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Molecular Characteristics of *Staphylococcus aureus* Strains to Carry Panton-Valentine Leukocidin Genes Isolated from Hospitalized Patients in Tehran, Iran

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Abstract

Background: *Staphylococcus aureus* with concurrent resistance to antibacterial agents is emerging globally. This emergence might be due to the production of different virulence determinants, notably Panton-Valentine leukocidin (PVL).
Objectives: This study aimed to investigate the genetic characteristics of PVL-positive *S. aureus* strains isolates from clinical samples.
Methods: An epidemiological study was conducted on 65 *S. aureus* isolates carrying *pvl* genes. An antibiogram test by the disk diffusion and broth microdilution methods was conducted to assess antimicrobial resistance profiles.
Results: All detected methicillin-resistant *S. aureus* (MRSA) isolates were confirmed by *mec*A polymerase chain reaction (PCR) assays. The PVL-positive isolates were characterized using multiplex PCR assay to detect staphylococcal cassette chromosome mec (SCC*mec*) and *agr* types. The PVL frequency was 19.5% and 17.6% in MRSA and methicillin-susceptible *S. aureus* (MSSA), respectively. Among the PVL-positive isolates, 66.2% and 33.8% were MRSA and MSSA, respectively. Multidrug resistance amounted to 84.6% of the isolates (MRSA: 61.5%, MSSA: 23.1%). Staphylococcal cassette chromosome mec III was dominated (55.8%; 24/43). The most commonly identified *agr* was type III (53.8%; 35/65). Resistance to vancomycin amounted to 12.3% of the isolates, and all belonged to *agr* type III and SCC*mec* type III. The frequency of inducible and constitutive clindamycin resistance among PVL-positive MRSA strains (12.3% and 26.1%) was higher than PVL-positive MSSA strains (7.7% and 15.4%). Most constitutive and inducible clindamycin resistance isolates belonged to *agr* type III (26.2% and 18.5%) and SCC*mec* type III (each 27.9%). In the present study, 32.3% of the isolates were confirmed as mupirocin resistant, and all were MRSA, 9 (42.9%) and 12 (57.1%) isolates of which exhibited high-level mupirocin resistant (HLMUPR) and low-level mupirocin resistant phenotypes. All HLMUPR MRSA isolates belonged to SCC*mec* III and recovered from wound samples.
Conclusions: The emergence of vancomycin-resistant *S. aureus* strains among PVL-positive *S. aureus* strains in Iran is a serious alarm and seems to be becoming the greatest concern in the treatment of staphylococcal infections in the healthcare setting. The present study reinforces plausible direct transfers between community and nosocomial PVL-positive *S. aureus* types.

Keywords: *Staphylococcus aureus*, Methicillin-Resistant *Staphylococcus aureus*, Polymerase Chain Reaction (PCR), PVL, Vancomycin

1. Background

Staphylococcus aureus, as one of the most important nosocomial pathogens, is known to be responsible for a diverse array of infections. Several studies have recently focused on understanding the importance of expressed *S. aureus* genes, which are involved both in virulence and antibiotic resistance (1). The pathogenesis of this bacterium is linked to the expression of various virulence determinants. An important virulence factor identified in this microorganism is Panton-Valentine leukocidin (PVL) (1, 2). These strains are particularly important because they are primarily associated with wound and life-treating infections. The role of PVL in the pathogenesis of *S. aureus*

is clear; however, whether the toxin affects disease severity, clinical presentation, and outcome is a matter of debate (1-3).

Most clinical and epidemiological studies have focused on PVL as a potential marker for community-associated methicillin-resistant *S. aureus* (MRSA) infections. Some studies have recently attempted to address that PVL genes are also related to healthcare-associated methicillin-resistant *S. aureus* (HA-MRSA) strains that indicate a change in the epidemiology of PVL-positive *S. aureus* isolates (4).

Following efforts made to map out the prevalence and spread of different PVL-positive *S. aureus* types in different parts of the world, Shahini Shams Abadi et al., in a systematic review and meta-analysis, indicated the prevalence of PVL among *S. aureus* isolates obtained from cutaneous infections within the range of 7.4 - 55.6% (3). However, there have also been reports of a concurrent worldwide increase in the prevalence of PVL among *S. aureus* isolates in different parts of the world (3, 5-9). Although the prevalence and distribution of hospital-associated (HA)-*S. aureus* carrying *pvl* genes have been well established, but there is a shortage of data on the characteristics of the PVL-positive *S. aureus* lineages in Iran.

2. Objectives

Given the high frequency of PVL in HA-*S. aureus* strains and their genetic diversity, this study set out to establish the frequency, antibiotic resistance, and genetic characteristics of PVL-positive HA-*S. aureus* strains isolated from clinical samples.

3. Methods

3.1. Sample Collection and Staphylococcus aureus Isolation

The 65 *S. aureus* carrying *pvl* genes used in the present study were obtained from 345 *S. aureus* isolated from five hospitals during the period of 2 years from July 2020 to June 2022. To initially identify the isolates, bacteriological and biochemical techniques were recruited (10). For definite identification, polymerase chain reaction (PCR) for the *nuc*A gene was performed, and for the next step, the presence of the *pvl* genes was assessed using PCR assay (11).

