Original Article

Identification of Hemolysine Genes and their Association with Antimicrobial Resistance Pattern among Clinical Isolates of *Staphylococcus aureus* in West of Iran

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Abstract

Background: *Staphylococcus aureus* is expressing a broad range of different hemolysins enhancing its ability to establish and maintain infection in humans. The aim of this study was to identify the types of hemolysins in different clinical isolates of *S. aureus* and their association with antibiotic resistance patterns. **Materials and Methods:** In this cross-sectional and descriptive study, clinical isolates of *S. aureus* were collected from Hamedan's hospitals during an 11-month period from June 2016 to January 2017 and identified by using biochemical tests. To determine the antibiotic resistance pattern, disk diffusion method and minimum inhibitory concentration (MIC) were conducted. Genomic DNA was extracted using extraction kit. The polymerase chain reaction was done with specific primers for identification of *hla, hlb, hld*, and *hld* genes. **Results:** Among a total of 389 clinical samples, 138 isolates (35.45%) of *S. aureus* were identified, which 87 isolates (63.04%) were cefoxitin MIC of >4 μg/ml and resistant to methicillin. The highest frequency of antibiotic resistance was observed against erythromycin in 108 isolates (78.26%) and penicillin in 133 isolates (96.37%) and the lowest resistance was against gatifloxacin in 50 isolates (36.23%) and Cefazolin in 11 isolates (97.7%). Furthermore, the *hla, hlb, hld,* and *hlg* genes were detected among 11 (7.97%), 7 (5.07%), 16 (11.59%), and 4 (2.89%) isolates, respectively. There was a significant relationship between the presence of alpha and delta hemolysin-encoding genes and the antibiotic resistance pattern of isolates ($P < 0.05$). **Conclusion:** The results exhibited that the association between the presence of the hemolysin genes and the antibiotic resistance pattern can be considered as a serious issue.

Keywords: *Alpha-hemolysin, antibiotic resistance, beta-hemolysin, delta-hemolysine, Staphylococcus aureus, virulence factors*

Introduction

Staphylococcus aureus is an important human pathogen that can cause a variety of diseases ranging from chronic biofilm-associated infection to life-threatening infection. Approximately 30% of a healthy human population carries *S. aureus* asymptomatically in the anterior mucosa of their noses.[1] This pathogenic agent can cause severe disease in bacteremia, childhood pneumonia, osteomyelitis, and venereal diseases by secretory exotoxins, toxic shock syndrome, and enterotoxins.[2]

Due to the development of drug resistance, especially, in methicillin-resistant *S. aurous* (MRSA), treatment of its infection has been a serious problem. In early 1940, penicillin was one of the most effective agents against *S. aureus* infections. However since 1952, penicillin-resistant *S. aureus* has increased by 70% to 80%.[3] Resistance to penicillin was fully developed in 1961, and next, *S. aureus* showed resistance to other drugs, including methicillin, nafcillin, and oxacillin, and a wide range of broad-spectrum antibiotic groups in addition to penicillin.[4] Methicillin is a beta-lactam antibiotic and inhibits bacterial peptidoglycans synthesis and destroys cell walls by binding and inhibiting the activities of penicillin-binding proteins (PBPs), finally leading to bacterial death.[5] The resistance of *S. aureus* to methicillin is caused by the *mecA* gene, which encodes the PBP 2a (or PBP2').[6] The *mecA* gene is located on a mobile genetic element called *Staphylococcal* Cassette chromosome

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mec types.[7] *S. aureus* is well-known for its ability to elaborate a wide range of virulence factors, including hemolysins, exotoxins, leukocidins, superantigens, capsules, and secreted enzymes, allows this organism to overcome host defenses.[8,9] Some of these toxins, including *alpha*-hemolysin, have been encoded in the genome, while others such as Panton-Valentine leukocidin (PVL) are encoded on mobile genetic elements such as prophage.[10,11] Lysis of red blood cells by *S. aureus* is mainly mediated by the different hemolysins known as alpha (α), beta (β), delta (δ) toxins, and *gamma* (PVL) hemolysins. The alpha-hemolysin is a 33 kDa toxin encoded by the *hla* gene and is secreted by clinical isolates of *S. aureus*, which can cause clinical symptoms in humans, including pneumonia, sepsis, and brain abscess.[12-14]

