

Original Article

# Evaluation of virulence factors among *Staphylococcus aureus* strains isolated from patients with urinary tract infection in Al-Najaf Al-Ashraf teaching hospital



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## ABSTRACT

*Staphylococcus aureus* contains numerous surface proteins called microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) that mediate binding to host tissues and initiate colonization leading to infection. Virulence genes such as enzymes, toxins, adhesin proteins, cell surface proteins play an important role in the pathogenicity of *S. aureus* strains. The distribution and prevalence of virulence genes vary among *S. aureus* strains in different regions. However, the highest frequency of virulence genes among *S. aureus* strains is related to toxin genes. There are many PCR methods for detecting these microorganisms such as conventional PCR, multiplex PCR, reverse transcription PCR (RT-PCR), and quantitative PCR (qPCR). Therefore, this study aimed to investigate the presence of virulence genes among methicillin-resistant *S. aureus* (MRSA) strains. In this study, multiplex PCR technique was applied to determine the presence of virulence genes among MRSA strains. Results showed the frequency of virulence genes among bacterial strains isolated from Al-Najaf Al-Ashraf teaching hospital. In addition, among the strains, *hla* gene with 91% frequency, exhibited the highest prevalence among pathogenic genes. *Sea*, *mecA*, *clfB*, *femA*, *fnbB*, *tsst*, *hly* genes with 88%, 65%, 54%, 45%, 39%, 27% and 13% were in the next ranks, respectively. This investigation showed *mecA* is a gene found in bacterial cells that allows them to be resistant to antibiotics such as methicillin and other penicillin-like antibiotics.

## 1. Introduction

Application of new methods to detect bacterial strains specifically antibiotic-resistant strains is a critical affair for finding effective ways to hinder and inactivate these microorganisms [1-4]. The antibiotic-resistance property of *Staphylococcus aureus* can be more complicated in the case of health-threatening bacterial infections [5, 6]. belongs to the Micrococcaceae family and is a member of the *Staphylococcus* genus, which contains more than 30 species including *S. epidermidis*, *S. saprophyticus* and *S. haemolyticus* [7]. Among *staphylococcal* species, *S. aureus* is the

most pathogenic to humans. *S. aureus* is one of the most important pathogenic bacteria that the anterior part of the nose is the source of this bacterium in adults and children. However, the prevalence of *S. aureus* nasal carriers varies in different regions [8].

Researchers have found that *S. aureus* is a major cause of lower respiratory tract infections and skin and soft tissue infections in all study areas [9]. *Staphylococcus aureus* is often responsible for toxin-mediated diseases such as toxic shock syndrome, and foodborne illness (SFD). This bacterium contains numerous surface proteins called microbial

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surface components recognizing adhesive matrix molecules (MSCRAMMs) that mediate binding to host tissues and initiate colonization leading to infection [10]. The FnBPA and FnBPB are involved in binding cells to an extracellular matrix component, fibronectin, and plasma clots. Clumping factors (*clfA* and *clfB*) mediate the clumping and attachment of bacterial cells to fibrinogen in the presence of fibronectin [11, 12].

Panton Valentine Leukocidin (*pvl*) is a prophage-encoded toxin that is associated with approximately 5% of *S. aureus* isolates and causes necrotic pneumonia and skin infections [13]. Protein A is a determinant of *S. aureus* that is encoded by the *spa* gene and is a cell wall-associated protein that binds to the Fc region of immunoglobulin G [14]. The  $\alpha$ -toxin is the most important cytolytic agent released by *S. aureus* and the first bacterial exotoxin known to form a pore. Alpha hemolysin, encoded by the *hla* gene, enters the membrane of eukaryotic cells to form barrel-shaped pores that cause osmotic cytolysis [15].

