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Molecular Detection and Characterization of Virulence and Antibiotic Resistance Genes in Clinical Isolates of *Staphylococcus aureus* Using PCR-Based Assays

Abeer Sami Kadhim[1]. Ahlam Ali soghi Al-Galebi[2*]
[1.2]Department of Biology, College of Education, University of Al-Qadisiyah, Iraq
abeer.sami@qu.edu.iq. ahlam.ali@qu.edu.iq
[*]Corresponding author email: ahlam.ali@qu.edu.iq

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Abstract **Background and Objective:** *Staphylococcus* (*S.*) *aureus* is a leading etiologic agent of sinusitis, and it is notorious for its multiple virulence factors and genes encoding antibiotic resistance. Molecular screening of those genes is important for elucidating its pathogenic role and for helping clinically effective intervention. In this study, the important virulence and resistance genes of *S. aureus* among the Staphylococcal Scalded Skin Syndrome patients was determined by PCR.

Methods: Out of 100 clinical specimens collected from patients with sinusitis, 48 strains were confirmed as *S. aureus* by both cultural media and biochemical tests and over VITEK 2 system. Genomic DNA was used for PCR to amplify the *16S rRNA* gene, *mecA*, *ermA*, *ermB*, and *ermC* (erythromycin resistance genes), and the *fnbA*, *fnbB*, *hlg* , and *tst* (virulence genes). The PCR products were examined by means of agarose gel electrophoresis.

Results: All 48 isolates were positive for the *16S rRNA* and *mecA* genes indicating identification of the bacterium and methicillin resistance. Furthermore, the macrolide resistance genes *ermA* and *ermC* were found in 50% of the isolates and the *ermB* gnen in 12.5%. Regarding virulence genes, *fnbA* was detected in 70.83%, *hlg and tst* in 66.66%, and *fnbB* in 50% of isolates.

Conclusion: Molecular characterization of *S. aureus* isolates from sinus infections uncovered a high prevalence of methicillin resistance and virulence factors, especially the *fnbA, hlg* , and *tst* genes. The PCR-based detection was proved to be efficient and dependable to provide rapid diagnosis of clinically important genes to the field of molecular epidemiological surveillance and antimicrobial stewardship.

Keywords: *Staphylococcus aureus*; MRSA; virulence genes; antibiotic resistance; PCR

Introduction

Staphylococcus aureus, a Gram-positive opportunistic pathogen, is a significant global health menace with the potential to cause a wide range of infections from hospital-acquired to community-acquired infections. It is a usual colonizer of skin and mucous membranes of humans and animals and is associated with a large number of clinical conditions, which vary from superficial impairments of the skin to severe diseases such as osteomyelitis, endocarditis, pneumonia, and septicemia, particularly in immunocompromised and hospitalized patients (1–3). The remarkable adaptability of the bacterium to environmental stress, escape from host immune responses and resistance to multiple antibiotics highlights its clinical significance.The emergence of methicillin-resistant *S. aureus* (MRSA) is a significant concern for *S. aureus* infection control. MRSA strains have become endemic worldwide and are closely related to available treatment options, longer hospitalization, more morbidity and mortality, and high cost of treatment (4–6). These strains present a special danger in hospitals, where they can start outbreaks and tend to be resistant to most of the antibiotics available, hindering efforts to control outbreaks and treat infections. StaphylococcuS. *aureus* mediates disease predominantly with an armamentarium of virulence factors. These factors include MSCRAMMs (microbial surface components recognizing adhesive matrix molecules) that allow the bacteria to adhere to host tissues, cytolytic toxins (or hemolysins) causing damage to host cells, and immune evasion products like protein A that disrupt the defense of the host (7–9). These processes are regulated by the global regulators accessory gene regulator (*agr*) and staphylococcal accessory regulator

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(*sarA*) systems controlling the switch from colonization to invasiveness (10–12). With regards to the expression of virulence factors, one of the best characterized is the gene coding for fibronectin-binding proteins A or B (*fnbA* and *fnbB*), involved in bacterial attachment to host tissues, the gene encoding gamma-hemolysin (*hlg*) with a cytotoxic activity, and the gene responsible for toxic shock syndrome toxin-1 (TSST-1) -producing, a superantigen associated with systemic inflammatory reactions and high lethality (13–15).In addition to its strong pathogenicity, *S. aureus* displays a broad spectrum of antibiotic resistance. The *mecA* gene that is carried of the staphylococcal cassette chromosome mec (SCCmec) is a major determinant of methicillin resistance. It produces an acquired penicillin-binding protein (PBP2a), which has a poor affinity for β-lactam drugs and hence does not respond to ordinary therapy (16,17). The macrolide-lincosamide-streptogramin B (MLSB) family of antibiotics is frequently inactivated through methylation of its target, the 23S rRNA, by erm genes, such as *ermA*, *ermB*, and *ermC*, which prevent antibiotic binding and cause cross-resistance within the MLSB group (18,19). These resistances are generally acquired by horizontal gene transfer, therefore, facilitating the rapid spread of multidrug-resistant *S. aureus*.In view of the emerging clinical complications due to *S. aureus* especially methicillin-resistant *S. aureus* (MRSA) resulting in treatment failure, prolonged hospital admission and higher mortality rates, rapid and accurate identification of its virulence and resistance markers are essential (20). These challenges have been reported in other countries as well and are attributed to the rise of multidrug resistant strains and limited treatment options (21). Traditional culture-based methods, while informative, are generally slow to renew and do not target particular resistance genes. Molecular methods including polymerase chain reaction (PCR) have gained an interest because of their specificity, sensitivity, and rapid turnaround time in identification of genetic resistance and virulence, which has hindered the development and spread of resistant strains (22–23). These tests are helpful for clinical diagnosis and are also important in epidemiological surveillance and infection control.Therefore, the aim of this study was to identify and identify the *mecA*, *ermA*, *ermB*, *ermC*, *fnbA*, *fnbB*, *hlg* , and *tst* genes, as well as to determine the antibiotic resistance of *S. aureus* strains isolated from patients with sinusitis in Al-Diwaniyah Governorate/Iraq. This study will contribute to providing information that can be used Specific primers were used to the virulence genes (*fnbA*, *fnbB*, *hlg* , and *tst*) and antibiotic resistance genes (*mecA*, *ermA*, *ermB*, and *ermC*) of *S. aureus* were selected from previously published studies based on validated gene sequences. The primer sequences, expected amplicon in making decisions regarding antibiotic use and infection control in hospitals.