Beta toxin or sphingomyelinase, with a molecular weight of 35 KDa, is coded by the *hlb* gene. One of the activities of this toxin in clinical conditions can be described as human lung, eye infection (cornea) and an ability to prevent the ciliary of nasal epithelium cells has been described.[14,15] The presence of a gap in the signal segment in the terminal region of the alpha and beta toxins indicates that they are secreted through the secretion system pathways.[16] Delta hemolysins is a 26 amino acid peptide encoded by the *hld* gene.[17,18] In contrast to alpha and beta toxin, delta toxin does not cause any split in the signal segment, and its secretion mechanism has not yet been fully understood.[17]

Because the genetic structure for delta toxin is encoded with polarized RNAIII molecules, the transcriptional activity of which is a group of virulence factors such as alpha toxin, enterotoxins and toxin shock-like syndrome, as well as transcriptional inhibition surface proteins, including superficial protein A (*Spa*), has not been elucidated to be accurately related to the delta toxin contamination with *S. aureus*.[19-21] The *S. aureus* gamma-toxins are β-barrel pore-forming toxins that are secreted from the bacteria as monomers. The gamma-toxin monomers are comprised S and F class subunits, corresponding to slow and fast elution from an ion exchange column.[22,23] The aim of this study was to identify the types of hemolysins in different clinical isolates of *S. aureus* and determine the association of these agents with antimicrobial resistance patterns.

Materials and Methods

Bacterial isolates

This study was conducted on 389 clinical samples including blood, urine, catheter, nasal swab, and ulcers of both outpatients and patients admitted to selected hospitals of the Hamadan University of Medical Sciences from June 2016 to January 2017 (during an 11-month period). The sample collection in this study was carried out with the Code of Ethics Committee No. IR. UMSHA. REC.1395.327 adopted by Hamadan University of Medical Sciences. The samples were cultured on Blood Agar (Merck, Germany), an enriched base with 5% sterile fresh blood of sheep (Sinapooyesh Iran), and incubated at 37°C for 24 h. Diagnostic tests were performed according to the diagnostic protocols. After observing the colonies, Gram-positive cocci were isolated using coloring forms. Slide catalase test was used to identify *staphylococci* from *streptococci*. The slide and tube coagulase test were used for the identification of *S. aureus* from other *Staphylococcus* species. The oxidative-fermentative test was used to detect *staphylococci* from *Micrococcus*. Coagulase test was used to distinguish coagulase-positive *staphylococci* from coagulase-negative isolates.[24,25]

Antibiotic susceptibility testing

For all *S. aureus* isolates, antibiotic susceptibility testing was carried out using Kirby-Bauer disk diffusion method according to the Clinical and Laboratory Standard Institute (2015) guidelines, Applied antibiotics (Mast, England) including cefoxitin (Fox) 30 μg, tetracycline (TE) 10 μg, gentamycin (GM) 10 μg, erythromycin (E) 15 μg, penicillin (10u), vancomycin (VAN) 30 μg, amikacin 30 μg, cefazolin (CZ) 10 μg, ciprofloxacin (CIP) 5 μg, gatifloxacin 10 μg, and norfloxacin (NR) 10 μg, Methicillin-resistance was examined using cefoxitin disk and cefoxitin E-test (Italy Liofilchem). The *S. aureus* ATCC25923 and *S. aureus* ATCC43300 were used as negative and positive controls, respectively.

DNA extraction

Total DNA was extracted using the DNA extraction Kit (Cina-gene Co., Iran), according to the manufacturer's instructions. Quality of extracting DNA was assessed spectrophotometrically by the Nanodrop ND-1000 (Nanodrop Technologies, Inc., Wilmington, DE, USA.[25]

Polymerase chain reaction detection of hemolysin genes

The reaction mixture for polymerase chain reaction (PCR) assay was 25 μL that was prepared as follows: 12.5 μl of 2x Taq premix Master mix (Ampliqon UK), 7.5 μl of sterile double distilled water, 1 μl of each forward and reverse primer [Table 1], and 3 μl of DNA sample. The DNA samples as well as a positive control (*S. aureus* ATCC43300) and a negative control *S. aureus* ATCC 25423) were amplified for *hla* and *hld* genes by an initial denaturation step for 5 min at 94°C followed by 35 cycles of 94°C for 30 s, 59°C for 60 s, and 72°C for 1 min and a final extension step at 72°C for 10 min in a Bio-Rad Thermal Cycler (Bio-Rad Laboratories, Inc., USA), and the PCR program for *hlb* and *hlg* genes was an initial denaturation step for 5 min at 94°C followed by 45 cycles of 94°C for 30 s, 65°C for 30 s, and 72°C for 30 s and a final extension step at 72°C for 10 min in a Bio-Rad Thermal Cycler. The products PCR were subjected to 2.5% Agarose gel electrophoresis. To control the quality and evaluate the results, *S. aureus* ATCC25923 was used as

