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Molecular characterizations of vancomycin and methicillin-resistant *Staphylococcus aureus* strains isolated from intensive care unit patients in Tehran, Iran

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ABSTRACT

Background: We investigated the prevalence, diversity, and antibiotic susceptibility profiles of methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *S. aureus* (VRSA) strains isolated from ICU patients in Tehran, Iran.
Materials & Methods: A total of 247 *S. aureus* isolates were collected from patients admitted to the ICU of a referral hospital in Tehran between June and August 2022. Antimicrobial susceptibility testing was done on all isolates using the disc diffusion method. MRSA was identified by cefoxitin screening and *mec*A gene detection. Vancomycin resistance was assessed by E-test and confirmed by the detection of *van*A and *van*B genes. A combination of Staphylococcal cassette chromosome *mec* (SCC*mec*), pulsed-field gel electrophoresis (PFGE), and *ccr* typing methods was used to measure the genetic diversity of the strains.
Findings: Our results revealed that 60 (24%) and 7 (3%) isolates were identified as MRSA and VRSA, respectively. *van*A and *van*B genes were detected in 100% and 29% of VRSA isolates, respectively. There was a high level of resistance to penicillin, ciprofloxacin, and tobramycin. Out of 205 (83%) strains showing SCC*mec,* 96 (47%) belonged to type III SCC*mec*. Typing of the isolates with PFGE showed the presence of 32 pulsotypes consisting of 19 common (CTs) and 13 single types (STs) among the studied strains, with CT3 comprising 43% of VRSA strains.
Conclusion: The detection of a dominant VRSA clone and a considerable prevalence of MRSA among ICU patients underscores the role of ICUs as potential reservoirs for MRSA dissemination.

Keywords: Intensive care units, *Staphylococcus aureus,* Methicillin resistance, Vancomycin resistance, Molecular typing

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Introduction

Over recent decades, *Staphylococcus aureus* has become increasingly prevalent in human infections, mainly because of its remarkable ability to develop resistance to multiple antimicrobial agents, particularly β-lactam antibiotics. Methicillin resistance in *S. aureus* arises from the production of an altered penicillin-binding protein, PBP2a, which is encoded by the *mec*A gene and carried on the staphylococcal cassette chromosome *mec* (SCC*mec*) [1]. This modified protein exhibits a low affinity for most β-lactam agents, allowing MRSA strains to bypass the inhibitory effects on native penicillin-binding proteins and sustain cell wall synthesis despite antibiotic exposure.

Based on the previous reports, patients admitted to intensive care units (ICU) are at elevated risk for MRSA infection due to factors such as severity of disease and usage of intravascular devices [2]. The high frequency of MRSA strains has forced clinicians to use vancomycin, a glycopeptide antibiotic, as the first line of treatment against MRSA. However, the abrupt and sustained increase in the clinical use of vancomycin has been associated with the emergence of two phenotypes of *S. aureus* with reduced susceptibility to glycopeptides. One phenotype, vancomycin-intermediate *S. aureus* (VISA), is characterized by a thickened cell wall with low cross-linking and reduced autolytic activity, which limits the access of vancomycin to its target site. The second phenotype, vancomycin-resistant *S. aureus* (VRSA), exhibits high-level resistance to this therapeutic agent, primarily through acquisition of the *van* gene cluster from the *Enterococcus* genus [3].

With the emergence of newly identified VRSA strains, epidemiological investigations have become essential for tracking their spread and for guiding effective infection control measures aimed at preventing successive outbreaks [4]. Among many typing methods, pulsed-field gel electrophoresis (PFGE) is the gold standard genetic typing technique for epidemiological studies of *S. aureus* strains. Despite its high discriminatory capacity, PFGE is limited by practical challenges, including labor-intensive procedures and difficulties in standardizing protocols and comparing results across different laboratories [5]. Also, SCC*mec* typing is an important method routinely used to evaluate the epidemiology of MRSA because the SCC*mec* has an important role in the antibiotic-resistance characteristics and evolution of MRSA clones [1]. SCC*mec* elements are categorized into various types and subtypes. To date, 15 SCC*mec* types have been distinguished and approved [6]. One of the drawbacks of SCC*mec* typing is the complexity of the typing system because SCC*mec* region is variable and newer types are permanently being defined [5]. Due to the limitations of each molecular technique, the combination of existing approaches can enhance the sensitivity and accuracy of the detection of pathogenic bacteria [7].

