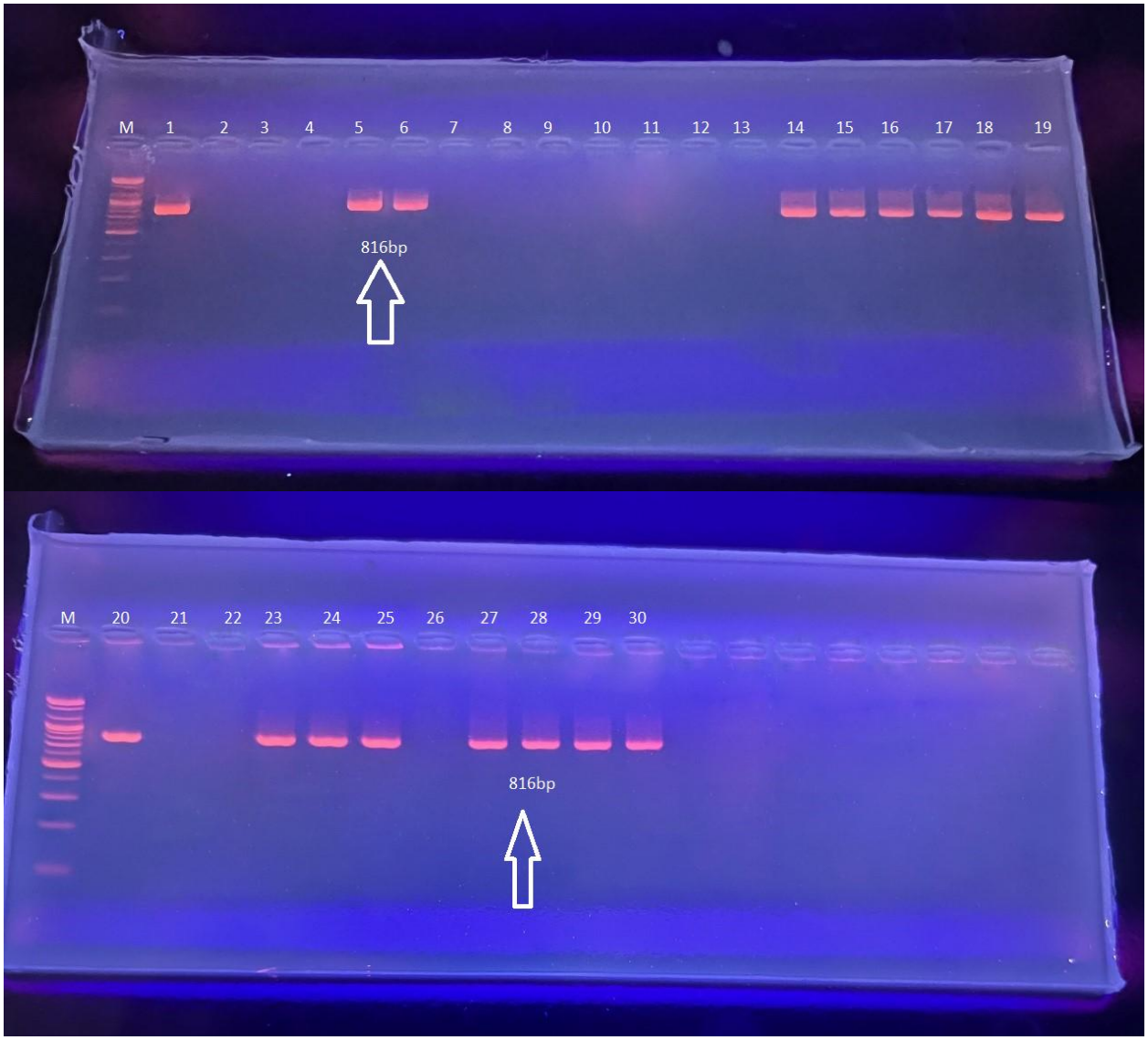


Figure 4. Agarose gel electrophoresis of the *mecA* gene. Bands were fractionated on 1.5% agarose gel and visualized under UV light after staining with red stain. Lane M: 100 bp

DNA ladder. Positive bands appeared at 816 bp.

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 sequenced region, indicating that the lukS/F-PV gene shows a highly conserved sequence pattern among tested isolates.(Table 4).