Materials and Methods

Ethical Approval

The present study complies with the ethical requirements and biosafety regulations in the manipulation of clinical bacterial isolates and molecular diagnosis. The College of Education Ethics Committee, University of Al-Qadisiyah, Iraq, approved the present study in the year 2023 (Approval No. EDU/BIO/2023/018). All clinical isolates of *S. aureus* were obtained, and in the course of the study, standard protocols guaranteed the confidentiality and anonymity of patient information.

Sample Collection

One hundred clinical samples were collected from patients diagnosed with sinus infections at Al-Diwaniyah Teaching Hospital between September 2024 and March 2025. Nasal swab samples were obtained using sterile cotton swabs and were immediately transported to the Microbiology Laboratory, College of Education, University of Al-Qadisiyah, for microbiological analysis.

Isolation and Identification of *S. aureus*

Swabs were placed in 5 mL of Tryptic Soy Broth supplemented with Yeast Extract (TSB-YE) and incubated at 37 °C for 24 h. After incubation, the enriched samples were first cultured on Mannitol Salt Agar (MSA), followed by subculturing on Blood Agar; both media were obtained from Himedia, India. Suspected colonies were evaluated based on their colony morphology and Gram staining.

Additional confirmation was performed using the VITEK 2 Compact system (BioMérieux, France), an automated identification system that analyzes biochemical profiles of bacterial isolates via reagent cards containing multiple substrates. The system interprets colorimetric changes and compares them against a reference database to identify bacterial species with high accuracy (24).

Molecular identification of *S. aureus*

DNA Extraction

Genomic DNA was extracted from the 48 *S. aureus* isolates using the Presto™ gDNA Bacteria Kit (Geneaid, USA) following the manufacturer's instructions. DNA purity and concentration were measured using a Nanodrop spectrophotometer.

Primers used for identification of *S. aureus*

16SrRNA universal gene was used to identification of *S. aureus* are mentioned in Table (1).

Primers used for detection of resistant gene of *S. aureus*

sizes, annealing temperatures, and corresponding references are summarized in Table (1). All primers were synthesized by Bioneer (Korea) according to the cited sources.

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Table 1. Primer sequences, product sizes, annealing temperatures, and references used for PCR amplification of virulence and resistance genes in *S. aureus*

Gene		Primer	Product Size (bp)	Reference
16S rRNA	F (5′–3′)	AGAGTTTGATCCTGGCTCAG	1500	(25)
	R (5′–3′)	GGTTACCTTGTTACGACTT		
mecA	F (5′–3′)	AAAATCGATGGTAAAGGTTGGC	533	(26)
	R (5′–3′)	AGTTCTGCAGTACCGGATTTGC		
ermA	F (5′–3′)	GTTCAAGAAC AATCAATACA GAG	421	(27)
	R (5′–3′)	GGATCAGGAA AAGGACATTT TAC		
ermB	F (5′–3′)	CCGTTTACGA AATTGGAACA GGTAAAGGGC	359	(28)
	R (5′–3′)	GAATCGAGAC TTGAGTGTGC		
ermC	F (5′–3′)	GAGGCTCATAGACGAAGAAA	375	(29)
	R (5′–3′)	AAGTTCCCAAATTCGAGTAA		
Hlg	F (5′–3′)	TTGGCTGGGGAGTTGAAGCACA	306	(30)
	R (5′–3′)	CGCCTGCCCAGTAGAAGCCATT		
fnbA	F (5′–3′)	CACAACCAGCAAATATAG	1362	(31)
	R (5′–3′)	CTGTGTGGTAATCAATGTC		
fnbB	F (5′–3′)	CAGAAGTACCAAGCGAGCCGGAAA	258	(30)
	R (5′–3′)	CGAACAACATGCCGTTGTTTGTTGA		
Tst	F (5′–3′)	AGCCCTGCTTTTACAAAAGGGGAAAA	306	(31)
	R (5′–3′)	CCAATAACCACCCGTTTTATCGCTTG		

PCR Amplification

PCR reactions were conducted for the 48 DNA samples using AccuPower® PCR PreMix (Bioneer, Korea). Each 25 µL reaction mixture contained 5 µL DNA template, 1.5 µL of each primer (10 pmol), 12 µL PCR master mix and 5 µL nuclease-free water to complete the final volume. The thermocycler was programmed as follows (Table 2 and 3):

Table 2. PCR cycling conditions (denaturation, annealing, extension) for amplification of target genes in *S. aureus*

Step	Temperature	Time	Cycles
Initial denaturation	95°C	3 min	1
Denaturation	95°C	30 sec	30
Annealing	50–60°C*	30 sec	
Extension	72°C	60–90 sec	
Final extension	72°C	5 min	1
Hold	4°C	∞	

Results

Classical Identification of *Staphylococcus aureus*

Out of 100 clinical isolates of patients diagnosed with sinus infections, 48 isolates were confirmed as *S. aureus* on the basis of conventional microbiological procedures. This entailed blood agar and mannitol salt agar cultures, colony morphologies and identification using the VITEK 2 Compact system (Biomerieux, France).