Table 1: The sequences of primers used to detect hemolysin genes in clinical isolates of *Staphylococcus aureus*

Gene targets	Primer sequences (5' to 3')	Amplicon/product size (bp)	References
Hla	F: CTGATTACTATCCAAGAAATTCGATTG	209	[26]
	R: CTTTCCAGCCTACTTTTTTATCAGT		
	R: CTTTCCAGCCTACTTTTTTATCAGT		
Hlb	F: GTGCACTTACTGACAATAGTGC	309	[26]
	R: GTTGATGAGTAGCTACCTTCAGT		
Hld	F: AAGAATTTTTATCTTAATTAAGGAAGGAGTG	111	[26]
	R: TTAGTGAATTTGTTCACTGTGTCGA		
Hlg	F: GTCAYAGAGTCCATAATGCATTTAA	535	[26]
	R: CACCAAATGTATAGCCTAAAGTG		
mecA	F: CATCCAGAACCAATCGAAGAC	184	[27]
	R: CCATTTACCACTTCATATCTTGTAACG		
	F: AAAGAACCTCTGCTCAACAAGT		

negative control and *S. aureus* ATCC 49775 was used as positive control[22] [Table 1].

Sequencing

One sample of each hemolysin PCR products (amplicons) was sequenced by Bioneer Co., Korea mediated by Pishgam Co., Iran, and the data were analyzed using the Chromas software.

Statistical analysis

Data were analyzed using SPSS software version 16 (SPSS Inc. Released 2007. SPSS for Windows, Version 16.0. Chicago, SPSS Inc, IBM, USA). Descriptive statistical methods were used to determine the frequency, percentage and mean, and Chi-square test was used to compare the qualitative results, and independent *t*-test to compare quantitative data. $P \leq 0.05$ was considered statistically significant in comparative data.

Results

Isolation and prevalence of *Staphylococcus aureus* Isolates

Among 389 clinical samples, 138 isolates (35.45%) of *S. aureus* were identified and 87 isolates (63.04%) were identified as MRSA and 51 isolates (38.95%) were identified as MSSA. The results of collected samples are shown in Table 2.

Of the 138 clinical isolates of *S. aureus*, 27 isolates (19.56%) from the wound, 39 isolates (28.26%) from the blood, 22 isolates (15.94%) of urine, 18 isolates (13.04%) from the trachea, 12 isolates (8.69%) of catheter, 14 isolates (10.41%) of swabs, and 6 isolates (34.4%) were isolated from outpatients.

Antimicrobial susceptibility patterns of *Staphylococcus aureus* isolated from clinical samples

The results of antimicrobial susceptibility patterns of the 138 *S. aureus* isolates examined, has been shown in Figure 1.

Table 2: Distribution of *Staphylococcus aureus* strains isolated from Hamadan hospitals based on the sample source

Samples	MRSA	MSSA	Total
Tracheal	6	12	18
Wound	18	9	27
Blood	29	10	39
Urine	15	7	22
Swabs	3	11	14
Catheter	11	1	12
Outpatient	5	1	6
Total	87	51	138

MRSA: Methicillin-resistant *Staphylococcus aureus*, MSSA: Methicillin-susceptible *Staphylococcus aureus*

Moreover, 108 (78.26%) isolates were resistant to E, 133 (96.7%) to penicillin, 81 (58.69%) to CIP, 99 (71.73%) to TE, 33 (23.91%) to gatifloxacin, 50 (36.23%) to gentamicin, 11 (97.7%) to CZ and 71 (45.44%) were resistant to NR and 87 (63.04%) isolates with a minimum inhibitory concentration of >4 μg/ml and were resistance to methicillin [Figure 2].

Prevalence of hemolytic genes

The hemolytic genes *alpha, beta, delta,* and *gamma* were detected as follows: 11 isolates (7.97%) with *hla* gene, 7 (5.07%) with *hlb* gene, 16 (11.59%) with *hld* gene, and 4 (2.89%) had the *hlg* gene [Table 3 and Figure 3].

