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Research article

Phenotypic and genotypic characterization of methicillin and vancomycin-resistant *Staphylococcus aureus* isolated from human clinical samples in Kerman hospitals, Iran

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Abstract

Today, methicillin-resistant *S. aureus* (MRSA) isolates are widely spread in both communities and hospitals, causing high morbidity and mortality worldwide. However, the emergence of vancomycin resistance among MRSA isolates has been perceived as a formidable threat to therapeutic management. Therefore, the present study aimed to determine the frequency and antibiotic susceptibility of MRSA and vancomycin-resistant *S. aureus* (VRSA) strains in Kerman hospitals in Iran. A total of 455 human clinical samples were collected from Kerman hospitals. The samples were examined for the presence of *S. aureus* and the isolates were tested for their susceptibility to antibiotics by disk diffusion method. MRSA and VRSA isolates were detected by a combination of phenotypic and genotypic methods. Sixty-five coagulase-positive *S. aureus* strains were isolated, and the higher isolation rate was from wound samples (47.7%) compared with blood (18.5%), respiratory tract secretions (10.8%), urine (10.8%), body fluids (9.2%), abscess (1.5%), and cerebrospinal fluids (1.5%). The isolates exhibited high resistance toward tetracycline (69.2%). Thirty-four isolates (52.3%) were categorized as MRSA (phenotypically resistant to cefoxitin). *mecA* gene was amplified in 97.1% of MRSA strains. Moreover, only one MRSA strain was resistant to vancomycin (MIC=128), and this isolate carried the *van*A gene. The frequency of MRSA was not significantly associated with gender, age, sample type, and sampling locality ($p>0.05$). Given the alarming rate of resistance among MRSA isolates, monitoring of antibiotic resistance should be performed to reduce treatment failure in patients with staphylococcal infections. Although vancomycin remains a drug of choice for MRSA, our study suggests that its efficacy may be limited by resistance development.

Keywords: *Staphylococcus aureus*, Methicillin, Vancomycin, Resistance, *mec*A gene, *van*A gene

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Introduction

Staphylococcus aureus (*S. aureus*) is a robust and versatile opportunistic pathogen that can survive in a diversity of environments (Sergelidis and Andelidis, 2017). It exists as a member of the normal microbiota of the nose and skin of both humans and animals, being isolated from food, food production systems, and the environment (Cuny et al., 2015; Dweba et al., 2018; Gajdács, 2019). *S. aureus* can cause several infections, such as skin and soft tissue infections (abscess, cellulitis, folliculitis, impetigo, and scaled skin syndrome), followed by invasive infections (bacteremia, sepsis, toxic shock syndrome, endocarditis, pneumonia, lung abscess, osteomyelitis, meningitis, and empyema), and food poisoning (Siddiqui and Koirala, 2018; Yamamoto et al., 2010). Toxic shock syndrome, sepsis, and endocarditis are potentially life-threatening infections that can quickly become fatal without antibiotic treatment. Methicillin-

resistant *S. aureus* (MRSA) is one of the ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* species) pathogens and is considered the most significant multidrug-resistant (MDR) bacteria in developed and developing nations worldwide (Correia et al., 2019; Denissen et al., 2022). The One Health approach to MRSA has been emphasized as being critical to successful intervention. MRSA strains are transmitted in both hospital and community settings and are isolated from humans, the environment, food, and animals (Aires-de-Sousa, 2017; Boswihi and Udo, 2018; Denissen et al., 2022). Cases of livestock-associated MRSA have been reported in human patients in contact with infected animals, resulting in invasive infections in humans, such as endocarditis, osteomyelitis, and ventilator-associated pneumonia (Goerge et al., 2017). Although the incidence of MRSA strains has recently declined in some regions, these strains pose a major threat to human health with persistent high morbidity and mortality due to their capacity for genetic adaptation and the emergence of epidemic strains (Turner et al., 2019).