Table 3: The Annealing temperatures varied by primer

Gene	Product Size (bp)	Annealing Temp
16S rRNA	1500	55°C
mecA	533	55°C
ermA	421	55°C
ermB	359	50.2°C
ermC	375	52.4°C
fnbA	1362	50°C
fnbB	258	55°C
Hlg	306	55°C
Tst	306	60°C

Agarose Gel Electrophoresis

Products were separated on 1.5% TBE agarose gels by electrophoresis followed by ethidium bromide staining. 0.5 µg/mL ethidium bromide was supplemented to the gel for DNA visualization. 100 bp DNA ladder was used as the molecular weight marker. Electrophoresis was performed at 100 V for 60 min and the gel was observed under a UV illuminator at 300 nm with UV light.

Detection of the *16S rRNA* Gene

The *16S rRNA* gene was used as a universal bacterial confirmation marker. All 48 isolates showed clear amplification at ~1500 bp.

Detection rate: 100% (48/48)

This confirms the identity of the isolates as bacterial, and PCR conditions were optimal for DNA amplification (Figure 1).

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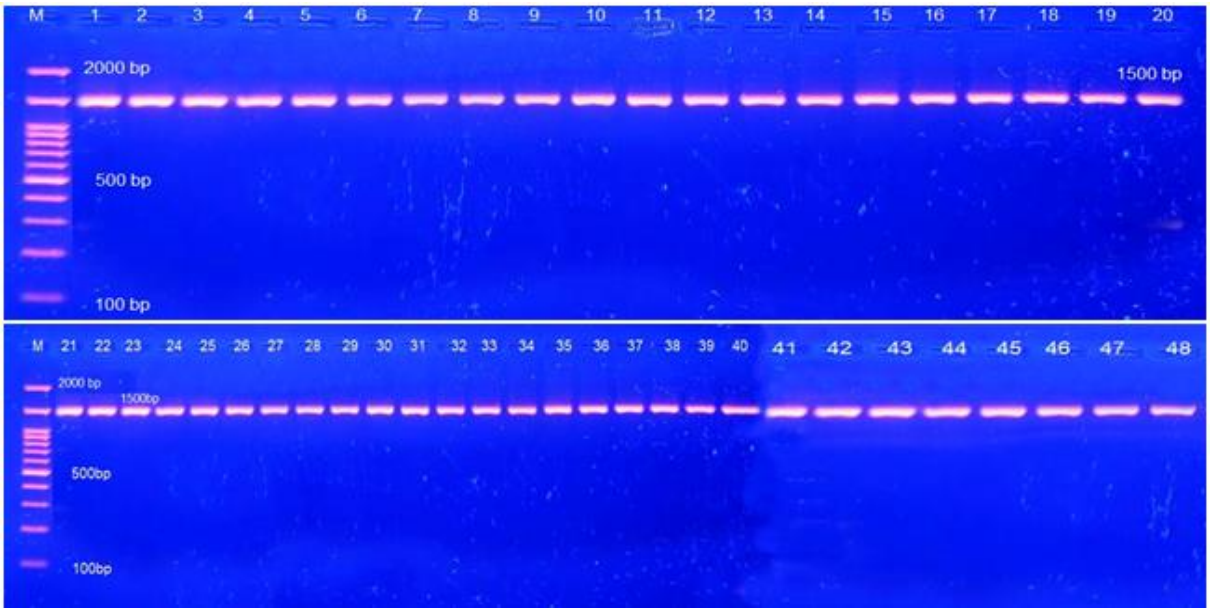

Figure 1: Amplification products of 16S rRNA gene of *S. aureus* isolates on 1.5% agarose gel at 1500 bp

Detection of Antibiotic Resistance Genes

The antibiotic resistance genes of the 48 *S. aureus* isolates were detected by polymerase chain reaction (PCR). All strains were positive for the *mecA* gene and therefore methicillin-resistant *S. aureus* (MRSA). *ErmA* and *ErmC* genes associated with ribosomal methylation of macrolide gave positive reactions to 24 isolates (n = 50%) each. On the opposite, the *ermB* gene was found in only 6 strains (12.5%) compared to the analyzed population (Table 4). These results underscore the prevalence of methicillin resistance and the difference in macrolide resistance gene distribution between isolates.

Table (4): Number and percentage of resistance genes of *S. aureus* isolates from sinusitis cases

Gene	No. of Isolates	No. of Positive Isolates	Detection Rate (%)
mecA	48	48	100
ermA	48	24	50
ermB	48	6	12.5
ermC	48	24	50

Detection of the *mecA* gene

The *mecA* gene responsible for methicillin resistance was detected in all isolates, producing a 533 bp band (Figure 2).

Detection rate: 100% (48/48)
This indicates that all *S. aureus* isolates are MRSA.