The  $\delta$ -toxin is a small amphipathic secretory peptide and a member of phenol-soluble modules (PSMs) that are produced only by members of the genus *Staphylococcus* [16]. All *S. aureus* have the *hld* gene and two loci for encoding the PSM  $\alpha$  and *psm $\beta$*  genes. PSMs play various roles in the pathogenesis of *S. aureus*, including facilitating biofilm diffusion, cytolytic activity, and pro-inflammatory activity [17]. *S. aureus* produces another group of exotoxins, which include toxic shock syndrome toxin 1 (TSST-1), *staphylococcal* enterotoxins including *sea*, *seb*, *sec*, *sed*, *see* and exfoliative toxins (*eta* and *etb*). These exotoxins are known to be potent immune-stimulating proteins involved in gastroenteritis and toxic shock syndrome [18, 19].

High pathogenicity of *S. aureus* causes recurrent nosocomial and community-related infections, so rapid isolation and identification of these strains are very important for timely treatment. Virulence genes play an important role in the pathogenicity of *S. aureus* strains (Figure 1) [20]. These virulence agents include enzymes, toxins, adhesin proteins, cell

surface proteins that attach to tissue and contribute to the formation of biofilms, many toxins and enzymes are involved in the spread and colonization of bacteria, tissue damage, and lysis of host cells [21].

Numerous reports indicate that the distribution and prevalence of virulence genes vary among *S. aureus* strains in different regions. However, the highest frequency of virulence genes among *S. aureus* strains is related to toxin genes [22-24]. Therefore, by identifying and determining virulence factors among the mentioned strains, the widespread prevalence of isolates with high-frequency pathogenic genes in different hospital wards can be prevented.

With the rapid development of molecular biology, polymerase chain reaction (PCR) has become a method for the rapid detection of *S. aureus*. Compared to traditional diagnostic methods, PCR technology is a faster, more efficient and more sensitive method. However, there are drawbacks. DNA must be extracted in this technology, and when PCR is used to detect *S. aureus*, it cannot detect living or dead cells, so the result may be false positive [25]. Thus, the results are prone to interference. There are many PCR methods for detecting foodborne microorganisms: conventional PCR, multiplex PCR, reverse transcription PCR (RT-PCR), quantitative PCR (qPCR) and so on. This study aimed to investigate the presence of virulence genes among methicillin-resistant *S. aureus* (MRSA) strains by multiplex PCR.

## 2. Materials and methods

### 2.1 Sample collection

In this study, clinical *S. aureus* samples (100 strains) were collected from hospitalized patients at an Iraq, Al-Najaf Al-Ashraf teaching hospital in 2021. Clinical specimens were isolated from throat, blood, wound, urine. Bacterial strains were identified using biochemical tests and conventional methods such as hemolysis and gram staining and it was determined that all strains belong to *S. aureus*.

### 2.2 Preparation of primers

To investigate the presence of virulence genes (*sea*, *clfB*, *femA*, *hla*, *hly*, *mecA*, *fnbB*,

tsst), multiplex polymerase chain reaction (multiplex PCR) was used [26]. The specific primers will be used to amplify each of the desired genes. For this purpose, primers used in previous studies were used to perform this experiment.

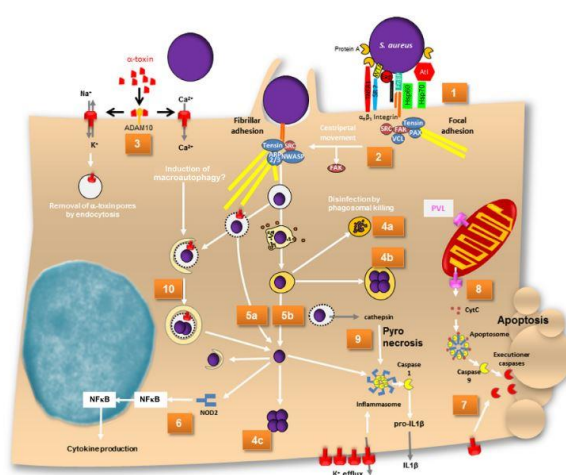
Table 1 shows the sequence of primers along with the size of each. The genomic DNA was extracted from *S. aureus* strains using a DNA extraction kit (Sigma-Aldrich). Because PCR reaction is a fast and reliable technique in which several genes are identified and amplified simultaneously using specific primers, it will reduce costs, time and energy. In order to perform the multiplex PCR reaction, specific primers related to virulence genes whose annealing temperatures were in the same range were grouped.