Relationship between the presence of *hla, hlb, hld,* and *hlg* genes and antibiotics resistance pattern in clinical isolates of *Staphylococcus aureus*

Considering the pattern of antibiotic resistance, there was a significant relationship between the presence of antibiotics and the genes of the alpha and delta hemolysines. In contrast to the alpha and delta hemolysine genes, there was no significant relationship between the presence of antibiotics studied and the agent genes for beta and gamma hemolsine. In all cases, $P \leq 0.05$ were considered as a statistically significant relationship between the variables under consideration [Table 4].

Table 3: Statistical analysis for determining possible relationship between antibiotic resistance pattern and hemolysin factor genes in methicillin-resistant *Staphylococcus aureus* and methicillin-susceptible *Staphylococcus aureus*

Antimicrobial agents	*MRSA*					*MSSA*				
	hla	*hlb*	*hld*	*hlg*	*mecA*	*hla*	*hlb*	*hld*	*hlg*	*mecA*
FOX	*P*=0/01	*P*=0/19	*P*=0/011	*P*=0/14	*P*=0/042	*P*=0/009	*P*=0/9	*P*=0/15	*P*=0/09	*P*=0/15
E	*P*=0/013	*P*=0/25	*P*=0/015	*P*=0/5	*P*=0/003	*P*=0/002	*P*=0/12	*P*=0/6	*P*=0/055	*P*=0/45
P	*P*=0/025	*P*=0/13	*P*=0/019	*P*=0/27	*P*=0/019	*P*=0/033	*P*=0/1	*P*=0/11	*P*=0/9	*P*=0/20
CIP	*P*=0/03	*P*=0/22	*P*=0/01	*P*=0/9	*P*=0/015	*P*=0/045	*P*=0/55	*P*=0/5	*P*=0/15	*P*=0/35
T	*P*=0/045	*P*=0/45	*P*=0/024	*P*=0/31	*P*=0/025	*P*=0/005	*P*=0/7	*P*=0/12	*P*=0/076	*P*=0/25
GAT	*P*=0/013	*P*=0/23	*P*=0/036	*P*=0/20	*P*=0/040	*P*=0/015	*P*=0/45	*P*=0/07	*P*=0/11	*P*=0/5
CEF	*P*=0/018	*P*=0/18	*P*=0/045	*P*=0/10	*P*=0/040	*P*=0/011	*P*=0/28	*P*=0/09	*P*=0/45	*P*=0/30
GEN	*P*=0/02	*P*=0/1	*P*=0/028	*P*=0/72	*P*=0/035	*P*=0/05	*P*=0/17	*P*=0/066	*P*=0/6	*P*=0/75
NOR	*P*=0/01	*P*=0/36	*P*=0/014	*P*=0/18	*P*=0/021	*P*=0/032	*P*=0/65	*P*=0/07	*P*=0/4	*P*=0/65
VAN	-	-	-	-	-	-	-	-	-	-

NOR: Norfloxacin, CIP: Ciprofloxacin, E: Erythromycin, FOX: Cefoxitin, VAN: Vancomycin, T: Tetracycline, GAT: Gatifloxacin, CEF: Cfazolin, GEN: Gentamicin, *P*: Probability value based on Chi-square test, MRSA: Methicillin-resistant *Staphylococcus aureus*, MSSA: Methicillin-susceptible *Staphylococcus aureus*

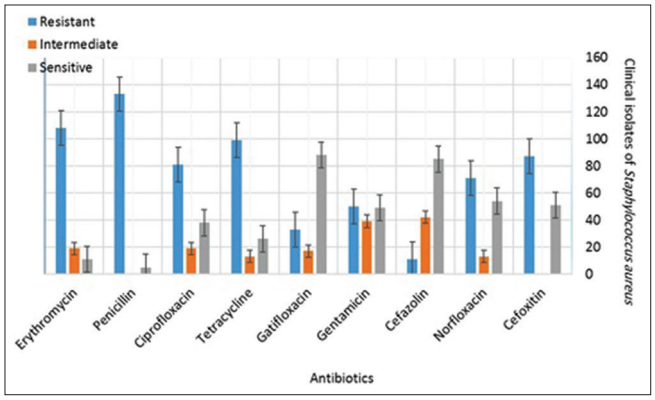

Figure 1: The prevalence of antibiotic resistance pattern for clinical isolates of *Staphylococcus aureus*