Despite the rising trend of VRSA infections in recent years, there is very little information about antibiotic resistance and clonal dissemination of VRSA strains in Iran.

Objectives: The present research aimed to investigate the prevalence, antimicrobial resistance patterns, and molecular characteristics of MRSA and VRSA isolates recovered from patients admitted to the ICUs of a referral hospital in Tehran, Iran.

Materials and Methods

Sample collection and characterization of MRSA isolates: Between June and August 2022, a total of 247 *S. aureus* isolates, recovered from various clinical samples of patients admitted to the ICUs, were collected from a referral hospital's diagnostic laboratory in the northern part of Tehran.

Iran. Only isolates recovered from clinical specimens of ICU patients with suspected infections were included in this study. Surveillance or colonization isolates were not included.

All specimens were transported to the bacteriology laboratory, Department of Microbiology, located at the University of Isfahan by maintaining the cold chain. Dark brown to black colonies grown on HiCrome aureus agar medium (HiMedia, India)were suspected as *S. aureus* and confirmed by amplification of the *nuc*A gene as described before [8].

Antimicrobial susceptibility testing: Based on the CLSI instructions, the disc diffusion method was done on all *S. aureus* isolates for susceptibility testing against 17 antibiotic disks (Mast Group Ltd., Merseyside, UK). These included kanamycin (30 µg), penicillin G (10 U), amikacin (30 µg), erythromycin (15 µg), teicoplanin (30 µg), clindamycin (2 µg), minocycline (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), linezolid (30 µg), chloramphenicol (30 µg), quinupristin-dalfopristin (15 µg), tobramycin (10 µg), rifampin (5 µg), nitrofurantoin (300 µg), tetracycline (30 µg), and trimethoprim-sulfamethoxazole (1.25-23.75 µg).

DNA extraction: Genomic DNA was extracted from all *S. aureus* isolates using the boiling method for initial molecular screening. For MRSA and VRSA isolates subjected to further molecular analyses, DNA was re-extracted using the QIAamp DNA Mini Kit (Qiagen, Germany) based on the manufacturer's instructions [9].

Phenotypic and molecular identification of MRSA strains: Screening for the MRSA strains was performed by assessing their susceptibility to cefoxitin using the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [10]. All cefoxitin-resistant strains were identified as MRSA using primers (Table 1) for the *mec*A and reaction conditions described previously [9]. In addition, oxacillin minimum inhibitory concentrations (MICs) were determined by E-test strips (bioMérieux, France) to further characterize the level of methicillin resistance among MRSA isolates. Isolates with oxacillin MIC values ≥4 µg/mL were considered methicillin resistant as recommended by CLSI [10].

Phenotypic and genotypic characterization of VRSA isolates: Vancomycin susceptibility among MRSA isolates was assessed by determination of MICs using E-test strips (bioMérieux, France). Isolates exhibiting vancomycin MIC values ≥16 µg/mL were regarded as VRSA. Phenotypically identified VRSA isolates were subsequently subjected to molecular confirmation by multiplex PCR targeting the vancomycin resistance genes *van*A and *van*B (the sequences of the primer are shown in Table 1). PCR amplification conditions were performed as previously described [11].

Amplification of the *pvl* gene and SCC*mec* and *ccr* typing: The presence of the lukS/F-*pvl* gene (encoding the PVL S/F bicomponent protein) among MRSA strains was tested by PCR using fragment-specific primers. Various types and subtypes of SCC*mec* (I, II, III, IVa, IVb, IVc, IVd, and V) as well as the types of *ccr* gene among *mec*A positive isolates were determined using a multiplex PCR. The sequence of each primer used in this study are provided in Table 1 and PCR amplification conditions were carried out according to previously published protocol [12].

Pulsed-field gel electrophoresis (PFGE): A PFGE analysis was done on all MRSA isolates employing the CHEF-DR III system (Bio-Rad, Hercules, CA, USA). PFGE was performed according to a previously validated protocol, using standardized electrophoresis conditions as described by Chung et al. [13]. A dendrogram was constructed using the unweighted pair group method with the

Table 1) Primer sequences and target genes used for PCR assays.