For each group of virulence genes, the reaction mixtures were Mastermix (9 µl), F primer (1 µl), R primer (1 µl), template DNA (1.5 µl), and water (12.5 µl). For this purpose, the reaction mixture of each group of virulence genes was added to sterile microtubes and after adjusting the temperature and time of the reaction, PCR was performed. After PCR amplification, PCR products were electrophoresed on 1 % (w/v) agarose gel in 1X TBE buffer for 60 min (Tables 2 and 3).

### 3. Results

In this study, 100 strains of *S. aureus* isolated from clinical samples were used in Al-Najaf Al-Ashraf teaching hospital, Iraq. Identification of clinical specimens using biochemical tests and conventional methods such as hemolysis and gram staining showed that all strains belonged to *S. aureus*. As shown in figure 2, most strains were isolated from the throat and wound, which accounted for 43 % and 36 % of the samples in each group, respectively.

Figure 2 shows the frequency of virulence genes among *S. aureus* strains isolated from Al-Najaf Al-Ashraf teaching hospital. Among the strains, *hla* gene with 91% frequency, showed the highest prevalence among pathogenic genes. *Sea*, *mecA*, *clfB*, *femA*, *fnbB*, *tsst*, *hly* genes with 88%, 65%, 54%, 45%, 39%, 27% and 13% were in the next ranks, respectively. Table 4 shows the frequency of virulence genes among clinical strains isolated from different parts of the body. As can be seen, among the strains isolated from the Al-Najaf Al-Ashraf teaching hospital, the prevalence of *hly* gene (61.5%) in wound samples is higher. In general, the frequency of virulence genes in samples isolated from the throat was higher than in other regions.



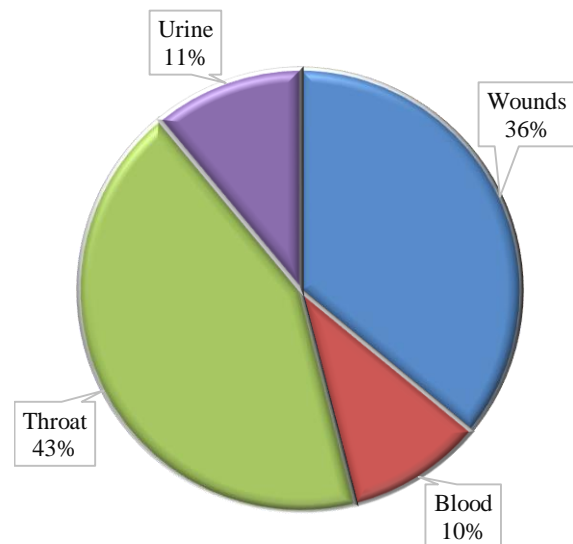
**Fig. 1.** Pathogenesis mechanisms of *S. aureus*. Copyright under the terms of the Creative Commons Attribution Non- Commercial License [27]. This figure is under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

**Table 1.** The sequence of primers with related sizes.

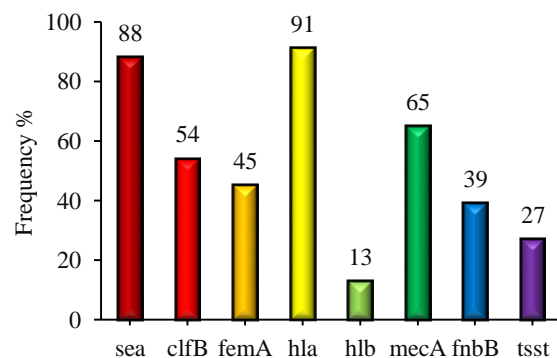
primer	Sequence	Size (bp)	reference
<i>clfB</i> -F	TGC AAG ATC AAA CTG TTC CT	596	[28]
<i>clfB</i> -R	TCG GTC TGT AAA TAA AGG TA		
<i>femA</i> -F	CTT ACT TAC TGC ACC	648	[28]
<i>femA</i> -R	ATC TCG CTT GTG TGC		
<i>hla</i> -F	CTG ATT ATC CAA GAA ATT CGA TTG	209	[29]
<i>hla</i> -R	CTT TCC AGC CTA CTT TTT TAT CAG T		
<i>hly</i> -F	GTG CAC TTA CTG ACA ATA GTG C	309	[30]
<i>hly</i> -R	GTT GAT GAG TAG CTA CCT TCA GT		
<i>mecA</i> -F	GTG AAG ATA TAC CAA GTG ATT	147	[31]
<i>mecA</i> -R	ATG CGC TAT AGA TTG AAA GGA T		
<i>sea</i> -F	GGT TAT CAA TGT GCG GGT GG	102	[26]
<i>sea</i> -R	CGG CAC TTT TTT CTC TTC GG		
<i>fnbB</i> -F	GGA GAA GGA ATT AAG GCG	813	[28]
<i>fnbB</i> -R	GCC GTC GCC TTG AGC GT		
<i>tsst</i> -F	ACC CCT GTT TTA TCA TC	329	[24]
<i>tsst</i> -R	TTT TCA TTT GTA GC		