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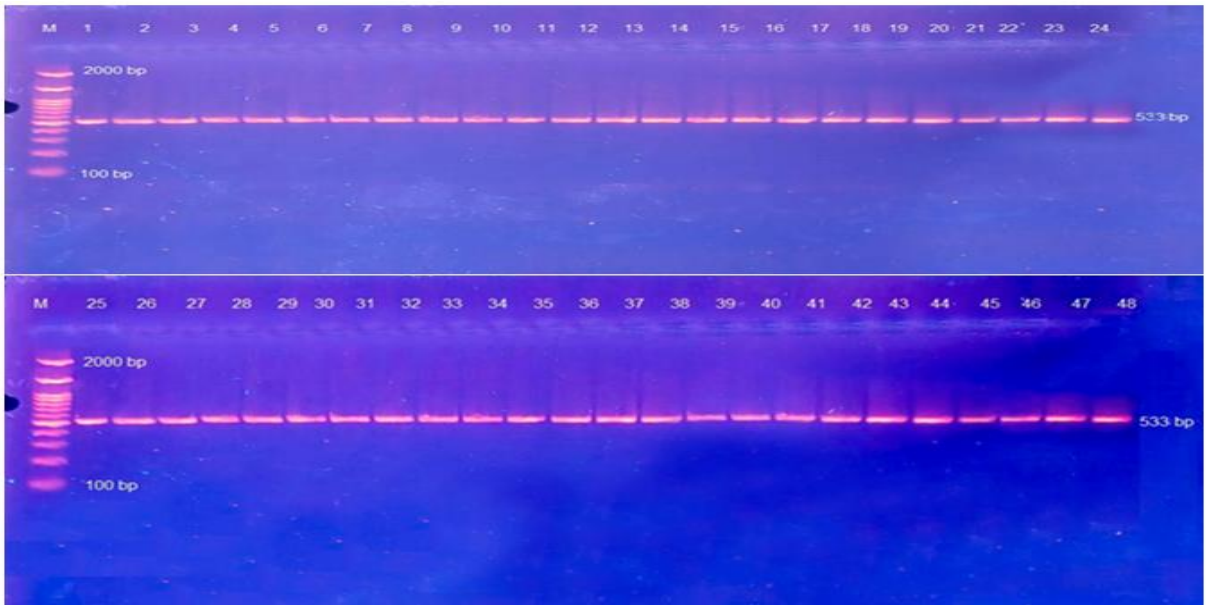

Figure 2: Amplification products of *mecA* gene of *S. aureus* isolates on 1.5% agarose gel at 533 bp

Detection of the *ermA* gene

Amplification of the *ermA* gene yielded a 190 bp band in 24 of the 48 isolates (Figure 3).

Detection rate: 50% (24/48)

This gene confers macrolide resistance through rRNA methylation.

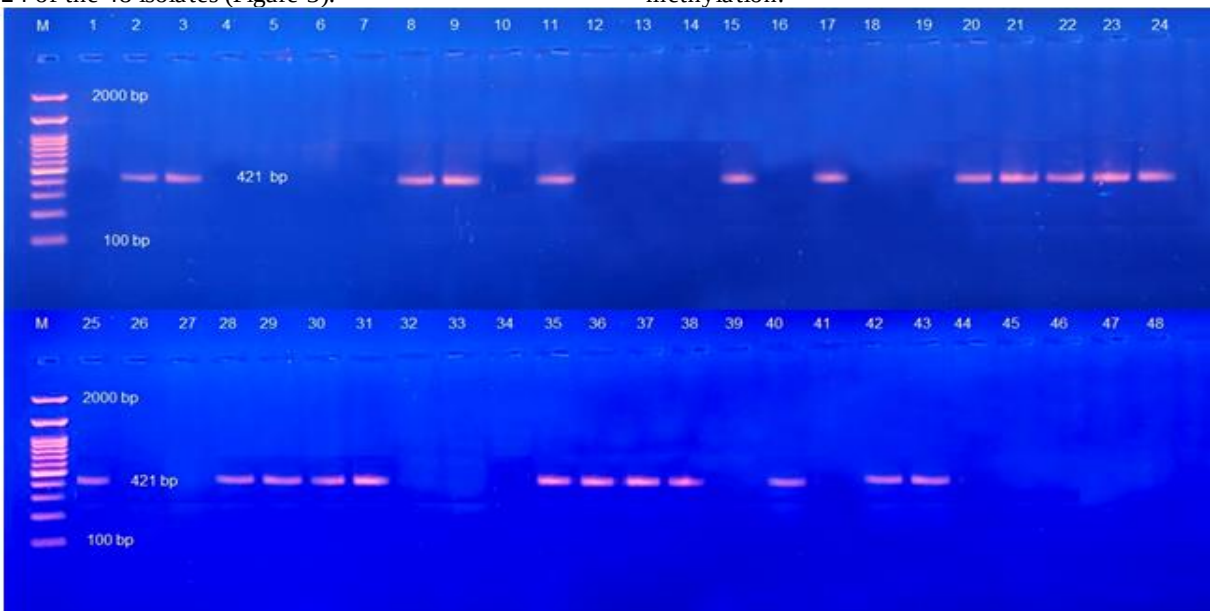

Figure 3: Amplification products of *ermA* gene of *S. aureus* isolates on 1.5% agarose gel at 421 bp

Detection of the *ermB* Gene (ermB414)

Only 6 isolates showed positive bands (~639 bp) for the *ermB* gene (Figure 4).

Detection rate: 12.5% (6/48)

Low distribution suggests limited dissemination of this resistance determinant in the population.

