١ Molecular investigation and virulence determination of methicillin and vancomycin resistant clinical
٢ *Staphylococcus aureus* isolates

٣ Abstarct:

٤ *Staphylococcus aureus* is an opportunistic pathogen that provides conditions for host invasion due to
٥ various virulence factors and plays a role in causing various infections. These bacteria may have different
٦ pathogenic functions depending on the susceptibility of the host. This study investigates the sensitivity of
٧ S. aureus strains isolated from clinical samples to methicillin and vancomycin and evaluates the presence
٨ of resistance, virulence and toxin-producing genes and their expression level in the methicillin-restsnat *S.*
٩ *aureus* (MRSA), vancomycin-resistant *S. aureus* (VRSA), and vancomycin-intermediate *S. aureus* (VISA)
١٠ isolates.

١١ In a cross sectional study, 502 *S. aureus* isolates were collected from different infections during one year.
١٢ Methicillin and vancomycin sensitivity of the isolates was checked by disk diffusion and microdilution
١٣ broth method, respectively. The presence of resistance, adhesion, and toxin-producing genes was checked
١٤ using the Multiplex PCR method. The expression level of the virulence and resistance genes was detected
١٥ in resistant and sensitive isolates using real-time qPCR.

١٦ Out of 502 *S. aureus* isolates, 168 isolates (33.6%) were MRSA. A total of 6 isolates (1.2%) were diagnosed
١٧ as VRSA and two isolates (0.4%) as VISA. Virulence and resistance-related genes showed different
١٨ frequencies. The results of the gene expression study showed that the expression of most of the studied
١٩ genes was significantly higher in resistant isolates (MRSA and VRSA) than in sensitive isolates.

٢٠ VRSA and MRSA are considered severe threats to human health. The present study showed that sanitary
٢١ measures are necessary to control this hospital pathogen more seriously due to the presence and expression
٢٢ of genes encoding virulence factors in *S. aureus* isolates.

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٢٤ 1.Introduction

٢٥ *Staphylococcus aureus* (*S. aureus*) is a type of Gram-positive bacteria that naturally resides in various parts
٢٦ of the human body. Its primary ecological niche in humans is the external areas of the nasal cavities.
٢٧ However, under certain conditions such as stress, viral infections, tissue damage, or any factor that weakens
٢٨ the immune system, this bacterium can lead to a wide range of infections. These infections can range from
٢٩ simple skin conditions such as pimples, boils, and abscesses to more serious diseases such as osteomyelitis,
٣٠ endocarditis, toxic shock syndrome, and septicemia (1).

٣١ The increasing resistance of *S. aureus* to antibacterial drugs is one of the main concerns of health experts.
٣٢ Thus, with the arrival of each new antibiotic, resistant strains of bacteria have emerged quickly and have
٣٣ made it difficult to treat infections caused by these bacteria (2).

٣٤ Methicillin is a penicillinase-resistant penicillin that was introduced in 1960. One year after the introduction
٣٥ of methicillin into the market, the first case of methicillin-resistant *S. aureus* (MRSA) was reported. After

٣٦ that, the prevalence of MRSA increased rapidly worldwide, and in 2003, the Clinical and Laboratory
٣٧ Standards Institute (CLSI) reported 64.4% MRSA worldwide (3). Vancomycin is a glycopeptide that
٣٨ disrupts the construction of the cell wall of Gram-positive bacteria and has been one of the best treatments
٣٩ against MRSA infections in the last three decades. However, the emergence of vancomycin-intermediate
٤٠ *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA) have led to global concern about the
٤١ treatment of staphylococcal infections (4).
٤٢ Identifying vancomycin-resistant strains, determining virulence factors, and studying the spread of these
٤٣ species are very important to determine the epidemiology and control of infections caused by these bacteria
٤٤ (6). This study investigates the sensitivity of *S. aureus* strains isolated from clinical samples to methicillin
٤٥ and vancomycin and evaluates the presence of resistance, virulence and toxin-producing genes and their
٤٦ expression level in the MRSA, VRSA, and VISA isolates.

٤٧ **2. Materials and methods**

٤٨ **2.1 Bacterial isolates**

٤٩ This cross-sectional study was conducted on 502 *S. aureus* isolates from different clinical samples
٥٠ (discharges, trachea, bronchi, wounds, blood, catheters). Isolates were collected from microbiology
٥١ laboratories of five hospitals (Loghman Hakim, Milad, Jam, Khatam, and Ebnesina) in Tehran, Iran, from
٥٢ 2019 to 2020. Isolates were confirmed as *S. aureus*, using previously described standard phenotypic tests.

٥٣ **2.2 Antimicrobial Susceptibility Testing**

٥٤ For detecting MRSA strains, all *S. aureus* isolates were evaluated by the Kirby-Bauer disk diffusion method
٥٥ on Mueller Hinton agar medium (Merck, Germany) and 30 µg cefoxitin disk (Padtan Teb, Iran) according
٥٦ to the CLSI recommended standard for methicillin resistance (7).
٥٧ Also, vancomycin minimum inhibitory concentration (MIC) was measured for all isolates using the broth
٥٨ microdilution method according to the CLSI recommendation. According to the CLSI standard, the MIC
٥٩ breakpoint for detecting the vancomycin-intermediate strains is a concentration between 4-8 µg/ml, and for
٦٠ detecting the vancomycin-resistant strains is greater than or equal to 16 µg/ml. *S. aureus* ATCC 29213 and
٦١ *S. aureus* ATCC 33592 were used as a negative and positive control for methicillin sensitivity, respectively.
٦٢ *E. faecalis* strain ATCC 51299 was used as a vancomycin-resistant strain for quality control investigation.