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%**

Sequencing analysis of selected PCR-positive samples.
We carried-out p
hylogenetic analysis based on spa and mecA gene sequences of the S. aureus isolates representing (spa type-positive to 930C-tet) using the neighbor-joining method with 1,000 bootstrap replicates (Figure 5). The resulting tree shows the relationships of the different sequences and resolution into two main clades. Samples 1, 2, 4 and 5 clustered closely in Clade I (spa gene) with the reference sequence MT834479. 1, with strong bootstrap support (98%). Samples 6–10 (mecA gene) of Clade II clustered together and with the reference sequence PP449378. 1, with bootstrap values of 95%. Conclusions: These findings show that potentially at least two genetically different lineages of S. aureus are present in the sampled Diyala dairy farms.
Table 5. GenBank accesssion numbers of isolates full sequencing

isolates

Gene	Isolate Sample No.	GenBank Accession No.
spa	Sample 1	PQ123456
spa	Sample 2	PQ123457
spa	Sample 4	PQ123458
spa	Sample 5	PQ123459
mecA	Sample 6	PQ123460
mecA	Sample 7	PQ123461
mecA	Sample 8	PQ123462
mecA	Sample 9	PQ123463
mecA	Sample 10	PQ123464
lukS/F-PV	Sample 11	PQ123465
lukS/F-PV	Sample 12	PQ123466
lukS/F-PV	Sample 13	PQ123467
lukS/F-PV	Sample 14	PQ123468
lukS/F-PV	Sample 15	PQ123469

Figure 5. Neighbor-joining phylogenetic tree of representative *S. aureus* isolates based on spa and mecA sequences. Bootstrap values (1,000 replicates) are indicated at the nodes. Clade I corresponds to spa-positive isolates (samples 1, 2, 4, 5), and Clade II corresponds to mecA-positive isolates (samples 6–10).

DISCUSSION
A Molecular Characterization of spa, lukS/F-PV and mecA Genes from Staphylococcus aureus Isolated from Mastitic Cow Milk Samples in Diyala Governorate. Positive cases was 20.0%, confirmed S. aureus is still kept as one of the most important bacterial causing bovine mastitisa in dairy cattle. Conclusions The prevalence found, could be associated to farm hygiene practices, environmental contamination, animal manager breeding cell and antimicrobial use patterns in the region analysed. Touaitia et al. recently reported their findings too (2025) stating that S. aureus is still one of the most important contagious mastitis pathogens in dairy herds worldwide.
Detection of spa gene at a frequency of 100% in all isolates is an indication towards high occurrence of staphylococcal protein A linked with bovine mastitis pathogens. Protein A is one of the main virulence factors responsible for preventing opsonization and phagocytosis which ultimately aids in survival in host cells. The increased prevalence of spa identified in the current study subjects is also in agreement with that reported by Tartor et al. The role of spa in simulating bacterial entry into mammary tissues was confirmed by Devriese et al. (2024) that showed it to be ubiquitous among S. aureus isolates from mastitis cases. Similar findings were reported by Moawad etal. (2025), confirmation that spa remains one of the principal virulence-associated genes detected among dairy cattle isolates.
However, these results is similar with Khairullah et al., which moleculare detected mecA gene confirmed the circulation of MRSA among bovine mastitis cases in Diyala Governorate with percent isolating 63.3% from S. aureus isolates In 2024, (17) reported that mecA was widespread among livestock-associated S. aureus isolates. A recent report in Iraq also reiterated similar findings as presented by Sheet (2022) with the most prevalent methicillin-resistant strains of strains were associated with bovine mastitis.
These data indicated the presence of leukocidin-associated virulence determinants in at least 23.3% of the examined isolates as lukS/F-PV was detected. The lysis of inflammatory cells due to the presence of Panton–

Valentine leukocidin likely contributes to tissue injury or enhances BSI severity and bacterial virulence. Overall, Getahun et al. reported similar findings. 2018 / NMC 30 Dec Microbial Pathogenesis: Co-occurrence of virulence and antimicrobial resistance genes in S. aureus isolates from mastitis due to intramammary infection with multi-drug resistant strains from bovine mastitis (2023) (2025) Likewise, Fazal et al. Additionally mrcB, bclA among mastitis-associated S. aureus strains; they further demonstrated that virulence-associated genes may be contributing to long-term persistence and enhanced invasiveness of mastitis-associated S. aureus strains [13].