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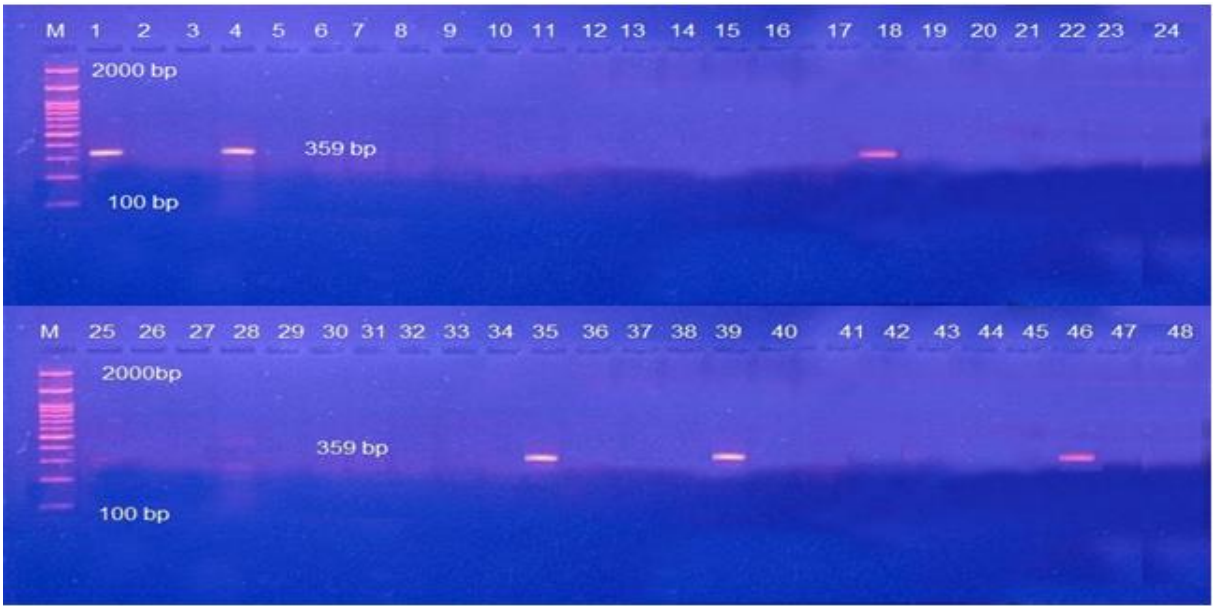

Figure 4: Amplification products of *ermB* gene of *S. aureus* isolates on 1.5% agarose gel at 359 bp

Detection of *ermC* Gene (ermC375)

The *ermC* gene was present in 24 isolates, with PCR product ~375 bp (Figure 5).

Detection rate: 50% (24/48)

Another common erythromycin resistance gene with moderate spread.

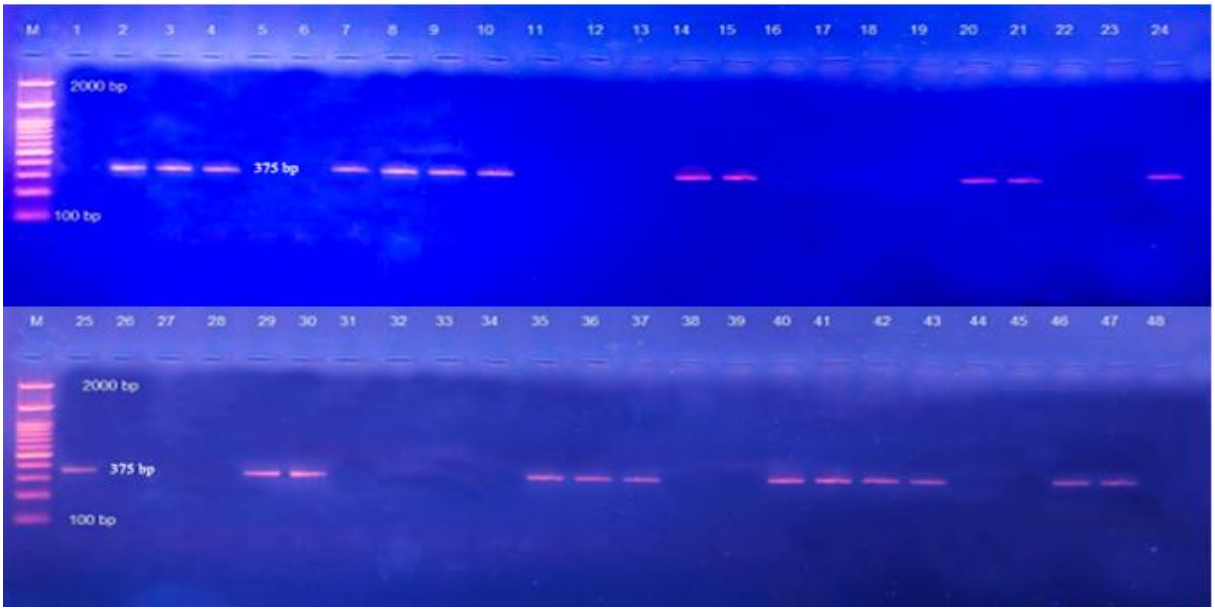

Figure 5: Amplification products of *ermC* gene of *S. aureus* isolates on 1.5% agarose gel at 375 bp

Detection of virulence genes of S. *aureus*

PCR analysis revealed variable distribution of virulence genes among the 48 *S. aureus* isolates. The *fnbA* gene, responsible for fibronectin-binding and host adhesion, showed the highest prevalence at 70.83%. Both the *hlg* gene, encoding gamma-hemolysin, and the *tst* gene, encoding toxic shock syndrome toxin-1, were detected in 66.66% of the isolates. The *fnbB* gene, involved in extracellular matrix attachment, was present in 50% of isolates (Table 5). These findings indicate a significant

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presence of virulence determinants contributing to pathogenicity and immune evasion among the studied strains.

Table 5. Number and percentage of virulence genes of *S. aureus* isolates from sinusitis cases

Gene	No. of Isolates	No. of Positive Isolates	Detection Rate (%)
hlg	48	32	66.66%
fnbA	48	34	70.83%
fnbB	48	24	50%
tst	48	32	66.66%

Detection of *hlg* Gene

The *hlg* gene, coding for gamma-hemolysin, was detected in 32 isolates with product size 306 bp (Figure 6). Detection rate: 66.66% (32/48), This virulence factor contributes to cytotoxicity and immune evasion.