٦٣ **2.3 Detecting the resistance, adhesion, and toxin-producing genes**

٦٤ DNA extraction was performed using SinaPure EX6021 extraction and purification kit (SinaClon, Tehran,
٦٥ Iran). All MRSA, VRSA, and VISA isolates were screened by PCR for the *S. aureus 16s rRNA* and *mec*A
٦٦ genes. For this purpose, Then, 25 µL of the final solution containing 1 µL of template DNA (50 ng/ml), 1
٦٧ µL of each primer (Table. 1) with a concentration of 25 pM, and 12.5 µL of master mix 2x (Ampliqon,
٦٨ Germany) was used to perform PCR. Deionized distilled water was used to dilute to the final volume.
٦٩ Multiplex PCR method in three sets was used to check the presence of adhesion genes (*ica*A, *ica*B, *ica*C,
٧٠ *ica*D, *fnb*A, *fnb*B, *clf*A, and *clf*B), toxin-producing genes (*tsst*1, *pvl*, *hla*, and *sec*), and vancomycin

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resistance genes (*van*A, *van*B, *van*C1-3). For this purpose, 12 µL of 2X PCR Master mix, 4 µL of template DNA, 1 µL of each pair of primers with the sequences shown in Table 1 were mixed in a DNase-free microtube of 0.2 ml, and the total volume of the mixture was adjusted up to 25 µL with RNase-free distilled water. DNA template was amplified in a Master cycler Eppendorf (Eppendorf, Germany) under the following conditions: initial denaturation for 10 min at 94°C; followed by 35 cycles at 94°C for 45 s, at specific annealing temperatures for 45 s, then at 72°C for 45 s; a final extension for 5 min at 72°C; and then maintenance at 4°C. Afterward, the microtubes were placed in a thermocycler, and the reaction was carried out with a specified temperature program. The strains *S. aureus* ATCC 25923, *S. aureus* ATCC 29213, *S. aureus* ATCC 33591, and *S. epidermidis* ATCC 35556 were used as positive controls, with distilled water as the negative control.

Table 1. Primer sequences and annealing temperature of investigated genes using multiplex and qPCR

Reactions	Genes	Primer sequences	Annealing Temp	PCR product size (bp)	References
Single reaction	*16S rRNA*	5′-GGGACCCGCACAAGCGGTGG-3′	60	191	(8)
		5′-GGGTTGCGCTCGTTGCGGGA-3′			
Single reaction	*mecA*	5′-GTAGAAATGACTGAACGTCCGATGA-3′	55	310	(9)
		5′-CCAATTCCACATTGTTTCGGTCTAA-3′			
Multiple reaction Set1	*icaA*	5′-GAGGTAAAGCCAACGCACTC-3′	60	151	(10)
		5′-CCTGTAACCGCACCAAGTTT-3′			
	icaB	5-ATACCGGCGACTGGGTTTAT-3′	60	140	
		5-TTGCAAATCGTGGGTATGTGT-3′			
	icaC	5′-CTTGGGTATTTGCACGCATT-3′	60	209	
		5′-GCAATATCATGCCGACACCT-3′			
	icaD	5′-ACCCAACGCTAAAATCATCG-3′	60	211	
		5′-GCGAAAATGCCCATAGTTTC-3′			
	fnbA	5′-AAATTGGGAGCAGCATCAGT-3′	60	121	
		5′-GCAGCTGAATTCCCATTTTC-3′			
	fnbB	5′-AAATTGGGAGCAGCATCAGT-3′	60	197	
		5′-GCAGCTGAATTCCCATTTTC-3′			
	clfA	5′-ACCCAGGTTCAGATTCTGGCAGCG-3′	60	165	
		5′-TCGCTGAGTCGGAATCGCTTGCT-3′			
	clfB	5′-AACTCCAGGGCCGCCGGTTG-3′	60	159	
		5′-CCTGAGTCGCTGTCTGAGCCTGAG-3′			
Multiple reaction Set2	*tsst-1*	5′-TTATCGTAAGCCCTTTGTTG-3′	60	398	(11)
		5′-TAAAGGTAGTTCTATTGGAGTAGG-3′			
	pvl	5′-GGAAACATTTATTCTGGCTATAC-3′	60	502	
		5′-CTGGATTGAAGTTACCTCTGG-3′			
	hla	5′-CGGTACTACAGATATTGGAAGC-3′	60	744	
		5′-TGGTAATCATCACGAACTCG-3′			
	sec	5′-GGGAATGTTGGATGAAGG-3′	60	900	
		5′-AGGCAAGCACCGAAGTAC-3′			
Multiple reaction Set3	*vanA*	5′-GTACAATGCGGCCGTTA-3′	54	732	(12)
		5′-GGGAAAACGACAATTGC-3′			
	vanB	5′-CCGACAATCAAATCATCCTC-3′	54	536	(13)
		5′-AAGCTATDCAAGAAGCCATG-3′			

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vanC1	5′-ATCGCATCACAAGGACCAATC-3′	54	796	(14)
	5′-GAAAGACAACAGGAAGACCGC-3′			
vanC2	5′-CGCAGGGACGGTGATTTT-3′	54	484	
	5′-CGGGGAAGATGGCAGTAT-3′			
vanC3	5′-GCTTGTTCTTTGACCTTA-3′	54	224	
	5′-GCCTTTACTTATTGTTCC-3′			

2.4 Gene expression analysis by Real-time reverse transcription (RT)-PCR

Real-time RT-PCR was performed on all isolates. The expressions of genes encoding the resistance, adhesion, and toxin factors were determined by real-time RT-PCR using the primers detailed in Table 1. First, total RNA was extracted using an EX6101-RNX Plus Solution for total RNA isolation kit (SinaClon, Tehran, Iran), and cDNA was then synthesized using a SinaClon First Strand cDNA Synthesis Kit (SinaClon, Tehran, Iran), according to the manufacturer's recommendations.