3.2. Determination of Isolates' Susceptibility

The Kirby-Bauer disk diffusion method was performed under the Clinical and Laboratory Standards Institute (CLSI) criteria for the susceptibility evaluation of isolates against penicillin, tetracycline, rifampin, clindamycin, quinupristin-dalfopristin, erythromycin, ciprofloxacin, nitrofurantoin, linezolid, gentamicin, and trimethoprim-sulfamethoxazole (12). Susceptibility to vancomycin and mupirocin (i.e., low-level mupirocin resistant (LLMUPR) and high-level mupirocin resistance (HLMUPR)) was determined by the broth microdilution test according to the CLSI guidelines. The D-zone test was performed to detect inducible clindamycin resistance *S. aureus* isolates. Reference strains of *S. aureus* ATCC 25923, ATCC 43300, and ATCC 29213 were used to control the experiment. Multidrug-resistant (MDR) isolates cover resistance to three or more unique antibiotic classes.

3.3. Extraction of Genomic DNA

DNA was extracted from the isolates identified as PVL-positive *S. aureus* using the phenol-chloroform method, along with adding lysostaphin (Sigma-Aldrich Co., USA) at a final concentration of 30 μg/mL for the cell wall lysis as previously described. The purity of DNA was monitored by a spectrophotometer (10).

3.4. MRSA Screening

The in vitro evolution of methicillin resistance was performed with a cefoxitin disc (30 μg) on Mueller-Hinton agar plates under the CLSI guideline (12). The *mec*A-mediated resistance was detected using PCR, as previously described (10).

3.5. Genotypic Characterization

3.5.1. Staphylococcal Cassette Chromosome mec Typing

A multiplex PCR was recruited for identifying staphylococcal cassette chromosome mec (SCC*mec*) types based on the oligonucleotide sequences and conditions described by Boye et al. (13). Obtained banding patterns were analyzed by comparing them to the banding patterns of reference stains as follow:

ATCC 10442 (SCC*mec* type I), N315 (SCC*mec* type II), 85/2082 (SCC*mec* type III), MW2 (SCC*mec* type IVa), and WIS (SCC*mec* type V) as reference strains

3.5.2. Detection of agr Alleles

To amplify the hypervariable domain of the *agr* locus and subsequently typing of isolates, multiplex PCR was performed based on the Gilot et al. method (14). The banding patterns of each isolate were compared to *agr* reference strains as follows:

The *agr* group-I strains with a 441-bp fragment, *agr* group-II strains with a 575-bp fragment, *agr* group-III strains with a 323-bp fragment, and *agr* group-IV strains with a 659-bp fragment

4. Results

4.1. Participants, Isolation, and Screening of PVL-Positive Strains

In the current survey, 345 *S. aureus* isolates were collected from five hospitals (A-E) during the study period, 18.8% (n = 65) of which were PVL-positive isolates. The studied isolates comprised mixed specimen types; the majority of PVL-positive isolates were obtained from a wound, representing 43.1% (28/65); however, other sources included blood (23.1%; 15/65), purulent discharge (15.4%; 10/65), urine (7.7%; 5/65), sputum (6.1%; 4/65), and body fluids (4.6%; 3/65). The majority of PVL-positive *S. aureus* strains were isolated from hospital C (41.5%, 27/65), followed by hospital B (23.1%; 15/65), hospital A (15.4%; 10/65), hospital E (12.3%; 8/65), and hospital D (7.7%; 5/65). The distribution of strains in different wards included 18 (27.7%), 15 (23.1%), 13 (20%), 12 (18.5%), and 7 (10.7%) isolates from the intensive care unit, surgery, internal, infectious, and oncology wards, respectively.

4.2. Antimicrobial Resistance Profiles of PVL-Positive Isolates

As observed in Table 1, all the isolates were susceptible to linezolid. None of the isolates under study was susceptible to all of the antibiotics. The resistance rate of penicillin (PEN) was the highest (93.8%), followed by erythromycin (ERY) (84.6%), tetracycline (TET) (72.3%), ciprofloxacin (CIP) (70.8%), rifampin (RIF) (64.6%), gentamicin (GEN) (63.1%), clindamycin (CLI) (49.2%), nitrofurantoin (NIT) (44.6%), trimethoprim-sulfamethoxazole (SXT) (35.4%), mupirocin (MUP) (32.3%), quinupristin-dalfopristin (SYN) (27.7%), and vancomycin (VAN) (12.3%). Most of the PVL-positive isolates were confirmed as MRSA (66.2%); nevertheless, methicillin-susceptible *S. aureus* (MSSA) was detected in 33.8% of the isolates. All *S. aureus* strains isolated from urine (n = 5) and sputum (n = 4) were MSSA. The MRSA isolates showed increased resistance rates over MSSA isolates to examined antibiotics. Regarding MSSA isolates, higher resistance rates were recorded against PEN (30.8%), RIF (24.6%), and ERY (23.1%). None of the MSSA isolates was resistant to MUP and VAN. Higher resistance rates among MRSA isolates belonged to PEN (63.1%; 41/65), TET (61.5%), and ERY (61.5%). In total, 84.6% of the isolates were found to be MDR, 40 MRSA and 15 MSSA isolates of which verified to be MDR accounted for 61.5% and 23.1%, respectively.

