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New Sequence Types of *Staphylococcus aureus* Strains Isolated from Hospitals and Community Settings in Southern Iran

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Abstract

Background: *Staphylococcus aureus* is a significant bacterial pathogen globally recognized as the primary cause of numerous uncomplicated skin infections and severe invasive infections. The emergence of methicillin-resistant *S. aureus* (MRSA) poses a serious threat, leading to severe infections in both hospitals and community settings.

Objectives: The aim of this study was to identify antibiotic resistance patterns and perform molecular classification of *S. aureus* strains isolated from both hospital and community settings in southern Iran.

Methods: In this cross-sectional study conducted in Bandar Abbas between 2020 and 2021, a total of 156 clinical strains of *S. aureus* were collected. Antibiotic susceptibility was determined using the disk-diffusion agar method. The presence of the *pvl* gene, Sccmec types, and Agr group was identified through PCR analysis. Additionally, Multilocus sequence typing (MLST) was performed on selected isolates.

Results: The study identified 156 strains, with 79 obtained from inpatients and 77 from outpatients, sourced from clinical samples. Among these isolates, 70 (44.8%) were classified as MRSA. The highest resistance was noted against azithromycin (83%), while the lowest resistance was observed for linezolid (5%) and gentamicin (7%). The presence of the *pvl* gene was detected in isolates from both hospital and community sources. Significant differences were noted in the occurrence of *agr* I and *agr* III genes between hospital and community isolates. Sccmec III was more predominant than other SCCmec types. Furthermore, MLST analysis revealed the presence of five distinct novel sequence types (STs): ST8634, ST8640, ST8650, ST8651, and ST8652.

Conclusions: The findings indicate that the potential spread of hospital-acquired *S. aureus* strains to the community and vice versa poses a significant public health risk. This underscores the urgent need for robust infection control strategies and the identification of potential environmental and hospital sources of resistant strains, particularly MRSA strains.

Keywords: Methicillin-Resistant *Staphylococcus aureus*,Multilocus Sequence Typing

1. Background

Staphylococcus aureus is a major bacterial pathogen known for causing both uncomplicated skin infections and severe invasive infections worldwide. The mortality rate from *S. aureus* bacteremia is believed to exceed those of tuberculosis, human immunodeficiency virus, and hepatitis B. Additionally, methicillin-resistant *Staphylococcus aureus* (MRSA) presents a significant threat, causing severe infections in healthcare-associated (HAI) and community-acquired (CAI) settings. Methicillin-resistant *S. aureus* is characterized by the presence of the *mec*A gene, which codes for the PBP2a protein located within a specific region of the chromosome known as the Staphylococcal cassette chromosome mec (SCCmec) (1-3).

Panton-Valentine leukocidin (PVL) is a toxin produced by some *S. aureus* strains. The presence of PVL in these strains can influence the severity and outcome of an infection. Infections caused by PVL-associated *S. aureus* (PVL-SA) are commonly linked with community-acquired methicillin-resistant *S. aureus* (CA-MRSA)

strains. However, the potential for nosocomial transmission poses a significant public health risk, as the presence of a PVL-positive clonal lineage of hospital-acquired MRSA (HA-MRSA) can rapidly spread, leading to more severe outcomes for infected patients (4).

The accessory gene regulator (agr) system is a global regulatory system in *S. aureus* that controls the expression of numerous virulence factors and regulatory molecules. This system comprises four genes (*agr*A, *agr*B, *agr*C, and *agr*D) that encode a quorum-sensing system responsible for coordinating the expression of virulence factors in response to cell density. The agr system plays a crucial role in regulating various virulence factors, including toxins, enzymes, and surface proteins, which contribute to the bacterium's pathogenicity. Recent studies have revealed a more complex understanding of the accessory gene regulator (agr) in *S. aureus* infection (5).