The reaction was performed in a StepOne™ Real-Time PCR System (Applied Biosystems, USA) .The sequences of primers are shown in Table 1. *16S rRNA* was used as a reference gene. A real-time qPCR was performed in a volume of 10 µL including 5 µL of SinaGreen HS-qPCR Mix, 2X with low Rox, 0.35 µL of each primer, 1 µL of synthesized cDNA, and 3.3 µL deionized, diethylpyrocarbonate (DEPC) water. The expression level of genes in MRSA isolates was compared to methicillin-susceptible *S. aureus* (MSSA) isolates.

2.5 Statistical analysis

Data were analyzed using SPSS version 19 software, and one-way ANOVA was applied to analyze variance between groups. An error rate of less than 0.05 was considered in this study.

3. Results

3.1. Bacterial isolates results

In this study, 502 *S. aureus* isolates were isolated from different clinical samples using microscopic and macroscopic methods. Most samples were isolated from Lughman Hakim Hospital (198 isolates) and followed by Milad (122 isolates), Ebnesina (78 isolates), Khatam (58 isolates), and Jam (46 isolates). Wound and bone aspiration samples were the most (260 samples) and the lowest clinical samples (2 samples), respectively.

3.2. Antimicrobial Susceptibility testing results

Based on the cefoxitin disk diffusion test from the 502 *S. aureus* isolates, 168 isolates (33.46%) were MRSA. In the vancomycin broth microdilution test, two isolates (0.39%) were identified as VISA, six isolates (1.19%) as VRSA, and the remaining isolates (98%) were identified as sensitive strains.

The highest percentage of MRSA compared to the number of isolates was assigned to Milad Hospital, with 36.88%, and the lowest resistance rate to Khatam Hospital, with 25.86%. Also, the highest percentage of VRSA (3/6; 50%) and VISA (1/2; 50%) was assigned to Ebnesina Hospital.

3.3 The results of detecting the resistance, adhesion, and toxin-producing genes

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All of the MRSA, VRSA, and VISA isolates harbored the *S. aureus 16srRNA* gene. Among the 168 MRSA isolates, the *mecA* gene was absent in only two isolates (1.19%), one of which was VRSA. The rest of the VRSA and VISA isolates had the *mecA* gene.

The presence of the *van* genes was investigated in 6 VRSA and 2 VISA isolates. Three (37.5%), 3(37.5%), and 1 (12.5%) isolates harbored *van* B, *van* C1, and *van* C3, respectively. Other *van* genes were not found. One hundred sixty-eight isolates were investigated for the presence of adhesion (Figure 1a) and toxin (Figure 1b) genes and the results are given in Table 2.

Among the adhesion genes, the highest frequency was related to *icaD* (148; 85.54%) and *fnbB* (105; 60.69%) genes, and the toxin-producing genes *hla* had the highest frequency (167; 96.53%).

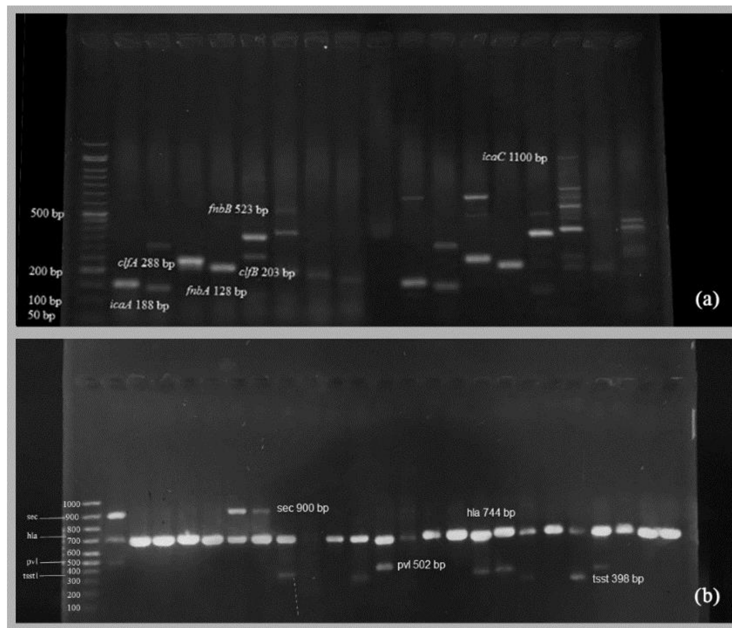

Figure 1. Electrophoresis image related to adhesion genes(a) and toxin-producing genes(b)

Table 2. Frequency of study sequence in *Staphylococcus aureus* isolates

Studied Genes		Total	MRSA*	VRSA**	VISA***
Adhesion Genes	*ica*A	40 (23.12)	16(9.52)	5(83.33)	1(50)
	*ica*B	28(16.18)	8(4.76)	4(66.66)	1(50)
	*ica*C	69(39.88)	55(32.73)	4(66.66)	1(50)
	*ica*D	148(85.54)	117 (69.64)	6(100)	2(100)
	*fnb*A	20(11.56)	12(7.14)	2(33.33)	2(100)
	*fnb*B	105(60.69)	92(54.76)	4(66.66)	1(50)
	clf A	33(19.07)	28(16.66)	1(16.16)	1(50)
	clf B	40(23.12)	21(12.5)	4(66.66)	1(50)