As shown in Table 1, 11 resistance profiles were detected, wherein PEN, TET, CIP, RIF, NIT, GEN, and ERY (20%; 13/65), PEN, TET, CIP, RIF, GEN, ERY, and CLI (15.4%; 10/65), and PEN, TET, CIP, RIF, NIT, GEN, ERY, CLI, SYN, SXT, and MUP (13.8%; 9/65) were the top 3 frequently detected profiles. The frequency of inducible and constitutive CLI resistance among PVL-positive MRSA strains (12.3% and 26.1%) was higher than PVL-positive MSSA strains (7.7% and 15.4%). In the present study, 32.3% of the isolates were confirmed as MUP resistant, and all were MRSA, 9 (42.9%) and 12 (57.1%) isolates of which exhibited HLMUPR and LLMUPR phenotypes.

4.3. Molecular Characterization

The SCC*mec* typing results showed that the most prevalent SCC*mec* type was III, representing 55.8% (24/43); nonetheless, 8 (18.6%), 6 (14%), and 5 (11.6%) isolates expressed SCC*mec* types II, IV, and I, respectively. Staphylococcal cassette chromosome mec type I was recovered from blood (40%; 2/5), wound (40%; 2/5), and body fluid (20%; 1/5). Staphylococcal cassette chromosome mec types II were recovered from blood (37.5%; 3/8), wound (12.5%; 1/8), and purulent discharge (50%; 4/8). Staphylococcal cassette chromosome mec types III were recovered from blood (33.3%; 8/24), wound (54.2%; 13/24), body fluid (4.2%; 1/24), and purulent discharge (8.3%; 2/24). Staphylococcal cassette chromosome mec types IV were recovered from the wound (83.3%; 5/6) and body fluid (16.7%; 1/6) samples. All 8 vancomycin-resistant MRSA isolates belonged to SCC*mec* III, 5 (62.5%) and 3 (37.5%) isolates of which had constitutive and inducible clindamycin resistance phenotypes, respectively. All HLMUPR MRSA isolates belonged to SCC*mec* III and recovered from the wound samples. The LLMUPR accounted for 6.2% (4/65), 1.5% (1/65), 4.6% (3/65), and 3.1% (2/65) of SCC*mec* types I, II, III, and IV, respectively. Figure 1 depicts a summary of the distribution of resistance profiles among different SCC*mec* types.

According to *agr* typing technique, 35 (53.8%), 12 (18.5%), 10 (15.4%), and 8 (12.3%) isolates harbored *agr* types III, I, II, and IV, respectively. The obtained results revealed that predominantly PVL-positive MRSA strains expressed *agr* type III (55.8%; 24/43), followed by types I and II (18.6%; every 8 isolates/43) and IV (7%; 3/43); however, PVL-positive MSSA strains predominantly harbored *agr* types III (50%; 11/22), IV (22.7%; 5/22), I (18.2%; 4/22), and II (9.1%; 2/22). Figure 2 depicts a summary of the distribution of resistance profiles among different *agr* types. The *agr* type I was not detected in any PVL-positive *S. aureus* strains recovered from blood and sputum samples. Most *agr* type III isolates were isolated from the wound (34.3%; 12/35) and blood (34.3%; 12/35) samples.

5. Discussion

This survey demonstrated several striking results, including a relatively high frequency of PVL-positive *S.*

Table 1. Resistance Combinations of Panton-Valentine Leukocidin-Positive Strains and Their Distribution Among Clinical Samples

Simultaneous Resistance to Antibiotics and Resistance Profile	Resistance Pattern	Type of Samples (n; % Indicated When Not 100%)	MRSA/MSSA (n; % Indicated When Not 100%)	Number of Isolates (%)
Eleven				
A	PEN, TET, CIP, RIF, NIT, GEN, ERY, CLI, SYN, SXT, MUP	W (6; 66.7), B (3; 33.3)	MRSA	9 (13.8)
B	PEN, TET, RIF, NIT, GEN, ERY, CLI, SYN, SXT, MUP, VAN	W (3)	MRSA	3 (4.6)
Seven				
C	PEN, TET, CIP, RIF, GEN, ERY, CLI	W (3; 30), PD (4; 40), U (2; 20), BF (1; 10)	MRSA (5; 50), MSSA (5; 50)	10 (15.4)
D	PEN, TET, CIP, GEN, ERY, SXT, VAN	W (1; 20), BF (1; 20), B (3; 60)	MRSA	5 (7.7)
E	PEN, TET, CIP, RIF, NIT, GEN, ERY	W (9; 69.2), B (3; 23.1), U (1; 7.7)	MRSA (6; 46.2), MSSA (7; 30.8)	13 (20)
Six				
F	PEN, TET, CIP, ERY, CLI, MUP	W (2; 28.6), PD (2; 28.6), B (2; 28.6), BF (1; 14.2)	MRSA	7 (10.8)
G	PEN, ERY, CLI, SYN, SXT, NIT	W (1; 33.3), B (2; 66.7)	MRSA	3 (4.6)
Four				
H	PEN, ERY, SYN, SXT	S (2; 66.7), U (1; 33.3)	MSSA	3 (4.6)
Three				
I	CIP, ERY, MUP	PD (2)	MRSA	2 (3.1)
J	PEN, NIT, GEN	U (1)	MSSA	1 (1.5)
Two				
K	PEN, RIF	W (3; 42.8), PD (2; 28.6), B (2; 28.6)	MRSA (3; 42.9), MSSA (4; 57.1)	7 (10.8)
Without				
L	-	S (2)	MSSA	2 (3.1)