Primer	Oligonucleotide sequence (5´ to 3´)	Amplicon size (bp)
*nuc*A-F *nuc*A-R	TAATGTACAAAGGTCAAC TGATAAATATGGACGTGGCT	310
*mec*A-F *mec*A-R	TGGCTATCGTGTCACAATCG CTGGAACTTGTTGAGCAGAG	195
*van*A-F *van*A-R	CATGAATAGAATAAAAGTTGCAATA CCCCTTTAACGCTAATACGATCAA	1030
*van*B-F *van*B-R	GTGACAAACCGGAGGCGAGGA CCGCCATCCTCCTGCAAAAAA	433
SCC*mec* type I-F SCC*mec* type I-R	GCTTTAAAGAGTGTCGTTACAGG GTCTCTCATAGTATGACGTCC	613
SCC*mec* type II-F SCC*mec* type II-R	CGTTGAAGATGATGAAGCG CGAAATCAATGGTTAATGGACC	398
SCC*mec* type III-F SCC*mec* type III-R	CCATATTGTGTACGATGCG CCTTAGTTGTCGTAACAGATCG	280
SCC*mec* type IVa-F SCC*mec* type IVa-R	GCCTTATTCGAAGAAACCG CTACTCTTCTGAAAAGCGTCG	776
SCC*mec* type IVb-F SCC*mec* type IVb-R	TCTGGAATTACTTCAGCTGC AAACAATATTGCTCTCCCTC	493
SCC*mec* type IVc-F SCC*mec* type IVc-R	ACATATTTGTATTATCGGAGAGC TTGGTATGAGGTATTGCTGG	200
SCC*mec* type IVd-F SCC*mec* type IVd-R	CTCAAAATACGGACCCCAATACA TGCTCCAGTAATTGCTAAAG	881
SCC*mec* type V-F SCC*mec* type V-R	GAACATTGTTACTTAAATGAGCG TGAAAGTTGTACCCTTGACACC	325
Type 1 *ccr*-βc Type 1 *ccr*-α1	ATTGCCTTGATAATAGCCITCT AACCTATATCATCAATCAGTACGT	695
Type 2 *ccr*- βc Type 2 *ccr*-α2	ATTGCCTTGATAATAGCCITCT TAAAGGCATCAATGCACAAACACT	937
Type 3 *ccr*- βc Type 3 *ccr*-α3	ATTGCCTTGATAATAGCCITCT AGCTCAAAAGCAAGCAATAGAAT	1791
Type 5 *ccr*- βc Type 5 *ccr*-α	ATGAATTCAAAGAGCATGGC GATTTAGAATTGTCGTGATTGC	336
Luk-*PV*-F Luk-*PV*-R	ATCATTAGGTAAAATGTCTGGACATGATCCA GCATCAAGTGTATTGGATAGCAAAAGC	433

arithmetic mean (UPGMA) method and the Dice similarity coefficient. The comparison between sets of variables in the dendrogram and determination of strain relatedness was done using Gelcompare II software version 4.0. Isolates that presented similarity between the banding profiles of equilibrium or more than 80% (fewer than six bands of difference) were considered as indistinguishable or clonally related and grouped into common types (CTs), whereas patterns with less than 80% similarity (six or more bands of difference) showed different clusters of unrelated strains [14, 15].

Statistical analysis: Statistical analysis was done using Fisher's exact test to assess differences between MRSA and VRSA isolates. A *p*-value <0.05 was considered statistically significant. Analyses were conducted using GraphPad Prism version 5.0 (GraphPad Software, USA).

Table 2) Antibiotic resistance patterns of MRSA isolates.