Toxin-producing genes	*tsst*1	37(21.38)	29(17.26)	2(33.33)	1(50)
	pvl	29(16.76)	28(16.66)	1(16.66)	1(50)
	hla	167(96.53)	159(94.46)	6(100)	2(100)
	sec	18(10.4)	16(9.46)	1(16.6)	1(50)

*Methicillin-resistant *S. aureus,* ** Vancomycin-resistant *S. aureus,* *** Vancomycin-intermediate *S. aureus*

3.4 Gene expression analysis results

The expression level of all studied genes in MRSA, VRSA, and VISA isolates is significantly higher than that of the reference gene. Statistical analysis was performed by Excel software using the Livak formula and Anova software based on the analysis of variance (Figure 2 (a-d) and Figure 3 (a-d)).

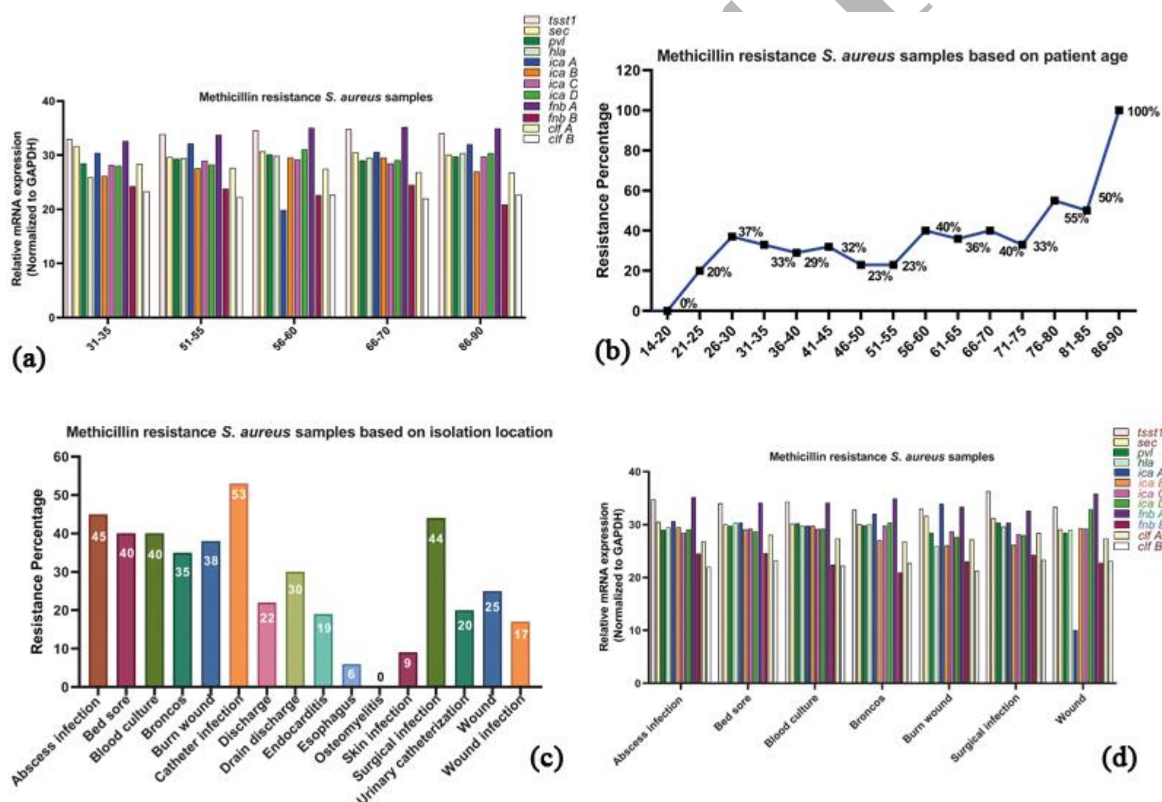

Figure 2. The expression level of all studied genes in MRSA isolates based on age group(a) and isolation sites(d). The percentage of MRSA isolates in relation to age group(b) and isolation sites(c)