MRSA strains resistant to almost all β-lactam antibiotics and other classes of antibiotics remain a global concern. A high frequency of resistant strains has occurred in hospitals and communities, and this high level of resistance has created many problems in the treatment of infections caused by this bacterium (Adhikari et al., 2017; Lobanovska and Pilla, 2017; Rasigade and Vandenesch, 2014). The emergence of vancomycin-resistant *S. aureus* (VRSA) strains is of great concern, as vancomycin was considered the most reliable last-resort treatment option for MRSA infections. The increased use of this drug has led to the emergence of VRSA-resistant strains that exhibit MDR status against a broad range of antimicrobials in recent years (Cong et al., 2020; Khan et al., 2018; Purrello et al., 2018). Vancomycin resistance in *S. aureus* is caused by *van* genes that encode for a ligase enzyme that catalyzes the production of peptidoglycan in the bacterial cell wall and has a lower affinity to bind to vancomycin (Asadpour and Ghazanfari, 2019). It was reported that VRSA isolates seem to have acquired the vancomycin resistance gene (*van*A) from enterococci (Askari et al., 2015; Shekarabi et al., 2017).

Hence, the resistance of *S. aureus* strains to methicillin and vancomycin in both hospitals and the community is a serious challenge that threatens public health worldwide. The present study aimed to determine the frequency and antibiotic susceptibility of *S. aureus* in human clinical samples collected from Kerman hospitals in Iran. The frequency of MRSA and VRSA strains was determined by phenotypic and genotypic methods.

Materials and methods

Sampling

A total of 455 human clinical samples were collected from Bahoner (n=255) and Afzalipour (n=200) University teaching hospitals in Kerman City, Iran. The samples were collected from patients of all age groups (n=65) who had been clinically diagnosed to have staphylococcal infections. They were comprised of wounds, blood, respiratory tract secretions, urine, body fluids, abscesses, and cerebrospinal fluids (CSF). The samples were labeled and transferred in an insulated ice box directly to the laboratory for further processing and bacteriological analysis.

Isolation and identification of *S. aureus*

The collected samples were processed according to standard microbiological procedures (Isenberg, 2004). The specimens were cultured on mannitol salt agar (Sigma-Aldrich, Gillingham, UK), followed by overnight incubation at 37°C. Presumptive *S. aureus* yellow colonies were sub-cultured onto nutrient agar plates (Thermo Fisher Scientific, Oxoid Ltd., Basingstoke, UK) and subjected to Gram staining and standard biochemical tests (catalase, slide, and tube coagulase tests, DNase test, mannitol fermentation test, and blood hemolysis test). A loopful of each biochemically confirmed *S. aureus* isolate was then inoculated into the nutrient broth (Thermo Fisher Scientific, Oxoid Ltd., Basingstoke, UK) containing 15% glycerol and frozen at -70˚C.

Antibiotic sensitivity test

All biochemically identified coagulase-positive *S. aureus* (CPS) isolates (n=65) were tested for their susceptibility to antibiotics that are commonly prescribed to treat staphylococcal infection. The antibiotic sensitivity was determined according to Clinical and Laboratory Standard Institute (CLSI) guidelines for disk agar diffusion (CLSI, 2021). The tested antimicrobials discs (Thermo Fisher Scientific, Oxoid Ltd., Basingstoke, UK)

were tetracycline (30 µg), erythromycin (15 µg), cefoxitin (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), clindamycin (2 µg), tobramycin (10 µg), gentamicin (10 µg), amikacin (30 µg), trimethoprime/sulphmethoxazole (23.75/1.25 µg), ceftaroline (30 µg), Quinupristin/dalfopristin (15 µg) and linezolid (30 µg). The inhibition zone diameters were measured and interpreted according to CLSI (CLSI, 2021). *S. aureus* ATCC 25923 was used for quality control of the disk diffusion test. MRSA and methicillin-sensitive *S. aureus* (MSSA) isolates were determined based on the disc diffusion test results for cefoxitin, which was used as a surrogate agent to methicillin and oxacillin as documented by CLSI (CLSI, 2021). All MRSA were tested for their susceptibility or resistance to vancomycin by broth microdilution method, and the minimum inhibitory concentration (MIC) breakpoints for vancomycin were interpreted following CLSI guidelines (CLSI, 2021). *S. aureus* ATCC 29213 was used for quality control of the broth microdilution method.