	Pattern	MRSA (%)	VRSA (%)
One antibiotic			
PG	1	10 (17)	-
Three antibiotics			
PG, CIP, TN	2	1 (1.7)	-
Five antibiotics			
PG, K, AN, TN, GM	3	1 (1.7)	-
Seven antibiotics			
PG, CIP, E, CD, T, RP, MN	4	1 (1.7)	-
PG, CIP, CD, TN, T, RP, MN	5	1 (1.7)	-
Eight antibiotics			
PG, CIP, E, CD, TN, T, K, SXT,	6	1 (1.7)	-
PG, CIP, E, CD, TN, T, K, AN, SXT	7	1 (1.7)	-
PG, CIP, E, CD, TN, K, RP, SXT	8	2 (3)	-
PG, CIP, E, CD, TN, K, AN, RP	9	1 (1.7)	-
Nine antibiotics			
PG, CIP, E, TN, T, K, AN, TS, MN	10	1 (1.7)	-
PG, CIP, E, CD, TN, T, K, AN, RP	11	1 (1.7)	-
PG, CIP, E, CD, TN, T, K, AN, SXT	12	1 (1.7)	-
Ten antibiotics			
PG, CIP, TN, T, NI, K, RP, MN, VAN, TEC	13	1 (1.7)	1 (14)
PG, CIP, E, TN, T, K, AN, GM, MN, SXT	14	2 (3)	-
PG, CIP, E, CD, TN, T, K, AN RP, MN	15	4 (7)	-
PG, CIP, E, CD, TN, T, K, AN, SXT, GM	16	5 (8)	-
Eleven antibiotics			
PG, CIP, E, CD, TN, T, K, AN, SXT, GM, MN	17	9 (15)	-
PG, CIP, E, CD, TN, T, NI, K, AN, RP, MN	18	1 (1.7)	-
PG, CIP, E, CD, TN, T, K, AN, SXT, RP, MN	19	1 (1.7)	-
PG, CIP, E, CD, TN, T, K, AN, SXT, RP, GM	20	3 (5)	-
Twelve antibiotics			
PG, CIP, E, CD, TN, T, NI, K, AN, SXT, GM, MN	21	1 (1.7)	-
PG, CIP, E, CD, TN, T, K, AN, SXT, RP, GM, MN	22	1 (1.7)	-
PG, CIP, E, CD, TN, T, NI, K, AN, RP, GM, MN	23	2 (3)	-
Thirteen antibiotics			
PG, CIP, E, CD, TN, T, NI, K, AN, SXT, RP, GM, MN	24	2 (3)	-
PG, CIP, E, CD, TN, T, NI, K, AN, VAN, TEC, RP, MN	25	1 (1.7)	1 (14)
PG, CIP, E, TN, T, NI, K, SXT, RP, VAN, TEC, GM, MN	26	1 (1.7)	1 (14)
Fourteen antibiotics			
PG, CIP, E, CD, TN, T, NI, K, AN, SXT, RP, MN, VAN, TEC	27	1 (1.7)	1 (14)
Fifteen antibiotics			
PG, CIP, E, CD, TN, T, NI, K, AN, SXT, RP, GM, MN, VAN, TEC	28	3 (5)	3 (43)

Abbreviations are PG, penicillin G, CIP, ciprofloxacin; TN, tobramycin; K, kanamycin; E, erythromycin; T, tetracycline; CD, clindamycin; AN, amikacin; SXT, trimethoprim-sulfamethoxazole; MN, minocycline; GM, gentamicin; RP, rifampin; NI, nitrofurantoin; VAN, vancomycin; TEC, teicoplanin

Table 3) Association between PFGE-based clonal groups, SCC*mec* types, *ccr* types, antibiotic resistance patterns, and presence or absence of *van*A, *van*B, and *pvl* genes among MRSA strains. The symbols (+) and (–) indicate the presence and absence of the corresponding genes (*van*A, *van*B, and *pvl*).