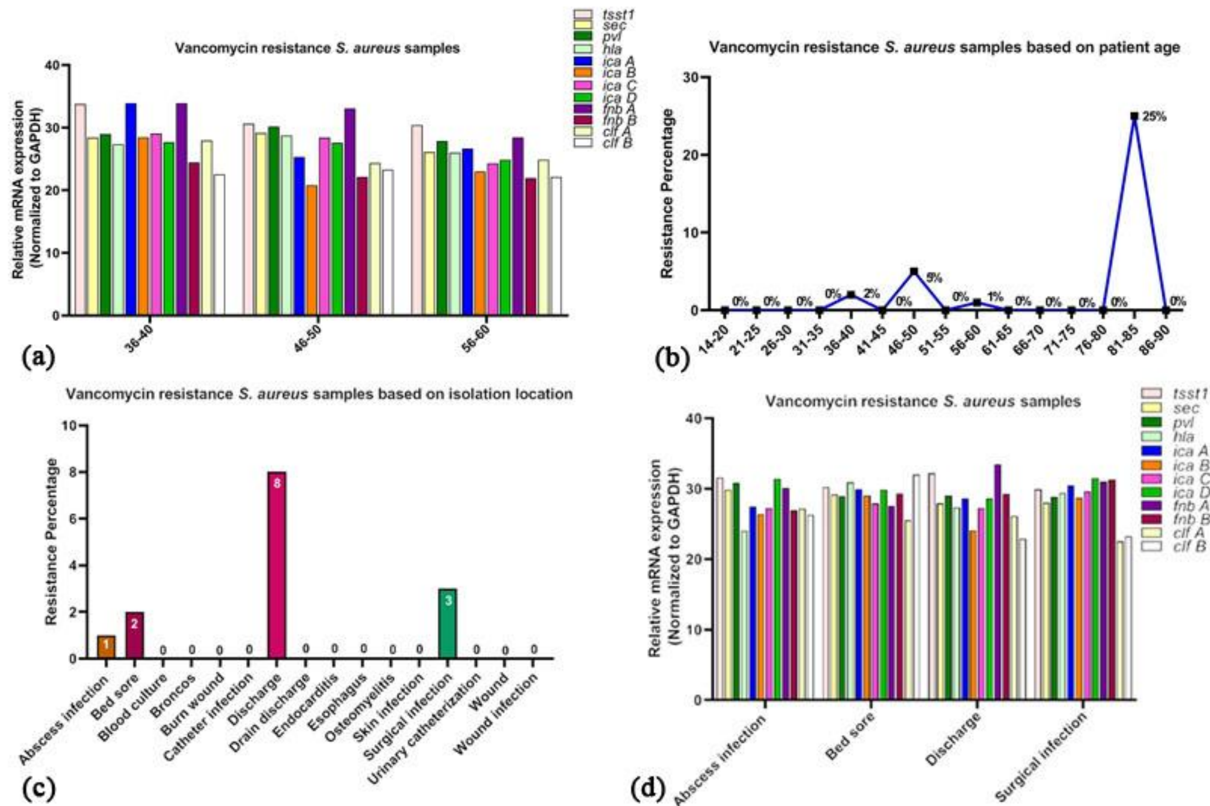

Figure 3. The expression level of all studied genes in VRSA isolates according to age group(a) and isolation site(d). Percentage of VRSA isolates according to age group (b) and location site (c)

4. Discussion

In the past three decades, the usage of vancomycin has increased significantly due to the increase in the prevalence of *S. aureus* resistance to methicillin (13). In the present study, 168 MRSA isolates (33.46%) were diagnosed which is a relatively high percentage. Since this antibiotic has the most clinical use, antibiotic resistance and ways to improve its effectiveness seem very important in the current situation. The reason is that due to the epidemic of the coronavirus and the epidemic of the disease of covid-19, hospital opportunistic infections such as *S. aureus* and *Acinetobacter baumannii* can lead to the death of hospitalized patients due to the weakening of the immune system of patients. The results of the Al Bshabshe et al. study (14) showed that the prevalence of MRSA strains increases over time. The results of other studies with agreement to the present study show the spread of MRSA strains in the world (15,16).

Regarding the frequency of MRSA strains, the current study shows a lower frequency compared to similar studies (10-12). Various factors are involved in the difference in the abundance of this strain and the level of resistance to methicillin maybe some of these factors include the hospital or sampling center, type of clinical sample, sampling time, and the performed test type (17). Various mechanisms have been identified for the resistance of *staphylococci* to methicillin. One of these mechanisms is the acquisition of a new

binding protein to methicillin (PBP2a), which also reduces the affinity of the antibiotic to bacteria. This type of resistance is related to the *mecA* gene and is carried by stimulating elements called SCC*mec*(18). *mecA* gene was detected in 166 of 168 MRSA isolates. In 2 MRSA isolates, this gene was not detected, and other factors causing resistance such as the presence of other *mec* gene types may have been involved. The present study determined the level of vancomycin resistance, and results showed that, fortunately, the level of resistance to this drug in the isolates was low. Based on the results obtained from 502 investigated isolates, 8 isolates (1.59%) were diagnosed as resistant to vancomycin or having intermediate vancomycin resistance by the broth microdilution test. In a report published by Huang et al. (19), among the 678 isolates 13 had intermediate resistance, and no vancomycin-resistant samples were found. Shariati et al. (20) examined the data recorded in scientific sources from 1997 to 2019 in a meta-analysis review. According to the studies registered in the databases, 1.5% of the 5855 studied strains had the VRSA phenotype. The rate of VISA phenotype among 22277 studied isolates was reported as 1.7%. Moreover, of the 47,721 investigated isolates, 4.6% had the heterogeneous VISA (hVISA) phenotype. This study found that the highest frequency of VRSA and hVISA was reported in the United States with 3.6% and 5.2%, respectively, while the highest frequency of VISA was 2.1% in Asia. Based on this study, vancomycin resistance increased in Asian and American countries, and preventive measures to control vancomycin resistance in these countries seem necessary. While the frequency of vancomycin-resistant isolates in our study was relatively low, the presence of strains showing intermediate resistance opens up intriguing possibilities for future research.The present study investigated the frequency of virulence genes, including adhesion and toxin-producing genes, and the expression level of these genes in methicillin and vancomycin-resistant strains compared to sensitive strains. The frequency of adhesion genes results showed that all studied genes are present in *S. aureus* isolates with different frequencies. The highest frequency related to *icaD* and *fnbB* genes was 85.54% and 60.69%, respectively, and the lowest frequency was related to *fnbA* (11.56%) and *icaB* (16.18%) genes. It is noteworthy that the expression of all adhesion-related genes (*clfA, clfB, fnbA, fnbB, icaA, icaB, icaC, icaD*) in methicillin-resistant isolates was significantly (P>0.05) more than the sensitive isolates. Mollaei and Reshki (21) conducted a study on 100 *S. aureus* isolates isolated from patients hospitalized in Zabul. They examined the presence of adhesion-related genes. The results of that study showed that 50% of the isolates examined had at least one of the studied genes.