Molecular characterization of methicillin and vancomycin-resistant *S. aureus*

DNA was extracted from all cefoxitin-resistant *S. aureus* isolates (n=34) and vancomycin-resistant *S. aureus* isolates (n=1) using the boiling method (Emaneini et al., 2013). The extracted DNA was subjected to a uniplex PCR for the detection of *mec*A (encoding methicillin resistance) and *van*A (encoding vancomycin resistance) genes using oligonucleotide primers supplied by Metabion, Germany. The primers' sequences, amplicon sizes, and cycling conditions are provided in Table 1. DNA amplification was performed in a thermal cycler (Applied Biosystems, Foster City, USA). The amplified PCR products, positive and negative control, were run on 1% agarose gel in 0.5 TBE × buffer and 0.5 µL green viewer dye at a voltage of 95 mV. The bands' sizes were visualized by the Gel Documentation system (Bio-Rad, California, USA).

Table 1: Primers' sequences, target genes, amplicon sizes, and cycling conditions.

Target gene	Primers' sequences (5´-3´)	Size (bp)	Initial denat.	Amplification cycles (30)			Final ext.	References
				Denat.	Ann.	Ext.		
*mec*A	F-CCAGATTACAACTTCACCAGG R-CCACTTCATATCTTGTAACG	162	94˚C 4min	94˚C 30sec	53˚C 30sec	72˚C 1min	72˚C 4min	Oliveira and Lencastre, (2002)
*van*A	F-CATGAATAGAATAAAAGT TGCAATA R-CCCCTTTAACGCTAATAC GATCAA	1030	94˚C 5min	94˚C 1min	57˚C 30sec	72˚C 1min	72˚C 10min	Tiwari and Sen, (2006)

Denat= Denaturation, Ann=Annealing, Ext=Extension

Statistical analysis

Statistical analysis was performed on the data by chi-square test using XLSTAT v. 16.77 Statistical Software for Excel version 2023. The differences between means were statistically significant at $p<0.05$.

Results

Frequency of *S. aureus* in the examined clinical samples

Sixty-five *S. aureus* strains were recovered from the examined clinical samples based on the culture and biochemical diagnostic tests. All *S. aureus* strains were positive for catalase, coagulase, mannitol, and DNase tests. The isolates had spherical and raised colonies on the blood agar medium, and the colonies' color was yellow to creamy due to the presence of pigment. Moreover, a faint halo was observed around the colonies due to the presence of hemolysin. *S. aureus* strains were isolated with a higher frequency from Afzalipour Hospital (n=45, 69.2%) than from Bahonar Hospital (n=20, 30.8%). A high percentage of *S. aureus* was recovered from wound samples (47.7%) compared to other sample types (Figure 1).

Antibiotic sensitivity

According to the obtained results, *S. aureus* showed a high resistance toward tetracycline (69.2%), followed by erythromycin (58.5%), cefoxitin (52.3%), ciprofloxacin and levofloxacin (47.7%) compared to other antibiotics. A high percentage of S. aureus isolates, 98.5%, 96.9%, and 95.4% were sensitive to linezolid, quinopristin/dalfopristin and ceftaroline, respectively. Based on CLSI interpretative criteria, 52.3% (34/65) of S. aureus isolates were phenotypically resistant to cefoxitin based on disk diffusion test results; these isolates were categorized as MRSA. Meanwhile, the other isolates, 47.7% (31/65), were sensitive to cefoxitin and categorized as MSSA. MRSA

isolates showed significant high resistance to tetracycline (83.3%), followed by erythromycin (80%), ciprofloxacin and levofloxacin (76.7%) each, clindamycin and gentamicin (70%), each, amikacin (63.3%) and tobramycin (60%) compared with MSSA isolates (p<0.05). Furthermore, MRSA isolates exhibited low resistance to linezolid (3.3%), quinupristin /dalfopristin, and ceftaroline (6.7%) each, and trimethoprim-sulphamethoxazole (23.3%). Based on the broth microdilution method for determining the MIC for vancomycin antibiotic in all phenotypically MRSA strains (n=34), the results showed that only one MRSA isolate, 2.9% (1/34), was resistant to vancomycin (MIC=128). Meanwhile, the other MRSA isolates (97.1%; n=33) were susceptible to vancomycin (MIC less than 2). 76.5% (26/34) of phenotypically MRSA isolates were MDR, i.e., resistance to at least one agent in more than three antimicrobial categories (Table 2).