No. of MRSA strain	PFGE type	Antibiotic resistance Pattern	*van*A	*van*B	SCC*mec*	*ccr*	*pvl*
1	CT1	15	-	-	III	3	-
2	CT1	17	-	-	III	3	-
3	CT2	17	-	-	III	3	-
4	CT2	1	-	-	IVc	2	+
5	CT2	12	-	-	II	2	-
6	CT2	14	-	-	I	1	-
7	CT3	21	-	-	III	3	-
8	CT3	16	-	-	III	3	-
9	CT4	17	-	-	I	1	-
10	CT4	17	-	-	III	3	-
11	CT5	27	+	-	III	3	-
12	CT5	13	+	-	I	1	-
13	CT5	25	+	+	III	3	-
14	CT6	17	-	-	III	3	-
15	CT6	17	-	-	III	3	-
16	CT6	17	-	-	II	2	-
17	CT7	1	-	-	IVa	2	+
18	CT7	1	-	-	IVa	2	+
19	CT8	4	-	-	I	1	-
20	CT8	11	-	-	III	3	-
21	CT9	1	-	-	IVa	2	+
22	CT9	24	-	-	III	3	-
23	CT10	1	-	-	IVc	2	+
24	CT10	17	-	-	III	3	-
25	CT11	23	-	-	III	3	-
26	CT11	20	-	-	I	1	-
27	CT12	18	-	-	III	3	-
28	CT12	19	-	-	I	1	-
29	CT13	16	-	-	I	1	-
30	CT13	16	-	-	II	2	-
31	CT14	15	-	-	III	3	-
32	CT14	23	-	-	I	1	-
33	CT15	15	-	-	III	3	-
34	CT15	15	-	-	III	3	-
35	CT16	19	-	-	I	1	-
36	CT16	3	-	-	III	3	-
37	CT16	14	-	-	I	1	-
38	CT16	24	-	-	I	1	-
39	CT16	17	-	-	III	3	-
40	CT17	8	-	-	III	3	-
41	CT17	8	-	-	III	3	-

No. of MRSA strain	PFGE type	Antibiotic resistance Pattern	*van*A	*van*B	SCC*mec*	*ccr*	*pvl*
42	CT17	7	-	-	I	1	-
43	CT18	5	-	-	III	3	-
44	CT18	22	-	-	I	1	-
45	CT19	16	-	-	III	3	-
46	CT19	9	-	-	II	2	-
47	CT19	20	-	-	III	3	-
48	ST1	6	-	-	III	3	-
49	ST2	1	-	-	IVc	2	+
50	ST3	1	-	-	IVa	2	+
51	ST4	1	-	-	V	5	+
52	ST5	1	-	-	V	5	+
53	ST6	1	-	-	V	5	+
54	ST7	16	-	-	II	2	-
55	ST8	2	-	-	III	3	-
56	ST9	28	+	-	I	1	-
57	ST10	26	+	+	II	2	-
58	ST11	28	+	-	II	2	-
59	ST12	28	+	-	III	3	-
60	ST13	10	-	-	I	1	-

Findings

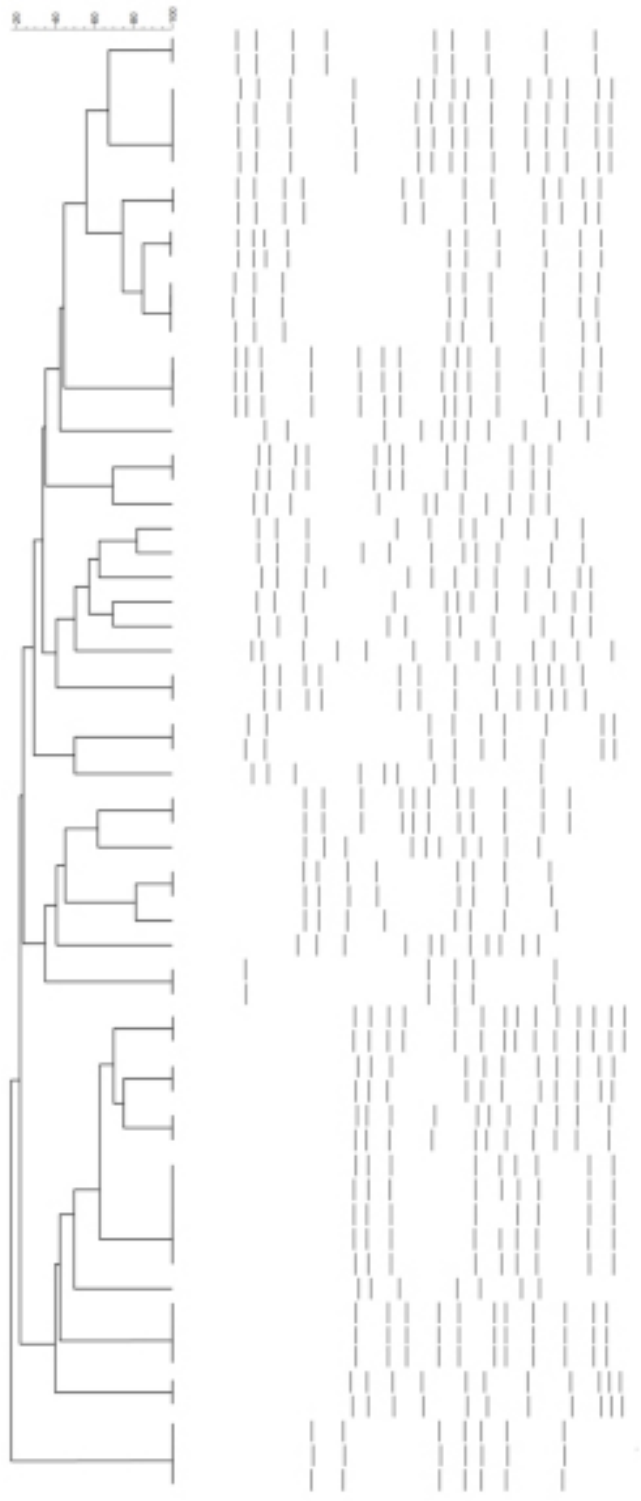
Identification of MRSA and VRSA strains: Among the 247 *S. aureus* isolates recovered from ICU patients, 60 isolates (24%) were identified as MRSA based on cefoxitin resistance and confirmation of the *mec*A gene. Among different types of clinical samples, most of the MRSA strains (35%) were isolated from wound specimens, followed by urine (22%), sputum (20%), blood (10%), abscess (10%) and CSF (3%). According to the oxacillin MICs, 63% of MRSA strains were assigned as high-level oxacillin-resistant (MIC ≥ 128 µg/mL).