In examining the frequency of toxin-producing genes, including *hla, pvl*, sec, and *tsst-1*, 96.53, 16.76, 10.40, and 21.38% frequencies were shown, respectively. In the study by Fathali et al. (10), out of 200 *Staphylococcus aureus* isolates investigated, 95 isolates were resistant to methicillin. The frequency of *hla*, *pvl*, *sec*, and *tsst-1* genes in the resistant isolates was 93.68, 4.21, 3.16, and 60%. Regarding the presence of *hla* gene, the results of the present study are consistent with the results of the above study; both have declared a frequency of nearly 95%.

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The frequency of the genes under study was also analyzed in relation to the age and location of the isolation. In this study, the highest rate of MRSA was related to the samples isolated from the catheter. The lowest was related to the samples isolated from the esophagus and skin infections. No resistance was observed in the samples isolated from the bone infections. Meanwhile, Nourbakhsh et al.(22) reported that the highest percentage of MRSA related to wound and catheter infection was 3.6%.

On the other hand, regarding the age factor, the results showed an increasing trend in the percentage of resistance at older ages, which also overlaps with the present study. Concerning the expression of toxin-producing genes and adherence to sensitive strains, the expression increased in all resistance strains (VISA, VRSA, MRSA, and VRSA-MRSA). The gene expression level differs in different age groups and does not follow a specific pattern regarding the expression of toxin-producing genes. However, most genes in all resistant categories had increased expression with an equal ratio in the tested age groups. Also, regarding the expression level of toxin-producing genes with the isolation location variable, among the MRSA isolates, the *tsst* 1 gene from surgical infections had the highest gene expression. However, the *hla* gene in burn wound infections and abscesses had the lowest gene expression. In Saeed Khan et al. study, from 10 samples isolated from the intensive care unit, all of which were MRSA, the *pvl* gene had the highest expression among the *hla*, *hlb, hld, hlg, sed*, *see*, *seg*, *she*, and *tsst* genes [23].

Regarding the expression of adhesion genes, it seems that the gene expression level is different in different age groups and does not follow constant changes. *fnbA* gene had the highest expression, and *clfB* and *fnbB* genes had the lowest expression in different age groups.

Based on the results of 502 clinical samples of *Staphylococcus aureus* obtained from Tehran hospitals, methicillin and vancomycin resistance rates were relatively low (33.46% and 1.2%, respectively). The important result of the present study was the significant increase in the expression of adhesion and toxin-producing genes in the studied isolates. Due to the presence of resistance to the vancomycin antibiotic, resistance genes, and virulence factor coding genes, it is suggested to seriously pursue control measures for this hospital pathogen. Also, the gene expression study results showed that the expression of most of the studied genes is significantly higher in resistant isolates (MRSA and VRSA) than in sensitive isolates. This opens up a promising avenue for further investigation in future studies.

Acknowledgments

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Author Contributions

FGh designed the study. NE and MMI collected the clinical data and performed experiments. MME analyzed the data. FGh and NE wrote the main manuscript. MMA and M.MI reviewed the manuscript. All authors accepted the final version of this manuscript.

Ethics

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٢٢٩ Not applicable.

٢٣٠ **Conflict of Interest**

٢٣١ The authors declare that they have no conflict of interest

٢٣٢ **Data Availability**

٢٣٣ The data that support the findings of this study are available on request from the corresponding author.

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Table 1. Primer sequences and annealing temperature of investigated genes using multiplex and qPCR

Reactions	Genes	Primer sequences	Annealing Temp	PCR product size (bp)	References
Single reaction	*16S rRNA*	5′-GGGACCCGCACAAGCGGTGG-3′	60	191	(8)
		5′-GGGTTGCGCTCGTTGCGGGA-3′			
Single reaction	*mecA*	5′-GTAGAAATGACTGAACGTCCGATGA-3′	55	310	(9)
		5′-CCAATTCCACATTGTTTCGGTCTAA-3′			
Multiple reaction Set1	*icaA*	5′-GAGGTAAAGCCAACGCACTC-3′	60	151	(10)
		5′-CCTGTAACCGCACCAAGTTT-3′			
	icaB	5-ATACCGGCGACTGGGTTTAT-3′	60	140	
		5-TTGCAAATCGTGGGTATGTGT-3′			
	icaC	5′-CTTGGGTATTTGCACGCATT-3′	60	209	
		5′-GCAATATCATGCCGACACCT-3′			
	icaD	5′-ACCCAACGCTAAAATCATCG-3′	60	211	
		5′-GCGAAAATGCCCATAGTTTC-3′			
	fnbA	5′-AAATTGGGAGCAGCATCAGT-3′	60	121	
		5′-GCAGCTGAATTCCCATTTTC-3′			
	fnbB	5′-AAATTGGGAGCAGCATCAGT-3′	60	197	
		5′-GCAGCTGAATTCCCATTTTC-3′			
	clfA	5′-ACCCAGGTTCAGATTCTGGCAGCG-3′	60	165	
		5′-TCGCTGAGTCGGAATCGCTTGCT-3′			
	clfB	5′-AACTCCAGGGCCGCCGGTTG-3′	60	159	
		5′-CCTGAGTCGCTGTCTGAGCCTGAG-3′			
Multiple reaction Set2	*tsst-1*	5′-TTATCGTAAGCCCTTTGTTG-3′	60	398	(11)
		5′-TAAAGGTAGTTCTATTGGAGTAGG-3′			
	pvl	5′-GGAAACATTTATTCTGGCTATAC-3′	60	502	
		5′-CTGGATTGAAGTTACCTCTGG-3′			
	hla	5′-CGGTACTACAGATATTGGAAGC-3′	60	744	
		5′-TGGTAATCATCACGAACTCG-3′			
	sec	5′-GGGAATGTTGGATGAAGG-3′	60	900	
		5′-AGGCAAGCACCGAAGTAC-3′			
Multiple reaction Set3	*vanA*	5′-GTACAATGCGGCCGTTA-3′	54	732	(12)
		5′-GGGAAAACGACAATTGC-3′			
	vanB	5′-CCGACAATCAAATCATCCTC-3′	54	536	(13)
		5′-AAGCTATDCAAGAAGCCATG-3′			
	vanC1	5′-ATCGCATCACAAGGACCAATC-3′	54	796	(14)
		5′-GAAAGACAACAGGAAGACCGC-3′			
	vanC2	5′-CGCAGGGACGGTGATTTT-3′	54	484	
		5′-CGGGGAAGATGGCAGTAT-3′			
	vanC3	5′-GCTTGTTCTTTGACCTTA-3′	54	224	
		5′-GCCTTTACTTATTGTTCC-3′			