**Table 2.** PCR program for genes (*clfB*, *hly*, *hla*, *fem*)

Stage	Time (s)	Temperature (°C)	Cycle
Denaturation	300	94	1
Denaturation	30	95	35
Annealing	45	52	
Elongation	60	72	
Final Elongation	350	72	1

**Fig. 2.** Frequency of *S. aureus* isolates in clinical specimens**Table 3.** PCR program for genes (*sea*, *mecA*, *fnbB*, *tsst*)

Stage	Time (s)	Temperature (°C)	Cycle
Denaturation	300	94	1
Denaturation	30	95	35
Annealing	45	56	
Elongation	60	72	
Final Elongation	350	72	1

**Fig. 3.** Percentage of virulence genes among *S. aureus* strains



**Table 4.** Percentage of virulence genes in *S. aureus* strains isolated from different organs of the body

Samples	sea	clfB	femA	hla	hly	mecA	fmbB	tsst
Wounds	34.1	40.7	37.8	37.4	61.5	32.3	33.3	29.6
Blood	11.4	7.4	13.3	9.9	0.0	10.8	7.7	7.4
Throat	42.0	42.6	42.2	44.0	30.8	44.6	46.2	51.9
Urine	12.5	9.3	6.7	8.8	7.7	12.3	12.8	11.1

#### 4. Discussion

*S. aureus* enterotoxins (SEs) belong to a large family of pyrogenic toxin superantigens that cause emetic. Classical SEs (sea, seb, sec, sed, and see) have been found in studies of *S. aureus* species involved in the prevalence of staphylococcal food poisoning (SFP) and are classified into distinct serological types. In the present study, the highest prevalence of virulence genes was related to *Sea* gene, which showed 88% frequency among bacterial samples isolated from patients. In the report of Kamarehei et al (2013) in Iran, the frequency of the mentioned gene among *S. aureus* strains was determined to be about 50.4%, respectively [32].

In the above report, the prevalence of this gene was relatively high, however, their frequency was lower than the results obtained in our study. The product of this gene is the main cause of toxic shock syndrome toxin 1 (tsst-1), which is produced in approximately 20% of *S. aureus* isolates. Thus, the production of this toxin is a variable genetic trait or trait. It is known as an acute illness that is associated with symptoms such as fever, skin rash, high blood pressure and dysfunction of multiple systems [33]. In the present study, the frequency of this gene among *S. aureus* strains was about 27%. Nagao et al. (Japan) found that the prevalence of this gene among 152 MRSA strains was about 75% [34].

Xie et al. estimated the frequency of this gene among 108 *S. aureus* strains isolated from 16 different hospitals in 14 provinces of China to be about 48.1%, which was the most common virulence gene among the studied genes [35]. In the study of Pakbaz et al., the frequency of the mentioned gene among 98 samples was obtained from Tehran University of Medical Sciences, a total of 21 strains containing *tsst* gene [36].