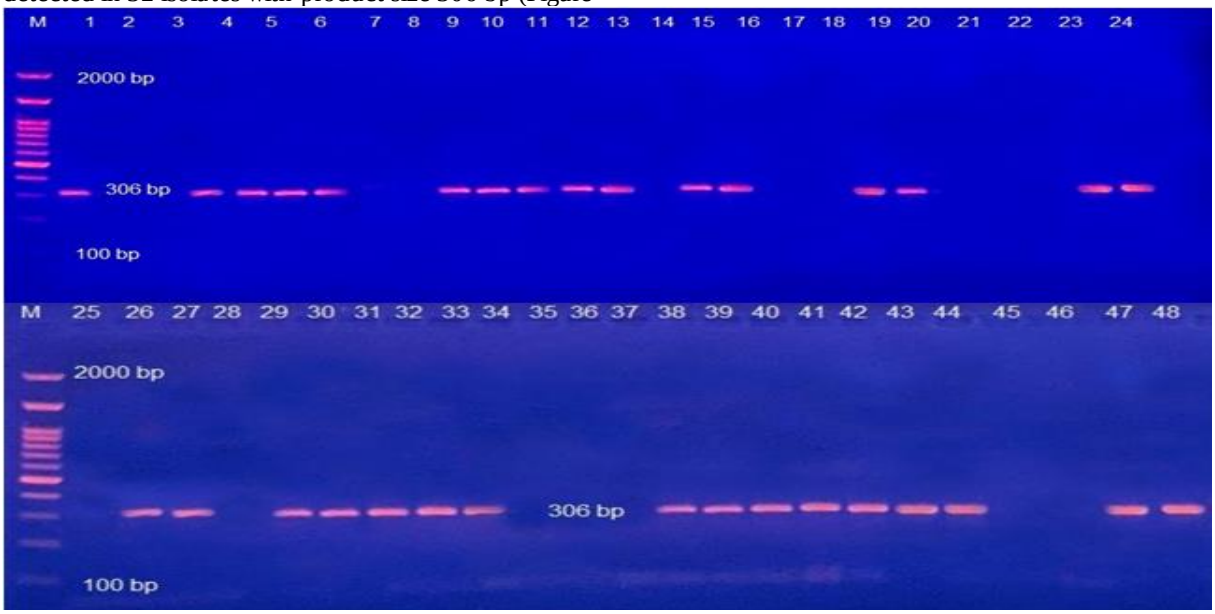

Figure 6: Amplification products of *hlg gene* of *S. aureus* isolates on 1.5% agarose gel at 306 bp

Detection of *fnbA* Gene

PCR analysis showed amplification of the *fnbA* gene (~1362 bp) in 34 isolates (figure 7). Detection rate: 70.83% (34/48), Encodes fibronectin-binding protein A, involved in host tissue adhesion.

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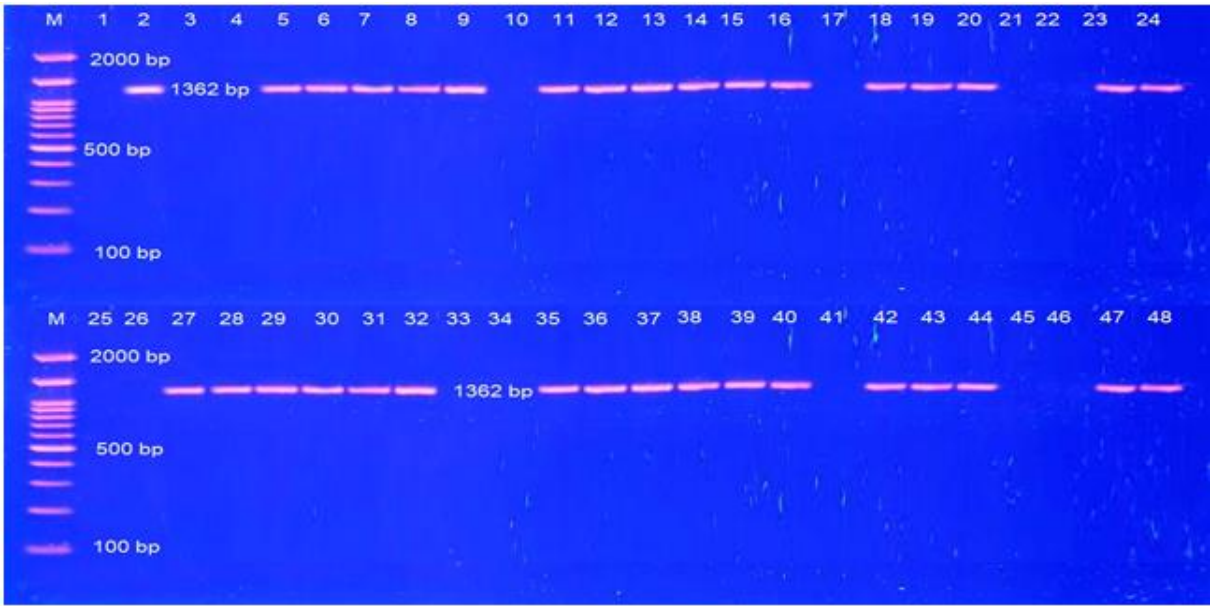
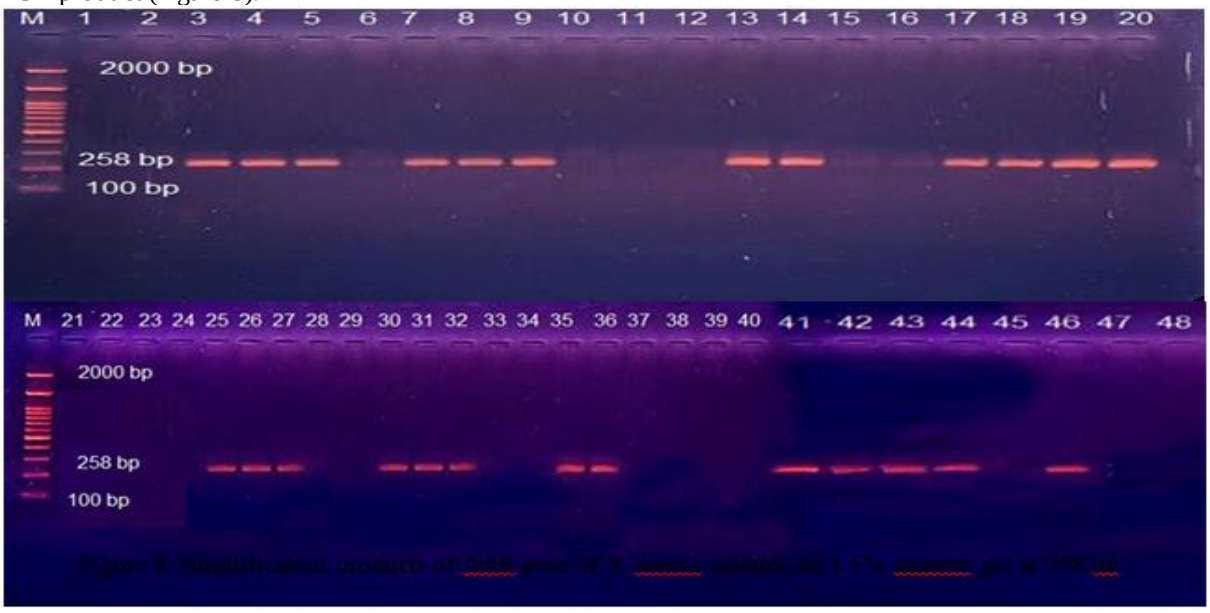