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۲۳۸ **Table 2.** Frequency of study sequence in *Staphylococcus aureus* isolates

Studied Genes		Total	MRSA*	VRSA**	VISA***
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	*ica*B	28(16.18)	8(4.76)	4(66.66)	1(50)
	*ica*C	69(39.88)	55(32.73)	4(66.66)	1(50)
	*ica*D	148(85.54)	117 (69.64)	6(100)	2(100)
	*fnb*A	20(11.56)	12(7.14)	2(33.33)	2(100)
	*fnb*B	105(60.69)	92(54.76)	4(66.66)	1(50)
	clf A	33(19.07)	28(16.66)	1(16.16)	1(50)
	clf B	40(23.12)	21(12.5)	4(66.66)	1(50)
Toxin-producing genes	*tsst*1	37(21.38)	29(17.26)	2(33.33)	1(50)
	pvl	29(16.76)	28(16.66)	1(16.66)	1(50)
	hla	167(96.53)	159(94.46)	6(100)	2(100)
	sec	18(10.4)	16(9.46)	1(16.6)	1(50)

۲۳۹ *Methicillin-resistant *S. aureus,* ** Vancomycin-resistant *S. aureus,* *** Vancomycin-intermediate *S.*
۲٤۰ *aureus*

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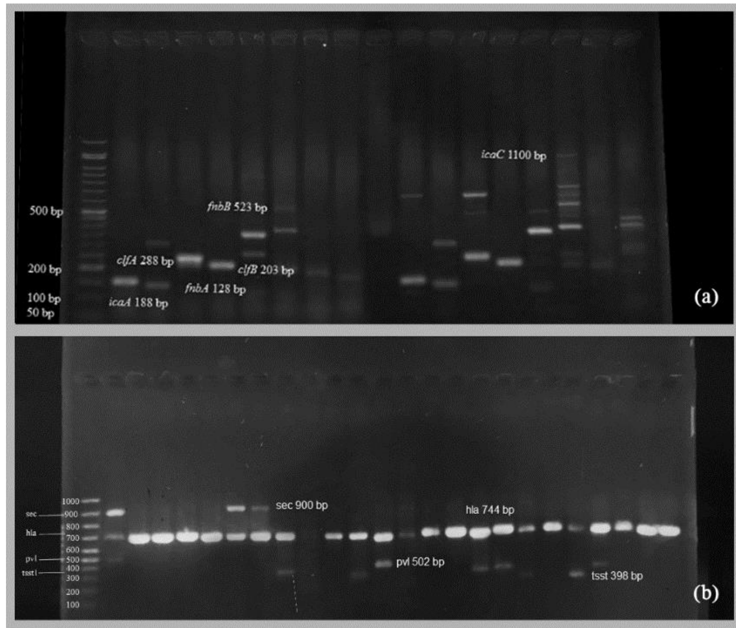

٢٤٥ **Figure 1.** Electrophoresis image related to adhesion genes(a) and toxin-producing genes(b)