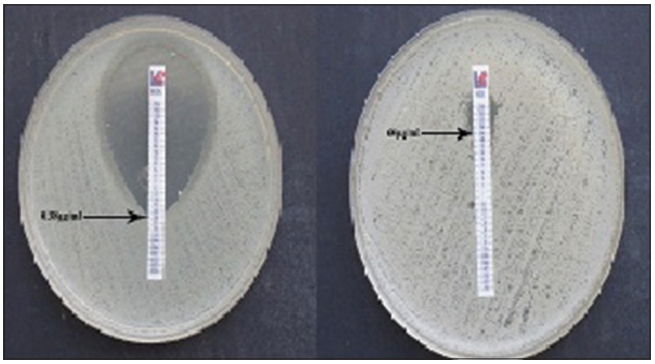

Figure 2: The primary screening of methicillin-resistant *Staphylococcus aureus* by the E-test (Liofelichem, Italy). The *Staphylococcus aureus* ATCC 43300 and *Staphylococcus aureus* ATCC 25923 were used as a positive and negative control

Results of gene sequencing

The results of the blast of the intended genes product indicated that it has the same DNA sequences and all PCR assay results were confirmed.

Discussion

MRSA is one of the most dangerous strains that have resistance to the wide range of antibiotics. MRSA can also produce various virulence factors along with resistance. The presence of pathogens in the body can threaten human health.[25] Besides the extraordinary capability to accumulate antimicrobial resistance determinants, *S. aureus* possess an enormous arsenal of virulence factors showing a unique ability to evade the host immune defenses.[5,6] The ability of *S. aureus* to cause disease depends on multiple strategies and on the redundancy of virulence factors. The group of hemolysins comprises toxins assembled out of one type of monomer like α-HL or the PSMs, bicomponent hemolysins such as PVL and γ-HL and the sphingomyelinase β-HL.[8,26] Although these toxins differ in their structure, all of them can lyze blood cells by the formation of transmembrane pores. The relationship between pathogenicity and antibiotic resistance in *S. aureus* depends on a variety of factors, the most important of which are environmental factors, adhesion molecules, host immune evasion, gene regulators, transcription factors, and bacterial toxin secretion.[27,28] Our research discusses this relationship and analyzes the production of various hemolysin in *S. aureus* and antibiotic resistance as an effective factor on each other.

In the present study, the highest antibiotic resistance was to penicillin and E being more than 90%, and the lowest resistance was to CZ which is similar to the finding of studies of Akia and Amini and Tafaroji *et al*.,[29,30] In studies by Arabestani *et al*., the highest antibiotic resistance in *S. aureus* was against methicillin, gentamicin, and CIP, with a frequency of >90% and increase of >90% of the resistance to ofloxacin, TE and clindamycin which is similar to the obtained results of the present study.[28,31] In addition, our results also demonstrated that all isolates were susceptible to vancomycin. However, in a study by Gitau *et al*., the prevalence of E, gentamicin, and cefocytosine antibodies was 33%, 13%, and 28%, respectively.[32] Naimi *et al*. also found that the resistance pattern to gentamicin

Table 4: Statistical analysis for determining possible relationship between samples type and hemolysin factor genes in Methicillin-resistant *Staphylococcus aureus* Methicillin-susceptible *Staphylococcus aureus*

Samples type	Hemolytic genes								*Staphylococcus aureus*	MRSA	MSSA	*P*>0.05	*P*≤0.05
	MRSA				MSSA								
	Hla	*Hlb*	*Hld*	*Hlg*	*Hla*	*Hlb*	*Hld*	*Hlg*					
Urine	2	1	1	0	1	0	0	0	6	5	1	**	*,§,¥
Tracheal	0	0	0	0	0	0	0	0	0	0	0	**	*,§,¥
Blood	3	4	5	3	2	0	3	0	20	15	5	**	*,§,¥
Wound	3	1	3	1	0	0	0	0	9	9	0	**	*,§,¥
Catheter	0	1	3	0	0	0	1	0	4	3	1	**	*,§,¥
Swabs	0	0	0	0	0	0	0	0	0	0	0	**,*,§,¥	-
Outpatients	0	0	0	0	0	0	0	0	0	0	0	**,*,§,¥	-
Total	8	7	12	4	3	0	5	0	39	31	8	-	-
	31				7								