Abbreviations: PEN, penicillin; CLI, clindamycin; NIT, nitrofurantoin; ERY, erythromycin; TET, tetracycline; CIP, ciprofloxacin; MUP, mupirocin; GEN, gentamicin; SYN, quinupristin-dalfopristin; VAN, vancomycin; SXT, trimethoprim-sulfamethoxazole; RIF, rifampicin; W, wound; B, blood; BF, body fluids; U, urine; S, sputum; PD, purulent discharge; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*.

aureus strains and a high diversity of their types. This study also showed a high frequency of PVL-positive *S. aureus* with simultaneous resistance to antibacterial agents. It manifests as a prominent threat in clinical settings. Another finding of the present study was the high resistance prevalence to vancomycin among *S. aureus* isolates.

The frequency of PVL among *S. aureus* in this study was 18.8%, a higher prevalence than that observed (4.9%) in *S. aureus*-related infections in the United Kingdom (7). However, various percentages of PVL-carrying *S. aureus* were reported by several studies worldwide (5-8). A high prevalence of PVL-carrying *S. aureus* strains has also been reported in Tunisia (79%) (8), Uganda (49.3%) (5), and Saudi Arabia (30%) (6). The findings of the current survey indicated that the prevalence of PVL-carrying *S. aureus* in Iran is quite similar to the previously reported rates of 21.4% and 19.5% in 2016 and 2017 in Iranian hospitals, respectively (11). The results pointed to a conclusion that despite the low prevalence of PVL positive, special attention for the laboratory routine detection of these isolates is needed because the mobility of the *pvl* gene across MRSA isolates might increase the morbidity of nosocomial infections caused by HA-MRSA (15-17).

Mupirocin is used to control the dissemination of *S. aureus* isolates in communities and healthcare settings and the occurrence of severe infections (18-21). In the present survey, the data showed a high prevalence rate of mupirocin-resistant MRSA isolates (32.3%). This finding is supported by the observations of Chamon et al. from Brazil (33%) (17) and Goudarzi et al. from Iran (30.5%) (11). In a recent meta-analysis study by Dadashi et al., various prevalence rates of mupirocin-resistant MRSA isolates were reported in different geographic areas (e.g., less than 1.0% in France, India, Iran, and Australia or more than 50% in India, the USA, and Egypt) (19). The reason for the

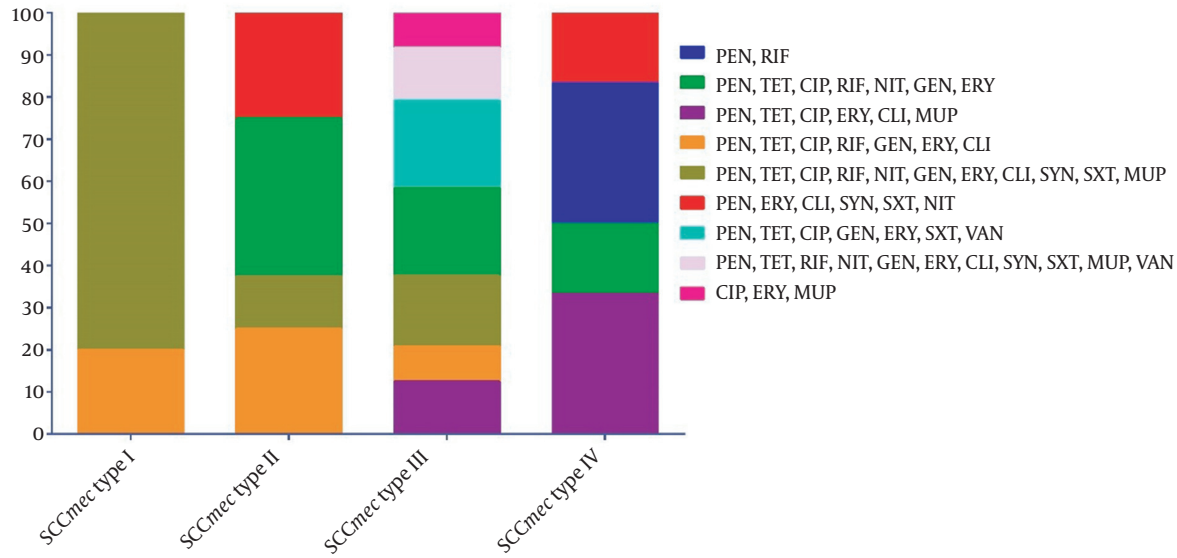

Figure 1. Distribution of resistance profiles in different staphylococcal cassette chromosome mec (SCC*mec*) types. Abbreviations: PEN, penicillin; RIF, rifampin; TET, tetracycline; CIP, ciprofloxacin; NIT, nitrofurantoin; GEN, gentamicin; ERY, erythromycin; CLI, clindamycin; MUP, mupirocin; SYN, quinupristin-dalfopristin; SXT, trimethoprim-sulfamethoxazole; VAN, vancomycin.