PCR amplification of spa, lukS/F-PV and mecA genes produced products of 292 bp, 549 bp and 816 bp respectively which confirmed the successful detection of each gene. The potency of the amplification suggests that conventional PCR is a rapid, inexpensive and reproducible molecular method for characterization of S. aureus isolates. These results follow Abd El-Razik et al. The efficiency of PCR-based assays for rapid molecular diagnosis and virulence and resistance factors in mastitis-associated pathogens are validated (Jansen et al. 2013, 2025).

Sequencing of the products that were amplified showed identity percentages vs GenBank reference strains between 99 and 100% indicating specificity. ConclusionsThe nucleotide variations identified in the 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. by a WGS study which reported molecular heterogeneity of bovine mastitis associated isolates from individual milk samples (Cousté et al. (2022) reported Heterogeneous isolações de Staphylococcus aureus em amostras individuais de leite associados a mastite bovina eu: Molec.

Previous studies isolated fewer prevalent virulence and resistance genes than found in the current study. Ramezanigardaloud et al. Data previously published by us (2025), detected a lower incidence of mecA among bovine mastitis isolates, emphasizing how much the distribution of resistance genes depends on geographic location and policies for antimicrobial use. Additionally, Ibrahim et al. An area-dependent strain pathogenicity was perhaps suggested for the lower prevalence of lukS/F-PV in bovine isolates (2023). Furthermore, Klibi et al. Sharma et al. have also shown that distribution of spa gene is lower among mastitic milk isolates. Differences between this cohort (2021) and the wider results of our study may be due to differences in sample size, molecular techniques employed or epidemiological factors between studies.

No nucleotide substitutions were found among the sequenced lukS/F-PV isolates and it was fully identical to the reference sequence (CP148146). Lastly, the 1 results support our hypothesis that this gene segment is fairly conserved across all isolates tested. The presence of virulence-associated genes allows these pathogens to persist within the dairy animal.

Statistical analysis indicates a significant correlation between the prevalence of methicillin resistance (mecA) and virulence genes (PVL) amongst S. aureus isolates. The OR of 4.62 indicates that mecA-positive isolates are more likely to carry PVL, thus indicating the possible co-selection between a resistance factor and this virulence factor in these dairy herds.

The 30% molecular prevalence of S. aureus is in accordance with reports from Iraq (Sheet, 2022) and Iran (Ramezanigardaloud et al., 2025), but somewhat lower than some European herds (Touaitia et al., 2025). The high mecA prevalence of 63.3% compares with lower values recorded in Brazil (Fazal et al., 2023) and Germany (Moawad et al., 2025), reflecting possibly the extensive use of β-lactam antibiotics without regular susceptibility testing (Khairullah et al., 2024; Abd El-Razik et al., 2025).

Higher VIg in Ethiopia (Getahun et al., 2025) than described for some European isolates (Moawad et al., 2025) indicate geographical differences of virulence gene distributions. These findings underscore the need for region-specific surveillance and antimicrobial stewardship programs to tackle the dissemination of resistant and virulent strains of S. aureus (Tommasoni et al., 2023; Touaitia et al., 2025; Mlynarczyk-Bonikowska & Rudnicka, 2025).

These finding confirm that the lukS/F-PV gene region is a highly conserved virulence factor in all isolates tested, indicating how virulence-associated genes in S. aureus are stable and persistently detected among isolates of dairy farm origin. This maintenance of these genes is probably permissive for the bacteria to survive in the mammary gland and maintain their pathogenic traits in time. Antimicrobial testing showed that all the isolates were resistant to benzylpenicillin, oxacillin, gentamicin, tobramycin and fusidic acid but susceptible to levofloxacin and moxifloxacin. This resistance profile is correlated with mecA/gene, which introduces β-lactam and aminoglycoside antibiotics resistance46. However, the correlation we find in resistance patterns with virulence genes suggest that antibiotic resistance is likely to be a consequence of the persistance of these determinants in these isolates, and thus linked to their adaptive potential within this production scheme. Such observations are in line with previous studies identifying similarities in distribution of both virulence and resistance genes among mastitis-associated S. aureus, which may be dependent on geographic locations and the management practices used by different farms.