Molecular typing methods in bacteria refer to a set of techniques used to characterize and differentiate bacterial strains based on their genetic makeup. These methods enable researchers to identify genetic variations within bacterial populations, track the spread of specific strains, and understand the epidemiology of bacterial infections. Common molecular typing methods include pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), whole-genome sequencing (WGS), and polymerase chain reaction (PCR)-based techniques targeting specific genes or genetic elements (6). MLST for *S. aureus* involves sequencing several housekeeping genes to determine the sequence type (ST) of the bacterium. This information is crucial for tracking the spread of specific strains and understanding the epidemiology of *S. aureus* infections (7).

2. Objectives

In this research project, our objective was to analyze the antibiotic resistance profiles and genetic relatedness of community-associated (CA) and hospital-associated (HA) MRSA, along with methicillin-sensitive *S. aureus* (MSSA) strains obtained from patients in both hospital and community environments in Bandar Abbas, located in southern Iran. To achieve this, we employed SCCmec typing and MLST techniques for strain characterization. Additionally, we aimed to explore the role of the accessory gene regulator (*agr*) genes in these clinical *S. aureus* isolates.

3. Methods

This study was conducted as a cross-sectional investigation from 2020 to 2021, during which a total of 156 strains of *S. aureus* were collected from both community (outpatients) and hospital (inpatients) settings in Bandar Abbas, southern Iran. The *S. aureus* strains were sourced from patients who had been hospitalized for a minimum of 72 hours, excluding duplicate samples from the analysis. Confirmation of *S. aureus* isolates was performed through gram staining and biochemical tests, including growth evaluation on blood agar medium, hemolysis, catalase activity, mannitol fermentation, and coagulase and DNase tests (8). Once identified, the *S. aureus* isolates were preserved for subsequent procedures using Trypticase Soy Broth (TSB) containing 30% glycerol.

3.1. Antimicrobial Susceptibility Testing

The susceptibility of *S. aureus* isolates to various antibiotics, such as azithromycin (15 µg), tigecycline (15 µg), gentamicin (10 µg), linezolid (30 µg), clindamycin (2 µg), ciprofloxacin (5 µg), and tetracycline (30 µg), was assessed using the Kirby-Bauer disc diffusion method following the Clinical and Laboratory Standards Institute (CLSI 2021) guidelines (9). The reference strain *S. aureus* ATCC25923 served as the control. Methicillin resistance was determined using a cefoxitin disc (FOX, 30 µg) according to the CLSI guidelines. The multidrug-resistant (MDR) phenotype was defined as acquired non-susceptibility to at least one agent in three or more antibiotic categories, and pandrug-resistance (PDR) was defined as non-susceptibility to all agents in all antibiotic classes (10).

3.2. Extraction of DNA and Amplification of Genes

The genomic DNAs from all *S. aureus* isolates were extracted using a previously established protocol. This process involved dissolving 20 µL of buffer containing 0.25% SDS (sodium dodecyl sulfate) and 0.05 M NaOH in 200 mL of deionized water, which was then placed into a microtube. Fresh bacterial colonies were dissolved in this solution and heated at 95°C for 10 minutes. Subsequently, the solution was centrifuged at 13,000 g for 1 minute, and 180 µL of deionized water was added to

it (11). To identify MRSA strains, all *S. aureus* isolates were screened for the presence of the *mec*A gene through PCR assays conducted using a SensoQuest Labcycler thermal cycler, following the method outlined by Mahmoudi et al. (12). Additionally, the *agr* and *pvl* genes were also detected using PCR with specific primers (13). PCR reactions were prepared with a total volume of 25 µL. The presence of various SCCmec types (I-V) among *S. aureus* isolates was investigated using specific primers designed for SCCmec types and subtypes I, II, III, IV, and V (14).