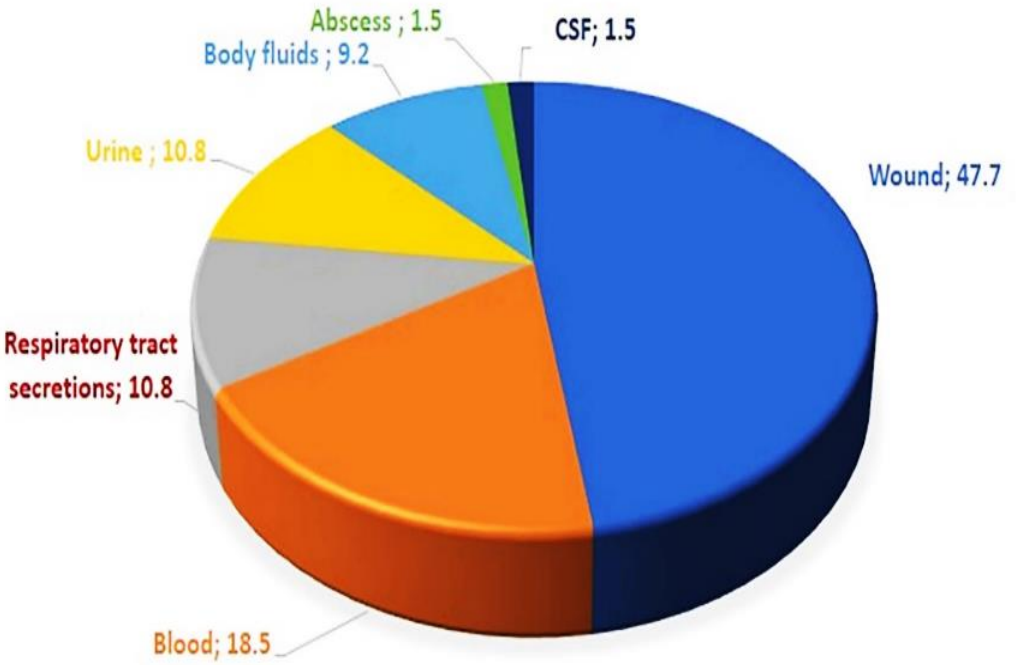

Figure 1: Frequency distribution of coagulase-positive *S. aureus* in human clinical samples.

Molecular characterization of *mec*A and *van*A genes in MRSA strains

All phenotypically MRSA strains (n=34), resistant to cefoxitin by disc diffusion method, were screened for the presence of the *mec*A gene (encoding methicillin resistance) using PCR.

Association between frequency of MRSA and patient demographic characteristics, sample type, and sampling locality

A higher frequency of MRSA in males (56.9%) than females (43.1%) was observed (Table 3). However, there was no significant association between gender and the frequency of MRSA (p-value= 0.745) (Table 3). Regarding the age of the patients, the results revealed that there was no significant association between age and the frequency of MRSA strains (p-value= 0.460). However, a high percentage of MRSA (48.5%) was observed in the age group >50 years compared to other groups. Concerning the type of clinical sample, wound samples had a high frequency of MRSA (45.5%) compared to other sample types (Table 3). No significant correlation was observed between the clinical sample type and frequency of MRSA strains (p-value= 0.593). Table 3 shows the frequency of MRSA for each hospital, and no significant association was observed between the frequency of MRSA and the type of hospital (p-value= 0.431). However, the samples collected from Afzalipour Hospital showed a high frequency of MRSA (72.7%) compared to Bahonar Hospital (27.3%).

*mec*A gene was identified in 97.1% (33/34) of the tested MRSA strains (Figure 2A). In addition, the vancomycin-resistant isolate was positive for the *van*A gene (encoding vancomycin resistance) (Figure 2B).