Vancomycin susceptibility testing of MRSA strains using E-test strips identified seven strains (3% of all strains and 11.7% of MRSA strains) with vancomycin MIC values ≥16 µg/mL, which were classified as VRSA. Molecular analysis showed that all VRSA strains carried the *van*A gene, while *van*B was detected in two strains (29%).

All subsequent phenotypic, antimicrobial resistance, and molecular analyses were therefore focused exclusively on MRSA and VRSA isolates.

Antibiotic susceptibility profile of MRSA and VRSA strains*:* Among MRSA strains, the highest level of antibiotic resistance was observed against penicillin (100%), followed by ciprofloxacin (82%), tobramycin (80%), kanamycin (78%), and erythromycin (77%). In contrast, all MRSA strains remained susceptible to linezolid, chloramphenicol, and quinupristin–dalfopristin. Furthermore, 28 antibiotic profiles were detected among the MRSA strains (Table 2), and 82% of the MRSA strains were resistant to at least one antibiotic in three or more classes of antibiotics and categorized as multidrug-resistant (MDR).

All VRSA strains were resistant to penicillin, cefoxitin, ciprofloxacin, rifampin, minocycline, nitrofurantoin, teicoplanin, tobramycin, kanamycin, and tetracycline (Table 3). Among VRSA, the most common resistance