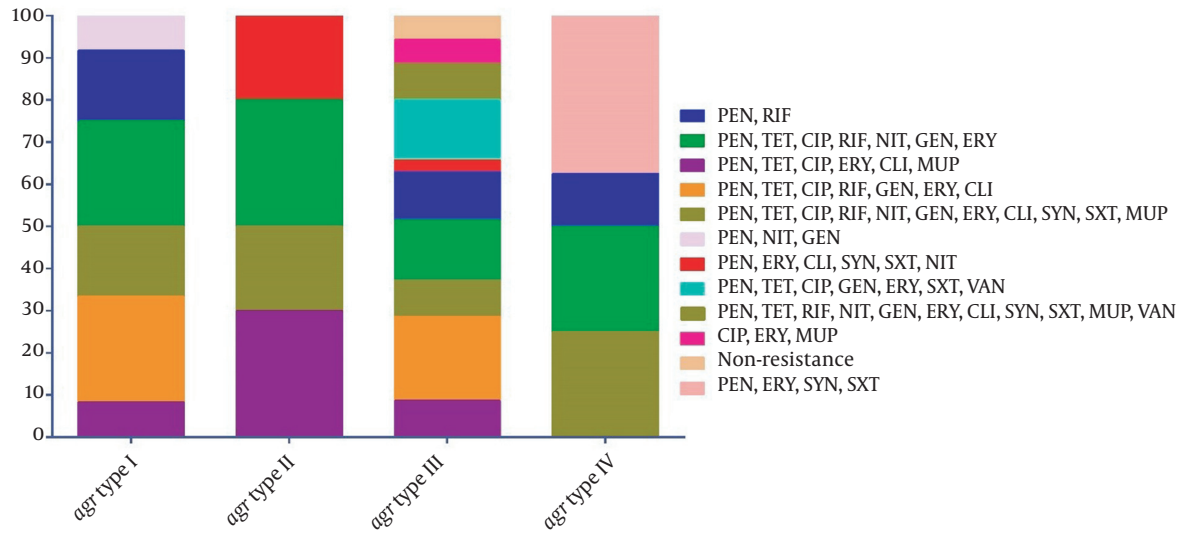

Figure 2. Distribution of resistance profiles in different *agr* types. Abbreviations: PEN, penicillin; RIF, rifampin; TET, tetracycline; CIP, ciprofloxacin; NIT, nitrofurantoin; GEN, gentamicin; ERY, erythromycin; CLI, clindamycin; MUP, mupirocin; SYN, quinupristin-dalfopristin; SXT, trimethoprim-sulfamethoxazole; VAN, vancomycin.

high mupirocin resistance rate is not well-understood but can be attributed to the ward, sample type, geographic or socioeconomic factors, and policies in the use of mupirocin in hospitals.

Furthermore, this study reported a high prevalence of HLMUPR and LLMUPR at 42.9% and 57.1% among MRSA isolates, respectively. A recent study in Egypt reported a high prevalence of HLMUPR and LLMUPR, with a proportion of 61.5% and 38.5% (22). In the present survey, the prevalence rates of high and low levels of resistance to mupirocin in MRSA isolates were reported as 13.5% and 18.5%, respectively. The reported rate of HLMUPR-MRSA in this study was higher than in France (0.8%) (20), Canada (4.3%) (18), and China (7%) (21). Overall,

the reason for this high mupirocin resistance rate is not well-understood; however, it appears to be related to a shortage in the implementation of antibiotic stewardship programs, incorrect policies unrestricted, and widespread use of this antibiotic. Therefore, before mupirocin therapy, it is essential to determine the susceptibility of isolates to mupirocin.

Methicillin-resistant *S. aureus* is important; nevertheless, the emergence of vancomycin-resistant *S. aureus* (VRSA) represents an additional challenge and concern for controlling staphylococcal infections (23). It is worth noting that VRSA was found in 12.3% of PVL-positive isolates. The results of a meta-analysis performed in 2020 depicted an upward trend in VRSA and vancomycin-intermediate *S. aureus* (VISA) worldwide. Shariati et al. demonstrated that VISA strains (1.7%) had a higher global rate than VISA strains (1.5%). Likewise, an increasing trend of 2 and 3.6-fold of VRSA and VISA after 2010, compared to before that, was noted. However, Asian countries, especially Iran and India, included the highest rates of VRSA incidence (67%) (23). This relatively high prevalence of VRSA in the two aforementioned countries, compared to American/European countries, can be due to the unrestricted and unscheduled administration of antimicrobials, geographic area, level of hygiene, poor health policies, and diverse attitudes toward antimicrobial protocols.