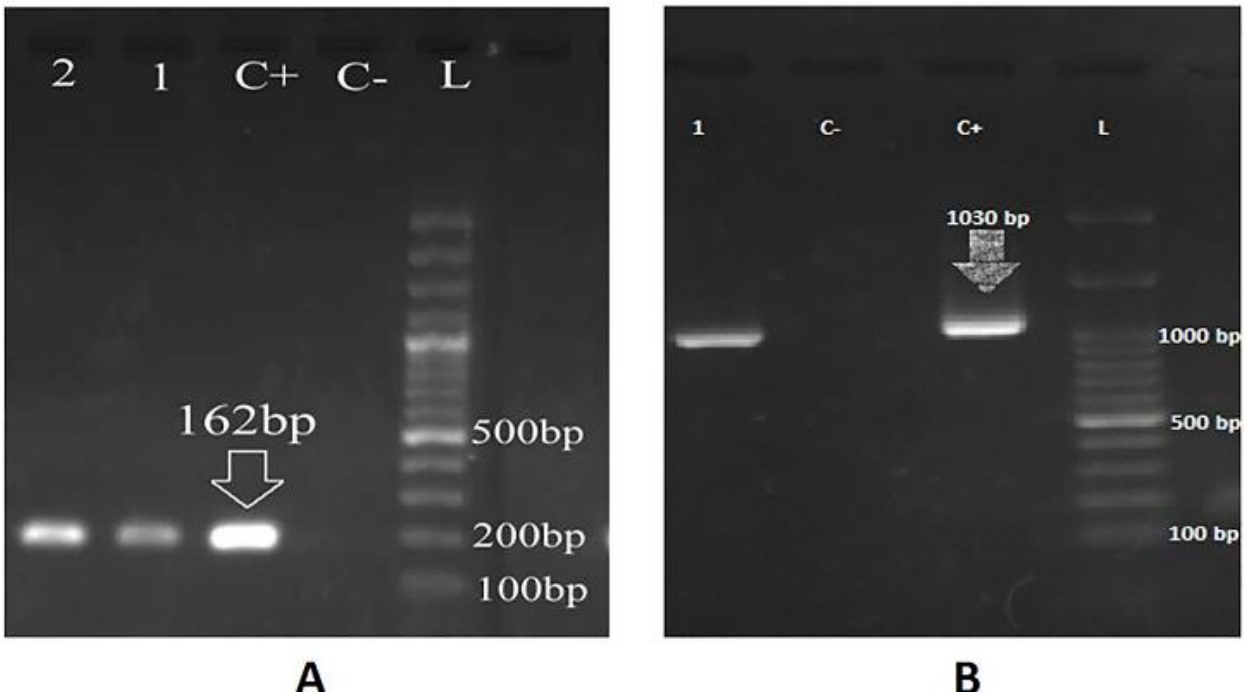

Figure 2: Agarose gel electrophoresis, showing amplification of the *mec*A gene (162 bp) in MRSA (A) strains and the *van*A gene (1030 bp) in VRSA stain (B). A) L: Ladder, C-: negative control, C+: positive control, 1 and 2: MRSA positive for the *mec*A gene. B) L: Ladder, C+: positive control, C: negative control, 1: VRSA positive for *van*A.

Table 2: Resistance patterns of multidrug MRSA were isolated in this study.