PFGE type	MIC-OXA	MIC-VAN	*vanA*/*vanB*	SCC*mec*	PVL
CT1	64	2	-/-	III	-
CT1	128	1	-/-	III	-
CT2	128	1	-/-	III	-
CT2	4	0.5	-/-	IVc	+
CT2	256	0.25	-/-	II	-
CT2	64	1	-/-	I	-
CT3	128	1	-/-	III	-
CT3	256	2	-/-	III	-
CT4	256	0.25	-/-	I	-
CT4	256	1	-/-	III	-
CT5	256	32	+/-	I	-
CT5	256	16	+/+	III	-
CT5	256	32	+/-	III	-
CT6	256	0.5	-/-	III	-
CT6	128	1	-/-	III	-
CT6	64	2	-/-	II	-
ST1	256	0.5	-/-	III	-
CT7	4	0.25	-/-	IVa	+
CT7	4	0.25	-/-	IVa	+
ST2	4	1	-/-	IVc	-
ST3	4	0.25	-/-	IVa	+
ST4	4	0.25	-/-	V	+
ST5	4	1	-/-	V	+
ST6	4	1	-/-	V	+
ST7	96	0.5	-/-	II	-
ST8	128	0.25	-/-	III	-
CT8	256	1	-/-	I	-
CT8	256	2	-/-	III	-
CT9	4	1	-/-	IVa	+
CT9	128	1	-/-	III	-
ST9	256	16	+/-	I	-
CT10	4	2	-/-	IVc	+
CT10	256	1	-/-	III	-
ST10	256	16	+/+	II	-
CT11	256	1	-/-	III	-
CT11	96	2	-/-	I	-
ST11	256	32	+/-	II	-
ST12	256	16	+/-	III	-
CT12	64	0.25	-/-	III	-
CT12	256	0.5	-/-	I	-
CT13	256	0.25	-/-	I	-
CT13	64	0.25	-/-	II	-
CT14	64	1	-/-	III	-
CT14	64	1	-/-	I	-
CT15	256	2	-/-	III	-
CT15	256	0.25	-/-	III	-
CT16	128	2	-/-	I	-
CT16	128	1	-/-	III	-
CT16	256	0.25	-/-	I	-
CT16	256	2	-/-	I	-
CT16	256	1	-/-	III	-
ST13	128	2	-/-	I	-
CT17	128	2	-/-	I	-
CT17	256	1	-/-	III	-
CT17	96	1	-/-	III	-
CT18	128	0.25	-/-	III	-
CT18	128	1	-/-	I	-
CT19	256	0.5	-/-	III	-
CT19	64	1	-/-	II	-
CT19	96	0.5	-/-	III	-

Figure 1) Dendrogram illustrating PFGE patterns of MRSA and VRSA strains isolated from ICU patients.

pattern was pattern 28 i.e. resistance to 15 different antibiotics (Table 2).

Based on the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines, 18 MRSA strains (30%) and all VRSA isolates met the definition of extensively drug-resistant (XDR) phenotypes.

SCC*mec* and *ccr* typing and screening for the *pvl* gene among MRSA strains: Overall, six SCC*mec* types (I, II, III, IVa, IVc, and V)

were identified among the MRSA strains, with 28 strains (47%) belonging to SCC*mec* type III (Table 3). Also, 17% of strains that harbored SCC*mec* types IVa (7%), IVc (5%), and V (5%) carried the *pvl* gene. Also, *ccr* types 1, 2, 3, and 5 were found among the MRSA strains.

PFGE typing of MRSA and VRSA strains: PFGE analysis grouped MRSA isolates into 32 pulsotypes, comprising 19 common types (CTs) and 13 single types (STs) (Fig 1). No PFGE type accounted for more than 8% of all strains (Table 3), giving a high diversity among the MRSA strains. Moreover, the prevalence of *pvl*-positive MRSA strains was limited to 4 CTs (CT2, CT7, CT9, and CT10) and 5 STs (ST2-ST6). The most common pulsotype was CT16 represented by five isolates (8%), followed by CT2 comprising four isolates (7%). Out of 7 VRSA strains, three strains belonged to CT3, and others belonged to four STs i.e. ST9-ST12 (Table 3).

Discussion

The findings of this study indicate a relatively high prevalence (24%) of MRSA strains among ICU patients compared with reports from several other countries. For example, a study conducted in the US showed that 11% of ICU patients were colonized with MRSA [16]. Similarly, the prevalence of MRSA has been reported to range from 13% to 47% in various regions of India [17]. Several factors, such as differences between surveillance systems, lack of uniformity in diagnostic criteria, variation in sample sizes, and duration of isolate collection period, could be the possible reasons for the different MRSA rates in various studies [18]. Other factors such as major surgery, previous hospitalization, history of chronic diseases as well as the gender and age of the patients admitted to the ICU can also contribute to differences seen in the prevalence of MRSA/VRSA in different studies [19].