The present study's observations about SCC*mec* types are in line with those of other studies that confirmed the relationship of SCC*mec* types I, II, and III with HA-*S. aureus* infections; nevertheless, IV and V are prominent types in community-associated (CA)-*S. aureus* infections (24, 25). Staphylococcal cassette chromosome mec typing illustrated a dominance for type III at 55.8%, which is similar to the results of several studies indicating PVL-positive HA-MRSA strains carrying SCC*mec* types II and III. This finding indicated that PVL-positive strains with SCC*mec* type III spread in different regions of Iran. Contrary to the finding of a study by Chamon et al. (17) from Brazil which reported a predominance of one SCC*mec* IV in PVL-positive *S. aureus* strains, representing 62% of the isolates, the current study's observations indicated SCC*mec* type IV at a low level (14%). This emergence has been reported regionally to higher levels in reports of the nearby countries, with dominance in SCC*mec* type IV ranging from 19% to 90% (26).

Although numerous studies have characterized *agr* types of HA- and CA-*S. aureus* isolates from the community and hospitals; narrow studies have focused on the features description of *agr* types among PVL-positive *S. aureus* strains (8, 10). The results of *agr* typing performed for strains also showed that *agr* type I was the second predominant genotype in PVL-positive strains. In a study in Thailand on 92 *S. aureus* strains, this type was more frequent in the tested isolates (27). Another study conducted by Javdan et al. on 150 *S. aureus* isolates showed that *agr* type I was predominant (54.7%), followed by type II (24.7%), type IV (14%), and type III (6.6%) (28). The present study observed relatively low infection rates of 15.4% and 12.3% of *agr* types II and IV, respectively. Similar rates were reported by Ghasemian et al. (29). It could be speculated that *agr* type I can have a crucial task in the regulation of staphylococcal toxins, especially PVL. The high prevalence of *agr* type III among PVL-positive isolates promoted us to understand its virulence.

5.1. Conclusions

Overall, this study indicated a diversity of *agr* and SCC*mec* types, easily transferred "from and to" hospitals. The widespread dissemination of MDR PVL-positive *S. aureus* strains was a wake-up call for researchers. Resistance to vancomycin in this study emphasized that using this and other antibiotics, especially mupirocin and clindamycin, and resistance to them should be carefully monitored.

Footnotes

Authors' Contribution: Mehdi Goudarzi conceived and designed the experiments performed the experiments, analyzed and interpreted the data, and wrote the paper. Chakameh Amini provided the materials, analysis tools, and data, performed the experiments, and wrote the paper. Maryam Fazeli analyzed and interpreted the data and wrote the paper. Mohammad Javad Nasiri conceived and designed the experiments. Sara Bahonar analyzed and interpreted the data and wrote the paper. Masoud Dadashi performed the experiments and wrote the paper. Mehrdad Haghighi contributed to reagents and analyzed and interpreted the data. Mirmohammad Miri analyzed and interpreted the data.

Conflict of Interests: The authors declare that they have no conflict of interest.

Data Reproducibility: The dataset presented in the study is available on request from the corresponding author during submission or after publication. The data are not publicly available due to privacy.