No.	Source	Sampling locality	Resistance profile
1	Wound	Afzalipour hospital	TE-E-CIP-LEV-DA-CN-TOB-AK-SXT-CPT-QD-LZD
2	Wound	Afzalipour hospital	TE-E-CIP-LEV-DA-CN-TOB-AK-SXT-CPT-QD
3	Wound	Afzalipour hospital	TE-E-CIP-LEV-DA-CN-TOB-AK-SXT
4	Wound	Afzalipour hospital	TE-E-CIP-LEV-DA-CN-TOB-AK-SXT
5	Wound	Afzalipour hospital	TE-E-CIP-LEV-DA-CN-TOB-AK-SXT
6	Wound	Afzalipour hospital	TE-E-CIP-LEV-DA-CN-TOB-AK-SXT
7	Wound	Afzalipour hospital	TE-E-CIP-LEV-DA-CN-TOB-AK-SXT
8	Wound	Afzalipour hospital	TE-E-CIP-LEV-DA-CN-TOB-AK-SXT
9	Wound	Afzalipour hospital	TE-E-CIP-LEV-DA-CN-TOB-AK-SXT
10	Wound	Afzalipour hospital	TE-E-CIP-LEV-DA-CN-TOB-AK
11	Wound	Afzalipour hospital	TE-E-CIP-LEV-DA-CN-TOB-AK
12	Wound	Afzalipour hospital	TE-E-CIP-LEV-DA-CN-TOB-AK
13	Wound	Afzalipour hospital	TE-E-CIP-LEV-DA-CN-TOB-AK
14	Wound	Afzalipour hospital	TE-E-CIP-LEV-DA-CN-TOB-AK
15	Wound	Afzalipour hospital	TE-E-CIP-LEV-DA-CN-TOB-AK
16	Wound	Afzalipour hospital	TE-E-CIP-LEV-DA-CN-TOB-AK
17	Blood	Afzalipour hospital	TE-E-CIP-LEV-DA-CN-TOB-AK
18	Respiratory tract secretions	Afzalipour hospital	TE-E-CIP-LEV-DA-CN-TOB-AK
19	Respiratory tract secretions	Afzalipour hospital	TE-E-CIP-LEV-DA-CN-TOB-AK
20	Blood	Afzalipour hospital	TE-E-CIP-LEV-DA-CN-TOB-AK
21	Urine	Afzalipour hospital	TE-E-CIP-LEV-DA-CN-TOB-AK
22	Urine	Afzalipour hospital	TE-E-CIP-LEV-DA-CN-AK
23	Body fluids	Afzalipour hospital	TE-E-CIP-LEV-DA-CN
24	Urine	Afzalipour hospital	TE-E-CIP-LEV-DA-CN
25	Blood	Afzalipour hospital	TE-E-CIP-LEV
26	Blood	Bahonar hospital	TE-E-CIP-LEV

TE: tetracycline, E: erythromycin, CIP: ciprofloxacin, LEV: levofloxacin, DA: clindamycin, CN: gentamicin, TOB: tobramycin, AK: amikacin, SXT: trimethoprim-sulfamethoxazole, CPT: ceftaroline, QD: quinupristin/dalfopristin, LZD: linezolid.

Table 3: Association of *S. aureus* (MRSA and MSSA) with patient demographic characteristics, sample type, and sampling locality.

Variable		MRSA (n=34) No (%)	MSSA (n=31) No (%)	Total (n=65) No (%)	*P*-value
Gender (sex)	Male	20 (58.8)	17 (54.8)	37 (56.9)	0.745
	Female	14 (41.2)	14 (45.2)	28 (43.1)	
Age group/year	< 20	3 (8.8)	6 (19.4)	9 (13.8)	0.460
	20 to 50	14 (41.2)	12 (38.7)	26 (40)	
	>50	17 (50)	13 (41.9)	30 (46.2)	
Type of clinical sample	Wound	16 (47.1)	15 (48.4)	31(47.7)	0.593
	Blood	4 (11.8)	8 (25.8)	12 (18.5)	
	Respiratory tract secretions	4 (11.8)	3 (9.7)	7 (10.8)	
	Urine	7 (20.6)	0	7 (10.8)	
	Body fluids	2 (5.9)	4 (12.9)	6 (9.2)	
	Abscess	1(2.9)	0	1 (1.5)	
	Cerebrospinal fluids (CSF)	0	1 (3.2)	1 (1.5)	
Sampling locality	Afzalipour hospital	25 (73.5)	20 (64.5)	45 (69.2)	0.431
	Bahonar hospital	9 (26.5)	11 (35.5)	20 (30.8)	

MRSA= methicillin-resistant *S. aureus*; MSSA= methicillin-sensitive *S. aureus*

Discussion

S. aureus is a significant bacterial pathogen responsible for infections acquired in both hospital and community settings. Resistant strains of *S. aureus*, particularly MRSA and VRSA, are a major public health concern. These bacteria have been classified as high-priority due to their potential to cause serious consequences such as prolonged hospitalization, recurrent septic infections, and significant mortality if adequate treatment options are not developed (WHO, 2017). Hence, MRSA and VRSA are widely spread in hospitals and environments, and the early detection of these strains is necessary for implementing serious measures to prevent and control their spread. The current work aimed to investigate the prevalence of MRSA and VRSA strains in clinical samples collected from patients in Kerman hospitals in Iran.