In most of the above reports, the expression of *tsst* gene in *S. aureus* strains was higher than in the present study. *mecA* is a gene found in bacterial cells that allows them to be resistant to antibiotics such as methicillin and other penicillin-like antibiotics. *mecA* is involved in the coding of PBP2A protein (a transpeptidase that promotes bacterial cell wall formation) and resistance to some antibiotics [37]. In the present study, the frequency of *mecA* gene among samples was about 65%. The frequency of this gene in the studies of Galdiero et al (Italy-2003) among 43 MRSA strains was estimated to be about 46.5% [38].

In a report published by Choi et al, the frequency of this gene among coagulase-negative *S. aureus* and MRSA was determined to be 98% and 81%, respectively [39]. In the study of Askari et al. the prevalence of this gene among 2690 strains was estimated to be about 52.7% [40]. The prevalence of *mecA* gene in the above reports was different from the results of the present study. *femA* is associated with high levels of resistance in MRSA because resistance levels decreased when *femA* was inactivated [41].

The results of PCR reaction showed that the frequency of *femA* gene in samples was 45%. So far, various studies have been performed to investigate the presence of this gene on *S. aureus* isolates. In a study by Kobayashi et al. (Japan-1994), the *femA* gene was detected in almost all 237 clinical specimens except for 5 methicillin-resistant strains [42]. In another study, Breves et al. (Brazil-2015) evaluated the prevalence of virulence genes among methicillin and vancomycin-resistant *S. aureus* strains isolated from medical devices and health care workers and showed that the *femA* gene there are more than 71.4% of these strains [43].

The high prevalence of *femA* gene in the above reports is inconsistent with the results obtained in our study, which is probably due to different sources of strain. Alpha hemolysin (*hla*) is involved in the development of osmotic phenomena, cellular depolarization and the loss of vital molecules (ATP). The frequency of this gene in samples obtained was 91%. Xiao et al. in China, by examining

the frequency of this gene among 47 *S. aureus* strains showed that a total of 28 *hla* genes can be detected [44]. Clumping factor B (clf B) mediates the clumping and attachment of bacterial cells to fibrinogen in the presence of fibronectin. Adhesion of *S. aureus* to the anterior regions during colonization is facilitated by Clumping factor B (clf B). The frequency of the gene among bacterial strains was about 54%. In the study of Eftekhari et al., the distribution of this gene among *S. aureus* strains isolated from patients admitted to health care in 7 hospitals of Tehran was determined to be about 78.6% [28].

In studies conducted by Nemati et al. (Iran), Zhang et al. (China) and Ren et al. (China), the prevalence of this gene was determined to be 100%, 89.29% and 100%, respectively [45-47]. According to the above studies, the frequency of this gene showed a close similarity with our data. Fibronectin-binding proteins fnBPs are *S. aureus* MSCRAMM proteins capable of binding to fibronectin, fibrinogen, and elastin [48]. In the present study, the frequency of *fnbB* gene bacterial samples was determined to be 39%. In the reports of other researchers such as Eftekhari et al. (Tehran), Ren et al. (China) and Kot et al. (Poland), the prevalence of this gene was 75%, 35.4% and 50.25%, respectively [28, 46, 49].

## 5. Conclusions

In the present study, the expression of *tsst* gene in *S. aureus* strains was lower than in previous studies. *mecA* is another gene found in bacterial cells that allows them to be resistant to antibiotics such as methicillin and other penicillin-like antibiotics. *mecA* is involved in the coding of PBP2A protein (a transpeptidase that promotes bacterial cell wall formation) with the ability of resistance to some antibiotics. This study was limited to Al-Najaf Al-Ashraf teaching hospital and therefore more investigations are required to evaluate virulence factors among other antibiotic-resistant strains of *S. aureus* isolated from a patient with urinary tract infection. Also, new efficient techniques should be applied to determine the presence of virulence genes among antibiotic-resistant strains.

## Conflict of interest

None of the authors have any conflict of interest to declare.

## Consent for publications

The author read and proved the final manuscript for publication.

## Availability of data and material

All data generated during this study are included in this published article

## Authors' Contribution

All authors had an equal role in study design, work, statistical analysis and manuscript writing.

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## Ethics approval and consent to participate

The study does not need ethical approval.

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