*MRSA, **MSSA, ¥Hemolytic genes, §Samples type. MRSA: Methicillin-resistant *Staphylococcus aureus*, MSSA: Methicillin-susceptible *Staphylococcus aureus*

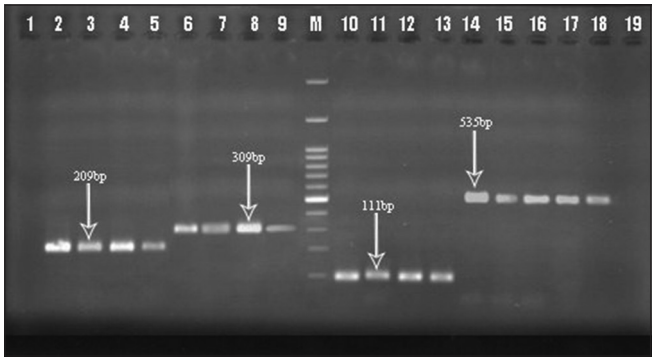

Figure 3: Gel electrophoresis for polymerase chain reaction assays products. Lane M: 100-bp DNA size marker (Fermentas, lituani), Lane: (1 and 19): Negative sample, Lane (2-5): *hla* (209 bp), Lane (6-9): *hlb* (309 bp), Lane (10-13): *hld* (111 bp), and Lane (14-18): *hlg* (535 bp)

was 23% and CZ 22%.[33] Despite, in our study, the frequency of resistance to antibiotics gentamicin, E, cefocytosine, and ceftazidine was 36.23%, 78.26%, 63.04%, and 45.44%, respectively. This can be attributed to the culture of drug use, the pattern of presentation by physicians, the nonproliferation of mutated strains as well as the weather. In the study of Grenni *et al*., ecological and environmental effects of antibiotic resistance patterns have been reported. They stated that these conditions alter the metabolism of bacteria and even interfere with the spread of new multiple drug resistant strains.[34]

In some studies, the association between antibiotic resistance and pathogenicity factors in *S. aureus* has been investigated. In the present study, the susceptible and resistant strains of methicillin were investigated for the presence of genes producing hemolysin.[26] The *hla, hlb, hld,* and *hlg* genes were detected among 11 (7.97%), 7 (5.07%), 16 (11.59%), and 4 (2.89%) isolates, respectively. Meanwhile, the frequency of hemolysin genes in MRSA strains was 63.35% and in MSSA strains was 72.13%. Furthermore, the prevalence of *hla*, *hlb*, *hld,* and *hlg* genes in MRSA strains was 9.19%, 8.04%, 13.79%, and 3.44%, respectively. Clinical information was also gathered about each patient. No difference was observed between MSSA and clinical specimen. On a hemolysin gene level, patients colonized with isolates encoding *hla, hlb, hld,* and *hlg* genes had significantly higher more frequent when compared to strains lacking the gene. However, methicillin-sensitive *S. aureus* strains also carry hemolysin genes in some cases, but the prevalence was higher in methicillin-resistant strains. Regarding the pattern of antibiotic resistance in methicillin-sensitive and methicillin-resistant strains, there was a significant relationship between the presence of hemolytic agent genes and the antibiotic resistance pattern. Studies done by Schroeder *et al*. indicate furthermore a role of virulence factors in the development of resistance to the antibiotic in humans because the presence of virulence genes was found to be significantly associated with antibiotic resistance in *S. aureus* and invasive Staphylococcal infections. In addition, in this study, it was found that there is a significant relationship between CA. MRSA isolates and the presence of certain *S. aureus* toxins. While in our review, there was no significant relationship between these two variables. Clinical isolates of hemolysin producing *S. aureus* are found most in blood and ulcers to cause more damage to the patient. Host immunity, regulatory activity genes, and the involvement of some environmental factors can produce hemolysin in CA. MRSA strains.[27] Studies by Remy *et al*. showed that MRSA strains could produce more levels of toxin and pathogens. The results of our studies showed that the prevalence of hemolytic agent genes in strains with multiple resistance was higher than susceptible strains.[35] The strains of superantigens toxins, particularly strains that produce toxic shock syndrome, can play an important role in drug resistance, can produce more intense toxins and ultimately, more damage potential. On the other hand, with the excessive consumption of antibiotics in recent decades, the emergence of resistant strains has been emerged. Hemolysins and their effects on the increasing of *staphylococcal* infections can be one of the most important issues that make the identification of

strains generating hemolysin more relevant. In the present study, hemolysin alpha and delta were the most frequent and beta-hemolysin and sometimes also had the lowest abundance.