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References

1. Cheung GYC, Bae JS, Otto M. Pathogenicity and virulence of Staphylococcus aureus. *Virulence*. 2021;**12**(1):547–69. [PubMed ID: 33522395]. [PubMed Central ID: PMC7872022]. https://doi.org/10.1080/21505594.2021.1878688.
2. Turner NA, Sharma-Kuinkel BK, Maskarinec SA, Eichenberger EM, Shah PP, Carugati M, et al. Methicillin-resistant Staphylococcus aureus: an overview of basic and clinical research. *Nat Rev Microbiol*. 2019;**17**(4):203–18. [PubMed ID: 30737488]. [PubMed Central ID: PMC6939889]. https://doi.org/10.1038/s41579-018-0147-4.
3. Shahini Shams Abadi M, Nikokar I, Hoseini Alfatemi SM, Malekzadegan Y, Azizi A, Sedigh Ebrahim-Saraie H. Epidemiology of Panton-Valentine Leukocidin harbouring Staphylococcus aureus in cutaneous infections from Iran: a systematic review and meta-analysis. *Infez Med*. 2017;**25**(3):217–23. [PubMed ID: 28956538].
4. Ozekinci T, Dal T, Yanik K, Ozcan N, Can S, Tekin A, et al. Panton-Valentine leukocidin in community and hospital-acquired Staphylococcus aureus strains. *Biotechnol Biotechnol Equip*. 2014;**28**(6):1089–94. [PubMed ID: 26019595]. [PubMed Central ID: PMC4433891]. https://doi.org/10.1080/13102818.2014.976457.
5. Asiimwe BB, Baldan R, Trovato A, Cirillo DM. Molecular epidemiology of Panton-Valentine Leukocidin-positive community-acquired methicillin resistant Staphylococcus aureus isolates in pastoral communities of rural south western Uganda. *BMC Infect Dis*. 2017;**17**(1):24. [PubMed ID: 28056833]. [PubMed Central ID: PMC5216539]. https://doi.org/10.1186/s12879-016-2124-8.
6. Bazzi AM, Rabaan AA, Fawarah MM, Al-Tawfiq JA. Prevalence of Panton-Valentine leukocidin-positive methicillin-susceptible Staphylococcus aureus infections in a Saudi Arabian hospital. *J Infect Public Health*. 2015;**8**(4):364–8. [PubMed ID: 25817805]. https://doi.org/10.1016/j.jiph.2015.01.010.
7. Holmes A, Ganner M, McGuane S, Pitt TL, Cookson BD, Kearns AM. Staphylococcus aureus isolates carrying Panton-Valentine leucocidin genes in England and Wales: frequency, characterization, and association with clinical disease. *J Clin Microbiol*. 2005;**43**(5):2384–90. [PubMed ID: 15872271]. [PubMed Central ID: PMC1153723]. https://doi.org/10.1128/JCM.43.5.2384-2390.2005.
8. Mariem BJ, Ito T, Zhang M, Jin J, Li S, Ilhem BB, et al. Molecular characterization of methicillin-resistant Panton-valentine leukocidin positive staphylococcus aureus clones disseminating in Tunisian hospitals and in the community. *BMC Microbiol*. 2013;**13**:2. [PubMed ID: 23289889]. [PubMed Central ID: PMC3544733]. https://doi.org/10.1186/1471-2180-13-2.
9. Pokhrel RH, Aung MS, Thapa B, Chaudhary R, Mishra SK, Kawaguchiya M, et al. Detection of ST772 Panton-Valentine leukocidin-positive methicillin-resistant Staphylococcus aureus (Bengal Bay clone) and ST22 S. aureus isolates with a genetic variant of elastin binding protein in Nepal. *New Microbes New Infect*. 2016;**11**:20–7. [PubMed ID: 27014464]. [PubMed Central ID: PMC4789347]. https://doi.org/10.1016/j.nmni.2016.02.001.
10. Nasirian S, Saadatmand S, Goudarzi H, Goudarzi M, Azimi H. Molecular Investigation of Methicillin-Resistant Staphylococcus aureus Strains Recovered from the Intensive Care Unit (ICU) Based on Toxin, Adhesion Genes and agr Locus Type Analysis. *Arch Clin Infect Dis*. 2018;**13**(2):e14495. https://doi.org/10.5812/archcid.14495.
11. Goudarzi M, Seyedjavadi SS, Nasiri MJ, Goudarzi H, Sajadi Nia R, Dabiri H. Molecular characteristics of methicillin-resistant Staphylococcus aureus (MRSA) strains isolated from patients with bacteremia based on MLST, SCCmec, spa, and agr locus types analysis. *Microb Pathog*. 2017;**104**:328–35. [PubMed ID: 28159661]. https://doi.org/10.1016/j.micpath.2017.01.055.
12. Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing*. Wayne, PA: Clinical and Laboratory Standards Institute; 2021.
13. Boye K, Bartels MD, Andersen IS, Moller JA, Westh H. A new multiplex PCR for easy screening of methicillin-resistant Staphylococcus aureus SCCmec types I-V. *Clin Microbiol Infect*. 2007;**13**(7):725–7. [PubMed ID: 17403127]. https://doi.org/10.1111/j.1469-0691.2007.01720.x.
14. Gilot P, Lina G, Cochard T, Poutrel B. Analysis of the genetic variability of genes encoding the RNA III-activating components Agr and TRAP in a population of Staphylococcus aureus strains isolated from cows with mastitis. *J Clin Microbiol*. 2002;**40**(11):4060–7. [PubMed ID: 12409375]. [PubMed Central ID: PMC139642]. https://doi.org/10.1128/JCM.40.11.4060-4067.2002.
15. Hussain K, Bandyopadhyay A, Roberts N, Mughal N, Moore LSP, Fuller LC. Panton-Valentine leucocidin-producing Staphylococcus aureus: a clinical review. *Clin Exp Dermatol*. 2022;**47**(12):2150–8. [PubMed ID: 36040400]. https://doi.org/10.1111/ced.15392.
16. Shore AC, Tecklenborg SC, Brennan GI, Ehricht R, Monecke S, Coleman DC. Panton-Valentine leukocidin-positive Staphylococcus aureus in Ireland from 2002 to 2011: 21 clones, frequent importation of clones, temporal shifts of predominant methicillin-resistant S. aureus clones, and increasing multiresistance. *J Clin Microbiol*. 2014;**52**(3):859–70. [PubMed ID: 24371244]. [PubMed Central ID: PMC3957793]. https://doi.org/10.1128/JCM.02799-13.
17. Chamon RC, Iorio NL, da Silva Ribeiro S, Cavalcante FS, Dos Santos KR. Molecular characterization of Staphylococcus aureus isolates carrying the Panton-Valentine leukocidin genes from Rio de Janeiro hospitals. *Diagn Microbiol Infect Dis*. 2015;**83**(4):331–4. [PubMed ID: 26431830]. https://doi.org/10.1016/j.diagmicrobio.2015.09.004.
18. Babu T, Rekasius V, Parada JP, Schreckenberger P, Challapalli M. Mupirocin resistance among methicillin-resistant Staphylococcus aureus-colonized patients at admission to a tertiary care medical center. *J Clin Microbiol*. 2009;**47**(7):2279–80. [PubMed ID: 19474267]. [PubMed Central ID: PMC2708468]. https://doi.org/10.1128/JCM.01834-08.
19. Dadashi M, Hajikhani B, Darban-Sarokhalil D, van Belkum A, Goudarzi M. Mupirocin resistance in Staphylococcus aureus: A systematic review and meta-analysis. *J Glob Antimicrob Resist*. 2020;**20**:238–47. [PubMed ID: 31442624]. https://doi.org/10.1016/j.jgar.2019.07.032.
20. Desroches M, Potier J, Laurent F, Bourrel AS, Doucet-Populaire F, Decousser JW, et al. Prevalence of mupirocin resistance among invasive coagulase-negative staphylococci and methicillin-resistant Staphylococcus aureus (MRSA) in France: emergence of a mupirocin-resistant MRSA clone harbouring mupA. *J Antimicrob Chemother*. 2013;**68**(8):1714–7. [PubMed ID: 23535880]. https://doi.org/10.1093/jac/dkt085.
21. Liu QZ, Wu Q, Zhang YB, Liu MN, Hu FP, Xu XG, et al. Prevalence of clinical meticillin-resistant Staphylococcus aureus (MRSA) with high-level mupirocin resistance in Shanghai and Wenzhou, China. *Int J Antimicrob Agents*. 2010;**35**(2):114–8. [PubMed ID: 19939636]. https://doi.org/10.1016/j.ijantimicag.2009.09.018.
22. Barakat GI, Nabil YM. Correlation of mupirocin resistance with biofilm production in methicillin-resistant Staphylococcus aureus from surgical site infections in a tertiary centre, Egypt. *J Glob Antimicrob Resist*. 2016;**4**:16–20. [PubMed ID: 27436387]. https://doi.org/10.1016/j.jgar.2015.11.010.
23. Shariati A, Dadashi M, Moghadam MT, van Belkum A, Yaslianifard S, Darban-Sarokhalil D. Global prevalence and distribution of vancomycin resistant, vancomycin intermediate and heterogeneously vancomycin intermediate Staphylococcus aureus clinical isolates: a systematic review and meta-analysis. *Sci Rep*.