Figure 7: Amplification products of *fnbA* gene of *S. aureus* isolates on 1.5% agarose gel at 1362 bp

Detection of *fnbB* Gene

The *fnbB* gene was detected in 24 isolates with 258 bp PCR product (Figure 8).

Detection rate: 50% (24/48), Supports bacterial colonization through extracellular matrix attachment.



9. Detection of *tst* Gene (Listed as tss in results)

The *tst* gene (toxic shock syndrome toxin-1) was detected in 32 isolates (~306 bp) (Figure 9).

Detection rate: 66.66% (32/48)

Indicates significant potential for toxin production among isolates.

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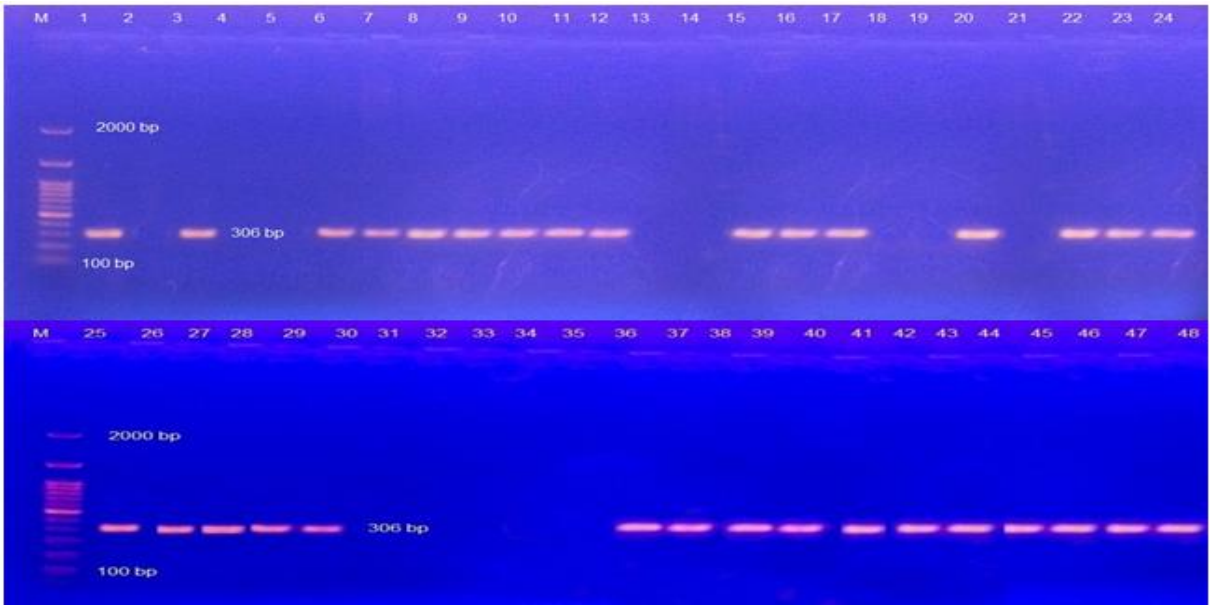

Figure 9: Amplification products of *tst gene* of *S. aureus* isolates on 1.5% agarose gel at 306 bp