3.3. Multilocus Sequence Typing

Five *S. aureus* isolates were selected for further characterization using MLST. The MLST for *S. aureus* was conducted following the scheme proposed by Enright et al (15). Internal fragments of seven housekeeping genes (*arcC*, *gmk*, *aroE*, *glpF*, *pta*, *tpi*, and *yqil*) were amplified using specific primers and conditions outlined in the online MLST database (https://pubmlst.org) and then subjected to direct sequencing. Allele numbers and sequence types (STs) were assigned based on the guidelines provided in the *S. aureus* typing section of the PubMLST database.

3.4. Statistical Analysis

The statistical software used for data analysis was SPSS version 22. Fisher's exact test was employed to determine significant associations between categorical variables. A significance level of $P < 0.05$ was considered significant.

4. Results

In this cross-sectional study, a total of 156 *S. aureus*-positive culture samples were collected from both outpatients and inpatients. Of these, 87 (56%) isolates were from female patients and 69 (44%) were from male patients, with ages ranging from under 15 to over 65 years. Demographic information for MRSA and MSSA is presented in Table 1. A total of 156 *S. aureus* strains were isolated, including 79 from inpatients (hospital-acquired: HA) and 77 from outpatients (community-acquired: CA). Among these, 70 (44.8%) were identified as MRSA based on both disc diffusion and PCR results. Of the MRSA isolates, 45 of 97 (46.3%) were from inpatients and 25 of 59 (42.3%) were from outpatients. Table 1 also compares MRSA and MSSA strains based on the sources of clinical samples and places of sampling (hospital wards). The statistical analysis showed no significant differences in age, gender, type of clinical sample, or hospitalization versus outpatient status between MSSA and MRSA strains. However, a significant difference was observed between MSSA and MRSA strains only in the orthopedic unit and other hospital units.

Table 2 presents the results of antimicrobial susceptibility testing against seven different antibiotics. According to disc diffusion results, the highest resistance was observed against azithromycin (83%) and the lowest was against linezolid (5%) and gentamicin (7%). Resistance rates were 44% for cefoxitin, 43% for tetracycline, 24% for clindamycin, 10% for ciprofloxacin, and 9% for tigecycline. All MRSA strains exhibited the MDR phenotype, and 4 out of 70 (5.7%) MRSA strains showed the pandrug-resistant (PDR) phenotype.

Table 3 also compares antibiotic resistance patterns between MRSA and MSSA strains, revealing significant differences in resistance to tetracycline, linezolid, and azithromycin between the two strains. Table 3 additionally displays the prevalence of various types of *pvl* and *SCCmec* genes in *S. aureus* isolates from hospital and community settings. The PCR results revealed that the *pvl* gene was present in three isolates: Two hospital-acquired (with SCCmec type IV and III and *agr* type III, I) and one community-acquired (with *agr* type I). The hospital strains were isolated from synovial fluid and bronchoalveolar lavage fluid samples, while the CA strain was isolated from a urine sample. The mec gene was identified in the PVL-positive strain obtained from bronchoalveolar lavage fluid, classifying it as an MRSA strain.

Table 3 presents the frequency of virulence genes and molecular typing of MRSA isolates. The PCR results for *agr* genes among strains from both hospital and community settings indicated that *S. aureus* isolates from inpatients and outpatients encompassed four distinct *agr* types. Among these, *agr* type I had the highest number of isolates originating from hospital settings. The findings also revealed that *agr* types I and III were more prevalent in strains isolated from hospitals compared to those from the community. However, the occurrence of the *agr* II group was higher in community isolates than in hospital isolates.