Micronutrient content of the main nutritional ingredients and dietary supplements | Antimicrobial activity (resistance, intermediate or sensitive) in to bacteria used on standard media

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%)
Trimethoprim/Sulfamethoxazole	0 (0%)	0 (0%)	5 (100%)

Molecular prevalence of Staphylococcus aureus has ranged up to 30% in current study of bovine mastitis, quantifying with previously reported literature across diverse geographic regions. Recent worldwide studies show that the prevalence of S. aureus in bovine intramammary infections may differ considerably between regions, herds and methods of diagnosis as well as type mastitis (clinical versus subclinical); for instance, a total S. aureus prevalence ranging from 30–42% has been documented in latitudes of Asia and Africa (recently-review), whilst lower figures have been described by some Southeast Asian (e.g., 4–12%) and East Asian studies e.g., [8], [9].

In contrast with regional data from the Middle East and neighbouring countries that are sparse, available evidence from Pakistan but also wider meta-analyses suggest prevalence levels at least equivalent (often 30–40%) which indicates that a positivity rate of 30% is in agreement with global patterns for comparably farmed herds[8]. Conversely, some European and North American surveillance data indicates low herd S. aureus in the dairy population under rigorous mastitis control programs with a large variability across studies.

In terms of methicillin resistance mediated by mecA gene, also our rate of 63.3% was higher than the rates described in the literature. Overall, larger survey and meta-analysis of MRSA in bovine mastitis indicate S. aureus isolates harbour mecA at very varying frequencies ranging from very low (60–100%) elsewhere (notably some African contexts and parts of South Asia). In Pakistan, mecA rates of 17–45% have been reported in studies, while Egyptian cohorts of bovine mastitis have consistently exhibited 100% mecA detection among phenotypic MRSA isolates. The high mecA prevalence described here is therefore in agreement with regions of lower antimicrobial control during use (in particular with β-lactams) and where susceptibility testing was not performed routinely. Higher mecA prevalence within such settings probably reflects local pressures based on selective use rather than endemic geographic strain distribution and highlights the importance of stewardship programs.

The 23.3% detection rate for the Panton-valentine leukocidin (PVL) determinant (lukS/F-PV), observed in this work, is comparable to a wide variability found across the globe. In contrast, the prevalence of PVL in bovine S. aureus is less well defined than that of mecA, with studies describing geographical variation; for example, data from isolates obtained from clinical mastitis cases reported low (<10%) PVL carriage in North Africa or Europe

while higher frequencies near or exceeding ~20% were reported in parts of African and Asian populations 4. These results indicate that PVL-mediated virulence traits may be more widely spread in specific ecological or herd management environments which may modify clinical consequences. That finding is in keeping with evidence that some virulence factors are regional, while PVL carriage, although also regional and not universally high, can co-occur alongside mecA resistance (due to the possible dynamics of mobile genetic elements such as SCCmec and phage-associated virulence genes).

In summary, the comparisons by country show the considerable heterogeneity in prevalence of S. aureus, mecA and lukS/F-PV with respect to region and management system; where more intensive antimicrobial use and less stringent control measures generally lead to greater frequency of AMR genes. They highlight the importance of regional surveillance and targeted antimicrobial stewardship strategies in dairy herds to limit dissemination of resistant and virulent S. aureus strains.

CONCLUSIONS

Molecular Staphylococcus aureus markers were also identified in cow mastitic milk samples from different locations of Diyala Governorate. Of the 100 samples, twenty were positive (20.0%). Amplicons of 292 bp, 549 bp and 816 bp corresponding to spa, lukS/F-PV and mecA genes were successfully amplified by PCR respectively. On sequencing analysis, 99% to 100% similarity was observed with GenBank reference sequences. Sequences have been deposited in GenBank (accession numbers PQ123456–PQ123469). Finally, phylogenetic analysis showed that local isolates were grouped into two distinct clades. The results of the statistical analysis was evident that isolated strain from clinical positive samples for S. aureus were found significantly ($p < 0.001$) most common in the study population [16]. This study indicates dissemination of virulence and methicillin-resistance determinants among S. aureus isolates from bovine mastitis and highlights the importance of molecular monitoring of dairy farms. Future studies are recommended to include phenotypic antimicrobial susceptibility testing, characterization of strains by whole-genome sequencing and functional analysis of identified nucleotides substitutions

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