Discussion

The molecular characterization of *S. aureus* isolates from sinus infection cases in this study revealed a high prevalence of both antibiotic resistance and virulence-associated genes, underscoring the clinical significance of this pathogen. All 48 isolates were confirmed as bacterial through successful amplification of the 16S rRNA gene, validating both the identity of the isolates and the quality of DNA extraction.Notably, the *mecA* gene was detected in 100% of the isolates, confirming that all strains were methicillin-resistant *S. aureus* (MRSA). The *mecA* gene encodes penicillin-binding protein 2a (PBP2a), which has a low affinity for β-lactam antibiotics, and is carried on the SCCmec mobile genetic element that facilitates horizontal gene transfer (32).These findings align with previous regional studies conducted in Saudi Arabia and Lebanon, where similarly high detection rates of *mecA* were observed in clinical MRSA isolates (33,34). In Iraq, Rahma and Jwher (2024) reported comparable results in a study conducted in Nineveh Governorate, where *mecA* and the virulence genes *clfA*, *clfB*, and *coa* were detected in MRSA strains isolated from the nasal cavities of cows, buffaloes, and their human handlers. This underscores the epidemiological overlap between human and animal reservoirs, and highlights the zoonotic potential of MRSA strains circulating in the region (35).The 100% *mecA* detection rate in the present study may be attributed to extensive β-lactam use in clinical practice, or possibly the spread of a dominant local MRSA clone. Compared to international studies reporting *mecA* prevalence between 70%–90%, the complete presence of *mecA* here suggests a localized pattern of resistance that warrants ongoing molecular surveillance.Resistance to macrolides was mainly due to the presence of *ermA* and *ermC* genes, which were observed in 50% of the MRSA strains. The *ermB* gene, on the other hand, was found in 12.5% of the isolates. This prevalence correlates with that observed worldwide in which *ermA* and *ermC* are the resistance genes most frequently found in human *S. aureus* isolates. They contain genes that confer methylation of the 23S rRNA molecule, resulting in a resistance to macrolides, lincosamides and streptogramin B antibiotics (MLSB phenotype) (36).Higher *ermB* may be considered as it is more associated with enterococci and animal-derived staphylococci than with clinical human MRSA strains (37). Similar results were also shown by Athliamai et al., who revealed that *ermA* and *ermC* predominated over *ermB* in MRSA strains from Lebanese hospitals (38).The simultaneous presence of *ermA* and *ermC* in various strains may be suggestive of clonal spread or the transfer of multiple resistance determinants by horizontal gene transfer, and suggests that molecular surveillance is necessary in the clinical setting.Among the investigated virulence genes, *fnbA* was the most frequently detected, appearing in 70.83% of the MRSA isolates. This gene encodes fibronectin-binding protein A, a critical adhesin that facilitates bacterial adherence to host extracellular matrix components and promotes colonization and invasion (39). The genes *hlg* (encoding gamma-hemolysin) and *tst* (encoding toxic shock syndrome toxin-1) were each detected in 66.66% of isolates, indicating their substantial contribution to cytotoxicity and immune evasion. The *fnbB* gene was found in 50% of isolates, further supporting the bacterial capacity for tissue adhesion and persistence.The co-expression of

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fnbA and *fnbB* has been strongly linked to enhanced biofilm formation, as these adhesins bind fibronectin and fibrinogen, allowing the bacteria to persist within host tissues (40). Supporting this, den Reijer et al. demonstrated that mutant strains lacking *fnbA* and *fnbB* exhibited significantly reduced virulence in experimental models of endocarditis and septic arthritis (41).A comparable investigation was carried out by Rahma and Jwher (2024), who examined MRSA isolates from the nasal cavities of cows, buffaloes, and their human breeders in Nineveh Governorate, Iraq. Their study reported a high isolation rate of MRSA, and detected several virulence genes, including *clfA*, *clfB*, and *coa*, which are functionally similar to *fnbA/B* in mediating adherence and immune modulation. The fact that those isolates were obtained from humans with occupational animal exposure reinforces the zoonotic dimension of MRSA and the potential overlap in virulence gene profiles between human and animal reservoirs (35).Taken together, the present findings emphasize the virulence potential of MRSA isolates in sinusitis cases, not only through classical cytotoxins such as *hlg* and *tst* , but also via potent adhesins like *fnbA* and *fnbB*, which enhance colonization and may contribute to treatment resistance through biofilm-associated persistence.Gamma-hemolysin encoding gene *hlg* was found in around two-third of isolates. This two componentize toxins causes hemolysis of red and white blood cells, tissue destruction and evasion of the immune system. The moderate to high frequency of this gene is in line with the result of clinical isolates reported in Africa and Asia (42). The participation of the toxin in sinus infections is especially remarkable because it will disrupt the epithelial barrier and potentiate the invasion of subjacent tissues.The *tst* gene coding toxic shock syndrome toxin-1 (TSST-1) was found in 66.66% of the isolates which was relatively high percentage. TSST-1 functions as a superantigen, inducing the overproduction of cytokines and leading to toxic shock syndrome. This gene is also commonly situated on pathogenicity islands and is often found with strains that cause serious systemic infections (43). The high incidence of *tst* in sinus isolates is worrisome for the invasive potential of these isolates and reinforces the need for routine genotyping in laboratories.The occurrence of several virulence and resistance determinants in a high fraction of isolates indicates genomic instability, and possibly implies participation of mobile genetic elements that can allow horizontal transfer of these traits. Strains harboring both *mecA* and *tst* genes have been reported to be associated with outbreaks and severe clinical conditions (44). This genetic overlap was suggested to be likely responsible for the predominance of highly virulent MRSA e isolates in the study population.The prevalence of these genes across isolates demonstrates a complex interplay between evolutionary pressure, antibiotic misuse, and strain selection. Both environmental and clinical sources may contribute to the ongoing existence of these strains, as comparative genomic analysis from food, livestock and human *S. aureus* has revealed (45). Additionally, PCR detection of these genes is a time-efficient, sensitive and inexpensive method for guiding clinical care and antimicrobial stewardship, especially in regions with a high prevalence of MRSA (46).

Conclusion

This study revealed a high prevalence of both virulence and antibiotic resistance genes in *Staphylococcus aureus* isolates from sinusitis patients. The universal detection of *mecA* confirms the widespread presence of MRSA, while the frequent detection of *ermA* and *ermC* indicates notable macrolide resistance. Additionally, the high rates of fnbA, *hlg* , and *tst* suggest significant pathogenic potential through adhesion, cytotoxicity, and toxin production. These findings underscore the need for routine molecular surveillance to guide effective therapy and monitor local resistance trends, especially given the risk of complications and treatment failure associated with multidrug-resistant, highly virulent strains.

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Conflict of interest

No conflict of interest is disclosed by the authors.

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