The higher isolation rate of coagulase-positive *S. aureus* strains from wound samples (47.7%) than other clinical samples from human patients in the current study indicates their role in pyogenic soft tissue and wound infection since *S. aureus* is a normal flora of the skin and can enter the body through abrasions, cuts, surgical incisions, burn, and intravenous catheter causing pyogenic infections. These findings coincided with a high frequency of *S. aureus* reported in wound and pus samples from human patients in Nepal (Adhikari et al., 2023; Kandel et al., 2020; Maharjan et al., 2021; Sapkota et al., 2019), Egypt (Elkhyat et al., 2020), Pakistan (Idrees et al., 2023), Iran (Rahimi, 2016), and Ethiopia (Dilnessa and Bitew, 2016). However, the frequency of *S. aureus* in wound and blood samples was nearly similar as previously reported (Abdelwahab et al., 2023; Kika et al., 2020). On the contrary to our results, Mwailunga et al. (2023) reported a high isolation rate of *S. aureus* from blood samples.

The increasing resistance of *S. aureus* to the various commonly used antibiotics is a problematic issue, making infections by this bacterium difficult to treat. Hence, the current study showed a high resistance rate of the isolated *S. aureus* strains to tetracyclines (tetracycline), macrolides (erythromycin), beta-lactams (cefoxitin), fluoroquinolones (ciprofloxacin and levofloxacin) which indicates the misuse of these antibiotics in the study area for treatment of Gram-positive organisms in hospital and community settings (Rahimi et al., 2014). These results correspond to other studies (Javidnia et al., 2013; Kandel et al., 2020; Maharjan et al., 2021; Mohammadi et al., 2014; Rahimi, 2016). A remarkable finding in this study is that *S. aureus* strains exhibited low resistance to linezolid (1.5%), quinupristin /dalfopristin (3.1%) and ceftaroline (4.6%), and this is probably due to lower consumption of these antibiotics in Iran. The high-cost burden and increased risk of adverse effects of these antibiotics have limited their consumption. Therefore, they can be useful when VRSA strains become more prevalent. Our results coincided with a low resistance rate (2.7%) of clinical *S. aureus* strains to linezolid in Nepal (Maharjan et al., 2021). However, Abdelwahab et al. (2023) reported 100% susceptibility of clinical *S. aureus*

strains to linezolid in Egypt.

In general, studies revealed that MRSA strains are increasing in some areas around the world, including Iran. The frequency of MRSA phenotypically resistant to cefoxitin (surrogate agent for methicillin and oxacillin) in this study (52.3%) coincided with other studies in Kerman City, Iran (Fasihi et al., 2018; Mahdiyoun et al., 2016; Sadeghi and Mansouri, 2014). These data show that MRSA strains, fortunately, have not increased in Kerman City during these few years, and the prevalence of infection caused by these strains has been successfully controlled. However, different methods (phenotypic or genotypic) may be used in these studies to detect MRSA strains, and consequently, the results should be compared with further considerations. A higher frequency of MRSA strains than reported in this study has been documented in several countries worldwide, including Iran (Yousefi et al., 2017), Egypt (Abdelwahab et al., 2023; Elkhyat et al., 2020), Pakistan (Idrees et al., 2023), India (Karmakar et al., 2016), Nepal (Maharjan et al., 2021; Sapkota et al., 2019) and Tanzania (Mwailunga et al., 2023). On the contrary, a lower frequency of MRSA strains in human clinical samples than our results have been reported in previous studies in Ethiopia (Dilnessa and Bitew, 2016), Albania (Kika et al., 2020), Nepal (Adhikari et al., 2023) and Iran (Rahimi, 2016). The variation in the frequency of MRSA isolates in different studies may be attributed to factors such as the number of patients studied, the geographical location of the hospitals involved, the antibiotics used (which can differ between hospitals), the type of samples studied, and the methodology employed (Rahimi et al., 2014).