A significant difference was observed in the frequency of *agr* group I and *agr* group III between hospital-associated (HA) and community-associated

Table 1. Characterization of MRSA and MSSA Strains According to Demographic Data, Clinical Specimens and Hospital Wards

Demographic Data	All Strains	MSSA	MRSA	PDR	P-Value
Age group					
Under 15	22	9	13	-	0.14
15 - 44	74	46	28	2	0.09
45 - 64	32	17	15	1	0.79
65 and above	28	14	14	1	0.54
Gender					
Female	87	47	40	2	0.75
Male	69	39	30	2	
Source					
Inpatients	97	52	45	2	0.62
Outpatients	59	34	25	2	
Clinical specimens					
Ascites	2	2	-	-	0.42
Blood	11	8	3	-	0.22
Tracheal tube	20	11	9	2	0.99
Personnel hand	8	-	8	-	0.00
Throat culture	3	2	1	-	0.68
Urine	65	37	28	2	0.70
Wound	42	22	20	-	0.67
Sputum	2	1	1	-	0.88
Bronchoalveolar lavage	2	2	-	-	0.42
Synovial	1	1	-	-	0.77
Hospital wards					
Outpatient	77	41	36	2	0.64
Internal ward	18	11	7	1	0.58
Infectious	14	5	9	1	0.12
Orthopedic	13	11	2	-	0.02
Surgery	8	4	4	-	0.76
Neurology	7	4	3	-	0.91
Burn	6	3	3	-	0.79
Intensive care unit	6	3	3	-	0.79
Emergency	3	2	3	-	0.68
Nephrology	2	1	1	-	0.88
ENT	1	1	-	-	0.77

Abbreviations: AZT, azithromycin; CIP, ciprofloxacin; CLI, clindamycin; GEN, gentamicin; LZD, linezolid; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-sensitive *S. aureus*; TE, tetracycline; TIG, tigecycline; PDR, pandrug-resistance.

(CA) isolates. Only one hospital strain exhibited the simultaneous presence of *agr* types I, III, and IV. The results of SCCmec typing indicated that SCCmec III was the most prevalent among the SCCmec types. The frequency of different SCCmec types was higher in hospital isolates than in community isolates. Statistical analysis revealed a significant difference in the frequency of SCCmec IV between hospital and community isolates.

The MLST results revealed that the five MRSA strains examined have new STs: ST8634, ST8640, ST8650, ST8651, and ST8652, which are being introduced and reported for the first time in this study. The number of alleles and the assigned STs for these five strains are listed in Table 4. The MLST findings demonstrate genetic diversity in MRSA strains isolated from both hospital and community settings. None of these identified STs belonged to clonal complexes; instead, they were considered singletons or unique STs.

5. Discussion

Table 2. Comparison of Antibiotic Resistance Percentage in MRSA and MSSA Strains Isolated from Inpatients and Outpatients [a]

	Antibiotics						
	AZT	TIG	GEN	LZD	CLI	CIP	TE
Inpatient (n = 79)	MSSA: 19 (4)	MSSA: 4 (5)	MSSA: 4 (5)	MSSA: 6 (7.5)	MSSA: 8 (10)	MSSA: 5 (8.4)	MSSA: 13 (16.4)
	MRSA: 17 (21)	MRSA: 2 (2.5)	MRSA: 2 (2.5)	MRSA: 1 (1.3)	MRSA: 9 (11.3)	MRSA: 4 (5)	MSSA: 13 (16.4)
Outpatient (n = 77)	MSSA: 26 (34)	MSSA: 2 (2.5)	MSSA: 2 (2.5)	MSSA: 1 (1.2)	MSSA: 10 (13)	MSSA: 4 (5)	MSSA: 13 (16.8)
	MRSA: 21 (27)	MRSA: 5 (6.4)	MRSA: 4 (5)	MSSA: 0 (0)	MRSA: 10 (13)	MRSA: 2 (2.5)	MRSA: 28 (36)
Total	83 (53)	13 (9)	12 (7)	8 (5)	37 (24)	15 (10)	67 (43)
P-value	0.01	0.36	0.44	0.02	0.38	0.15	0.01

Abbreviations: TE, tetracycline; TIG, tigecycline; AZT, Azithromycin; CIP, ciprofloxacin; CLI, clindamycin; GEN, gentamicin; LZD, linezolid; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-sensitive *S. aureus*; PDR, pandrug-resistance.

[a] Values are expressed as No. (%).