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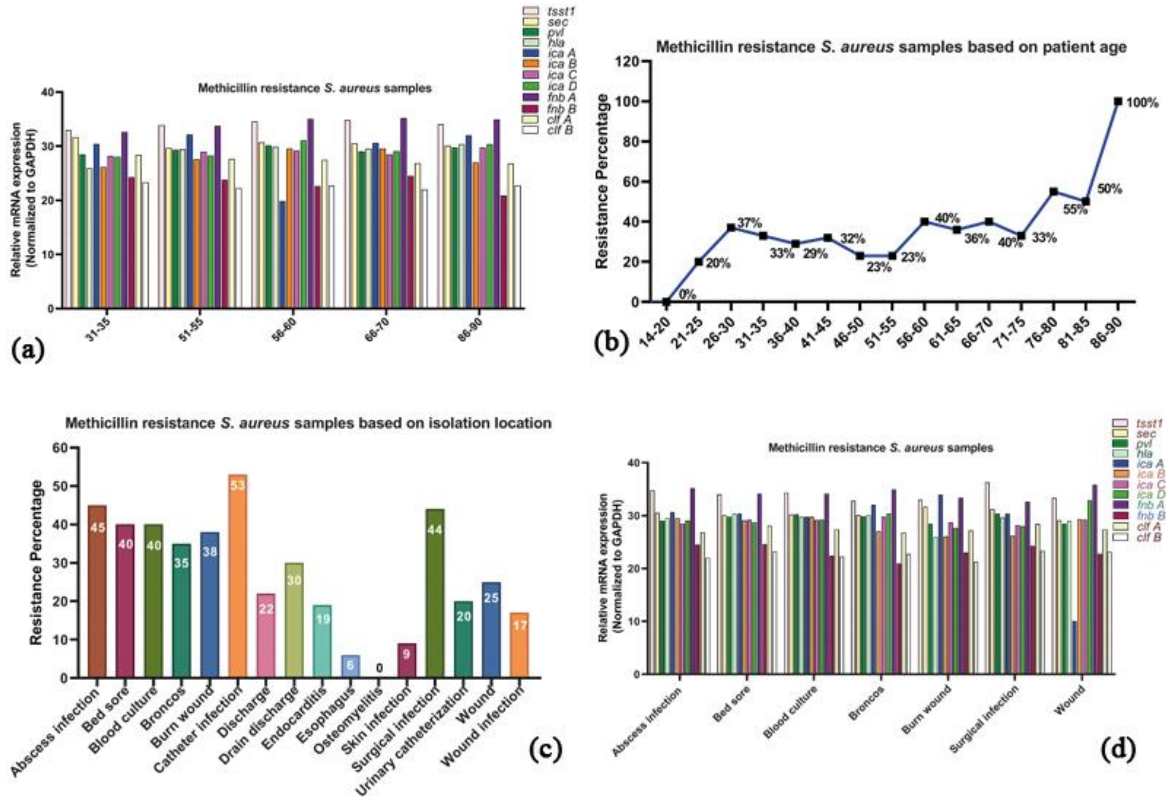

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٢٤٩ **Figure 2.** The expression level of all studied genes in MRSA isolates based on age group(a)
٢٥٠ and isolation sites(d). The percentage of MRSA isolates in relation to age group(b) and
٢٥١ isolation sites(c)

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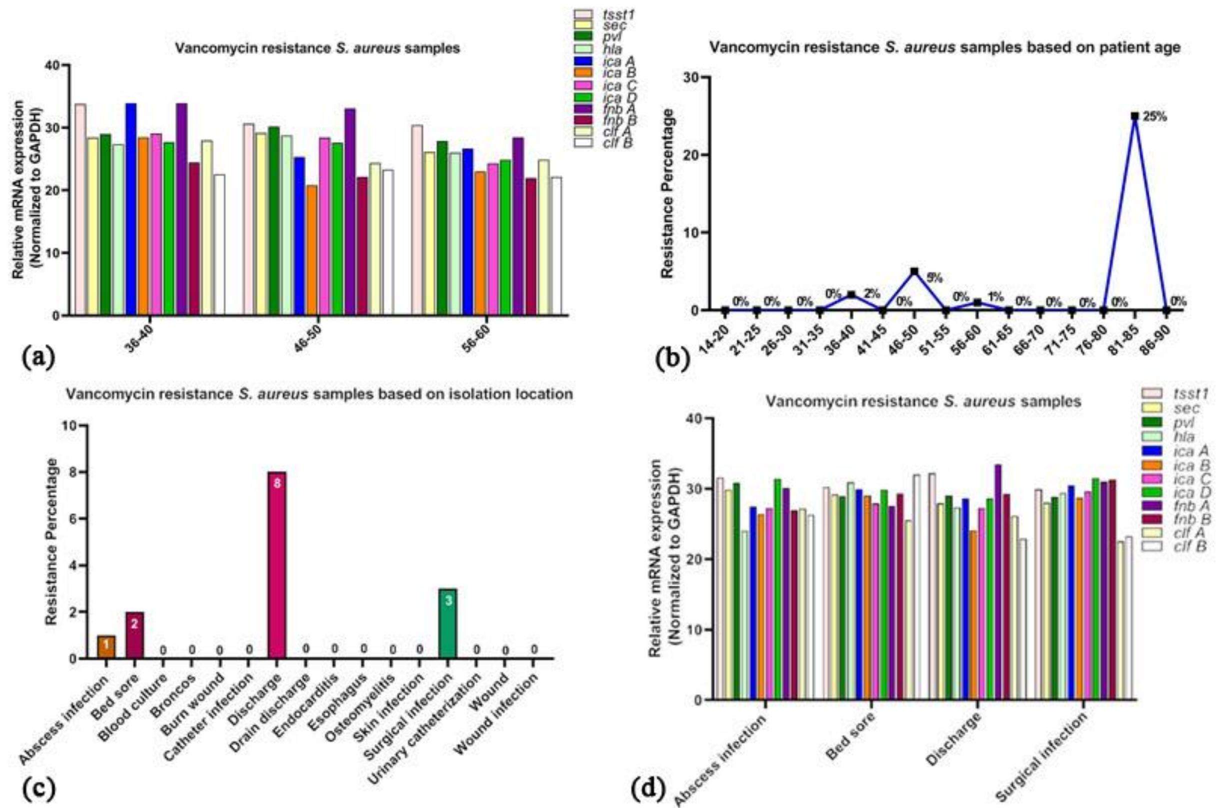

٢٥٣ **Figure 3.** The expression level of all studied genes in VRSA isolates according to age group(a)
٢٥٤ and isolation site(d). Percentage of VRSA isolates according to age group (b) and location site
٢٥٥ (c)
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