In this study, 33 out of 34 MRSA isolates (97.1%) were carrying the *mec*A gene as genotypically confirmed by PCR. This is not surprising because MRSA strains harbor the *mec*A gene, which encodes a penicillin-binding protein (PBP2a) with a low binding affinity for methicillin and other beta-lactam antibiotics. The *mec*A gene is carried on a mobile genetic element called Staphylococcal cassette chromosome mec (SCCmec). Consequently, MRSA strains are resistant to all members of extended-spectrum beta-lactam antibiotics (Peacock and Paterson, 2015). The high percentages of MRSA isolate possessing the *mec*A gene and exhibiting high resistance to different classes of antibiotics such as tetracyclines, macrolides, fluoroquinolones, lincosamides, and aminoglycosides coincides with other studies (Elkhyat et al., 2020; Emaneini et al., 2013; Idrees et al., 2023; Kandel et al., 2020; Karmakar et al., 2016). This indicates that these antibiotics are no longer effective in the treatment of MRSA infections. It has been reported that the gene cassettes carrying the *mec*A gene in many MRSA strains also carry the genes resistant to other antibiotics, such as tetracycline-resistant (tet) and aminoglycoside-resistant genes (AME), which explains the high resistance of MRSA to antibiotics other than beta-lactams (Emaneini et al., 2013).

A noteworthy observation was that only one MRSA strain (2.9%) in this study was phenotypically resistant to vancomycin and harbored the vancomycin resistance gene (*van*A). Although it might sound like this resistance is ignorable, this indicates that VRSA strains harboring the *van*A gene are already emerging in this study area, constituting a great threat to public health since vancomycin is the highly effective last-resort antibiotic used for the treatment of MRSA infections. In comparison to this study, the frequency of VRSA among MRSA strains in other countries is variable and ranged from 0-6.6% in Iran, 0-25.5% in Egypt (Abdelwahab et al., 2023; Elkhyat et al., 2020), 1.6% in Pakistan (Idrees et al., 2023), 54.3% in India (Karmakar et al., 2016), 0-11.1% in Nepal (Maharjan et al., 2021; Sapkota et al., 2019), and 0% in South Korea (Park et al., 2019). Such differences in the frequency of VRSA may be due to differences in antibiotic policies and infection control measures (Asadpour and Ghazanfari, 2019).

The current study showed that the frequency of MRSA was not significantly associated with patient demographic characteristics such as gender, age, sample type, and sampling locality. However, the frequency of MRSA was higher in males than in females in the age group of over 50 years compared to other age groups. This result is consistent with previous studies (Adhikari et al., 2023; Dilnessa and Bitew, 2016; Kandel et al., 2020; Kika et al., 2020), indicating that gender and age are not risk factors for the acquisition or colonization of MRSA in human patients. Remarkably, the frequency of MRSA showed no significant correlation with sample type despite the fact that wound samples showed a higher frequency of MRSA than other sample types. This result is in line with the findings of other studies

(Adhikari et al., 2023; Dilnessa and Bitew, 2016; Kandel et al., 2020).

Conclusions

In this study, although the frequency of MRSA strains has not increased in Kerman in recent years, there are concerns about the effective treatment of MRSA infections in patients. These strains have shown high resistance to other antibiotics. The presence of VRSA among MRSA strains in this study, despite its low prevalence, is a serious warning about increasing the resistance to vancomycin and reducing its effectiveness in the treatment of future MRSA infections. The detection of the vancomycin resistance gene (*van*A) in the VRSA strain in this study is alarming and indicates the risk of *van*A spreading among *S. aureus* and other bacterial pathogens. Therefore, it is crucial to conduct antibiotic sensitivity tests before prescribing antibiotics, implement intensive surveillance of antibiotic resistance, and prudently use antibiotics in both hospital and community settings to combat the spread of MRSA and VRSA strains.

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Author Contributions. FS: Conceptualization, methodology, funding acquisition, investigation, project administration, data curation, writing – original draft, reviewing and editing; RG, PMD, and SST: Conceptualization, investigation, data curation, supervision, writing–original draft, reviewing and editing, all authors read and approved the final manuscript.

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