In the present study, most MRSA isolates were obtained from wound specimens. Although biofilm formation was not experimentally evaluated, previous studies have reported that bacterial strains recovered from wound infections tend to exhibit stronger biofilm-forming capacity compared with isolates from other clinical sources. Therefore, the predominance of wound-derived isolates in this study may be partly attributable to this characteristic, as supported by findings from earlier reports [20, 21]. Here, we found a lower level of resistance to ciprofloxacin compared to what we saw before [8, 9] but still higher than those reported in China [22], India [23], and Nepal [24]. Nonetheless, the high level of resistance to different classes of antibiotics in our study could be partly due to the misuse of these antibiotics in this country. Consistent with previous findings in Pakistan [25], Egypt [26], and Saudi Arabia [27], our results indicated a high sensitivity of MRSA to linezolid, chloramphenicol, and quinupristin/dalfopristin, suggesting some alternative treatment choices against multidrug-resistant MRSA and VRSA strains. The frequency of VRSA in Iran before 2010 was reported to be 1.2% [28]. In our study, we found a more than 2-fold increase (2.83%) in the frequency of VRSA among hospitalized patients. A similar prevalence of VRSA has been reported among MRSA strains in southwest Iran [29], which could be in part due to the horizontal transfer of resistance genes among the MRSA strains. In the present study, the frequency of *van*A and *van*B among VRSA strains was 100% and 29%, respectively. Due to the small number of VRSA isolates, direct quantitative comparisons with previous studies should be interpreted cautiously. To the best of our knowledge, this is the first report of *van*B-positive VRSA strains in Iran, despite numerous studies reporting a failure to detect the *van*B gene [30, 31].

In this study, we used three methods to identify clonal relationships among the MRSA strains. With the PFGE, we identified the presence of diverse genetic populations of both the MRSA and VRSA strains within the studied ICU. This could be partially attributed to evolutionary genetic variation via point mutations, acquisition/deletion of mobile genetic elements, and recombination, resulting in the emergence of several novel MRSA clones [32]. The SCC*mec* typing method showed that almost half of the strains belonged to SCC*mec* type III. Such a high frequency of MRSA isolates with SCC*mec* type III in different studies highlights the fact that there are is dominant clonal group of MRSA strains carrying SCC*mec* type III that are circulating within hospitals in Iran [33]. Based on these findings we postulate a further increase in antibiotic resistance profile of these clonal groups over time causing major outbreaks in healthcare settings. We also speculate that this SCC*mec* type could be related to the unique lineage of MRSA strains in Iran [34]. Since SCC*mec* III is located on large genetic mobile elements, it usually cannot transfer among *S. aureus* strains by horizontal gene transfer and has probably been transferred from healthcare workers to patients [-]. Also, in this study, a subset of MRSA isolates carrying SCC*mec* types IVa, IVc, and V harboured the *pvl* gene and exhibited low-level oxacillin resistance (MIC = 4 μg/mL). These SCC*mec* types and resistance profiles are commonly associated with community-associated MRSA lineages. The presence of such *pvl*-positive strains within the ICU suggests a possible epidemiological overlap between community- and hospital-associated MRSA populations, which may contribute to the introduction and persistence of these clones in healthcare settings.

The present study has several limitations. First, it was conducted at a single referral hospital, which may limit the generalizability of the findings to other healthcare settings. Second, detailed patient-level clinical data, including underlying conditions, antibiotic exposure, and treatment outcomes, were not available, preventing assessment of clinical correlations. Third, sampling was restricted to ICU patients, which may introduce selection bias and does not reflect the epidemiology of MRSA and VRSA in other hospital wards or community settings. Finally, the relatively small number of VRSA isolates limits the strength of comparative analyses and epidemiological inferences.

Conclusions

From an epidemiological perspective, the identification of clonally related MRSA and VRSA isolates in ICU patients indicates the potential for intra-hospital dissemination, particularly in settings with high antibiotic pressure. This finding highlights the importance of continuous molecular surveillance and strict infection control measures to limit the spread of resistant *S. aureus* strains within critical care units.

From a clinical standpoint, the high prevalence of multidrug-resistant MRSA and the detection of VRSA strains may adversely affect treatment outcomes by restricting effective therapeutic options and complicating empirical therapy in ICU patients. However, the preserved susceptibility of the isolates to linezolid, chloramphenicol, and quinupristin–dalfopristin suggests that these agents may remain suitable alternatives for the treatment of severe infections caused by resistant strains.

In terms of hospital antibiotic policy, the observed resistance patterns emphasise the need for rational antibiotic stewardship programmes, particularly the prudent use of glycopeptides and broad-spectrum agents in ICU settings. Regular review of

local resistance data and optimisation of empirical treatment guidelines may help reduce selective pressure and prevent further emergence and dissemination of highly resistant MRSA and VRSA strains.

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