Alpha-hemolysin is a cytolytic and cytotoxic toxin implicated in severe skin infections, pneumonia, and sepsis. In the present study, *hlg* and *hla* genes have the highest frequency in MRSA strains and have a significant association with the antibiotic pattern. A study of Luisa *et al*. showed that there is a significant relationship between antibiotic resistance and pathogenicity of *staphylococci*. The frequency of genes producing toxins in *S. aureus* isolates collected of blood and urine was higher than wound and catheter, also, there was a significant relationship between the types of the clinical sample with the presence of toxin-producing genes. Furthermore, in studies conducted by Alenizi and Beceiro *et al*., studies, there was a significant correlation between pathogenicity and antibiotic resistance.[36,37] Further indication that hemolysins are important virulence factors are also provided by studies which investigated the expression levels of hemolysins in different strains: High levels of α-PSMs are mainly produced by common MRSA strains like USA300 and are associated with enhanced virulence of these strains compared to MSSA strains. Similar to what is observed for most of the other *S. aureus* secreted proteins, alpha-hemolysin is not expressed constitutively.[3] The *hla* expression is activated during the postexponential-early stationary phase of growth, and toxin production is coordinately controlled by several regulators, including the *Agr* and *SarA*. These two regulators play a very important role in the formation of biofilms and quorum sensing.[8] Hemolysins indirectly increase the bacteria against antibiotics by acting on their activity and cause the emergence of resistant strains. Our research showed that the isolates producing hemolysin had the highest resistance to different antibiotic groups. The reason is the combination of various hemolysin, especially *hla* and *hlg* in these strains. Koch *et al*. showed that the presence of genes responsible for resistance and genes of pathogenicity as well as antimicrobial resistance patterns can be significantly correlated. In this study, among *S. aureus* isolates, it became clear that strains with antibiotic resistance had more invasion and pathogenicity. The similarity between these results and our observations showed that there is a significant relationship between antibiotic resistance and pathogenicity in *S. aureus* isolates.[38] This makes it increasingly important to identify strains that produce different hemolysin and can help identify resistant strains.

A central principle in effective regulation of both antibiotic resistance and virulence is the ability to sense and respond to the bacteria's external environment, specifically, the presence and concentration of ambient antibiotic.[8] In blood and ulcer samples, the bacteria may be affected by various factors, such as fever and body temperature, to alter its metabolic structure to cope with this increase in temperature. A change in the activity of regulating genes such as *agr* may change the production of hemolysin in *S. aureus*.[23] Our results showed that there is a significant relationship between clinical isolates and hemolysin secretion. Our results showed that there is a significant relationship between clinical isolates and hemolysin secretion. Although Corredor Arias *et al*., reported no significant correlation between the type of clinical sample and the virulence factors,[7] Osman *et al*., Chen *et al*., and Arabestani *et al*. reported a significant relationship between pathogenicity and antibiotic resistance.[10,20,28]

Our knowledge of this study suggests that the presence of hemolysin in *S. aureus* can lead to different resistance patterns. The interference of many factors, such as biofilm formation, RNAIII activity, temperature, environmental variables, and many other factors, can affect the relationship between resistance and pathogenesis. In this study, despite the lack of examination of these cases, this connection was raised for various reasons. Taken together, epidemiological and experimental data suggest an important role for hemolysins such as α-HL, β-HL, γ-HL, and PSMs in *S. aureus* infections, based on antibiotics resistance. However, the exact impact of some hemolysins remains to be elusive since the relevance of the existing data for the *in vivo* situation in humans is conflicting.

Conclusion

The results obtained in the present study concluded that the growing resistance to antibiotics in MRSA strains is increasing. However, increasing the prevalence of MRSA strains can play an important role in the development of various human infections. Hence, due to the association between the pattern of antibiotic resistance and the production of hemolysin in MRSA strains, identification of isolates with hemolytic genes is of great importance. Furthermore, this work describes that staphylococcal hemolysins are important virulence factors for *S. aureus* and explains the significant relationship between different hemolysin and antimicrobial resistance pattern.

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Conflicts of interest

There are no conflicts of interest.

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