2020;**10**(1):12689. [PubMed ID: 32728110]. [PubMed Central ID: PMC7391782]. https://doi.org/10.1038/s41598-020-69058-z.

24. Gulmez D, Sancak B, Ercis S, Karakaya J, Hascelik G. [Investigation of SCCmec types and Panton-Valentine leukocidin in community-acquired and nosocomial Staphylococcus aureus strains: comparing skin and soft tissue infections to the other infections]. *Mikrobiyol Bul*. 2012;**46**(3):341–51. Turkish. [PubMed ID: 22951646].
25. Liu J, Chen D, Peters BM, Li L, Li B, Xu Z, et al. Staphylococcal chromosomal cassettes mec (SCCmec): A mobile genetic element in methicillin-resistant Staphylococcus aureus. *Microb Pathog*. 2016;**101**:56–67. [PubMed ID: 27836760]. https://doi.org/10.1016/j.micpath.2016.10.028.
26. Al-Saleh A, Shahid M, Farid E, Bindayna K. Trends in methicillin-resistant Staphylococcus aureus in the Gulf Cooperation Council countries: antibiotic resistance, virulence factors and emerging strains. *East Mediterr Health J*. 2022;**28**(6):434–43. [PubMed ID: 35815875]. https://doi.org/10.26719/emhj.22.042.
27. Indrawattana N, Sungkhachat O, Sookrung N, Chongsa-nguan M, Tungtrongchitr A, Voravuthikunchai SP, et al. Staphylococcus aureus clinical isolates: antibiotic susceptibility, molecular characteristics, and ability to form biofilm. *Biomed Res Int*. 2013;**2013**:314654. [PubMed ID: 24069597]. [PubMed Central ID: PMC3773402]. https://doi.org/10.1155/2013/314654.
28. Javdan S, Narimani T, Shahini Shams Abadi M, Gholipour A. Agr typing of Staphylococcus aureus species isolated from clinical samples in training hospitals of Isfahan and Shahrekord. *BMC Res Notes*. 2019;**12**(1):363. [PubMed ID: 31248448]. [PubMed Central ID: PMC6598336]. https://doi.org/10.1186/s13104-019-4396-8.
29. Ghasemian A, Peerayeh SN, Bakhshi B, Mirzaee M. Detection of accessory gene regulator groups genes and cassette chromosome mec types among Staphylococcus aureus isolated from intensive care unit patients. *Asian Pac J Trop Dis*. 2015;**5**(2):153–7. https://doi.org/10.1016/s2222-1808(14)60643-5.