Table 3. The Frequency of *pvl* and *mec*A Genes and *agr* and SCCcmec Types of MRSA Strains Isolated from Hospitals and Community Settings [a]

	Inpatient (n = 79)	Outpatient (n = 77)	Total (n = 156)	P-Value
***mec*A gene**	34 (43)	36 (47)	70 (45)	0.32
pvl	2 (2.5)	1 (1)	3 (2)	0.3
Agr I	19 (24)	6 (8)	25 (16)	0.005
Agr II	4 (5)	10 (13)	14 (9)	0.07
Agr III	9 (11)	1 (1)	10 (6)	0.005
Agr IV	1 (1)	0 (0)	1 (0.6)	0.34
Agr I, III, IV	1 (1)	0 (0)	1 (0.6)	0.34
Sccmec I	5 (6)	4 (5)	9 (6)	0.38
Sccmec II	3 (4)	0 (0)	3 (2)	0.09
Sccmec III	10 (13)	6 (8)	16 (10)	0.15
Sccmec IV	6 (8)	1 (1)	7 (4.5)	0.02
Sccmec IV, III	3 (4)	0 (0)	3 (2)	0.09

[a] Values are expressed as No. (%).

This study evaluated antibiotic resistance, the prevalence of genes such as *mec*A, *pvl*, *agr*, SCCmec, and the genetic relatedness between MRSA and MSSA strains in isolates from healthcare-associated and community-associated infections. Our findings indicate that 43.6% of both outpatients and inpatients in Bandar Abbas, southern Iran, were infected with MRSA. This rate aligns with other studies conducted in Iran, underscoring the significance of MRSA presence in the community, which exceeds 40% and demands special attention for control and management from a public health perspective.

Analysis of multiple studies in Iran shows that the rate of MRSA infections among confirmed *S. aureus* isolates is approximately 43.0%. Detailed analyses have shown that the prevalence of MRSA was higher in studies conducted after 2000 (16). The high prevalence of MRSA in healthcare settings in Iran could be attributed to various factors such as indiscriminate antibiotic use, insufficient implementation of prophylactic hygiene measures, inadequate staff training, and lack of hospital infection control programs. Furthermore, the association of multidrug resistance with MRSA has compounded the challenges of managing MRSA in hospitals across Iran. issue the prevalence of MRSA in Iran, at 43.0%, does indeed appear to be higher than rates reported in other studies (16).

The overall prevalence of clinically isolated MRSA in Egypt is notably high at 63% (17). Variations in MRSA prevalence across different regions of Iran may be due to differences in infection control practices, healthcare conditions, antibiotic prescription practices, selective antibiotic pressure in hospitals, and underlying clinical conditions. These factors can contribute to the varying

Table 4. Characterization of New Sequence Types of *S. aureus* According to Source and Allele Numbers

Strain	In/Out	Source	Sequence Type	*arc*C Allele	*aro*E Allele	*glp*F Allele	*gm*k Allele	*Pta* Allele	*tpi* Allele	*ygi*L Allele
1	Out	Urine	8634	853	37	19	89	805	665	32
6	In	Wound	8640	12	4	1	437	1	724	87
15	In	Trachea	8650	3	1	319	475	787	8	879
24	In	Blood	8651	620	653	1	78	504	698	96
44	In	Trachea	8652	79	1	14	23	691	497	398

rates of MRSA prevalence in different regions of the country (18).

Recent evidence indicates that CA-MRSA accounts for a significant portion of all MRSA infections, with these strains also being detected in hospitalized patients, suggesting intra-hospital movement of CA-MRSA. The prevalence of CA-MRSA strains varies by country, with the lowest reported in France and the highest in the United States (19). In our study, the frequency of CA-MRSA strains was 42%, aligning with results reported from Tehran, Shiraz, and Hamadan in Iran (20-22). However, some studies in Iran have reported lower frequencies of MRSA than those found in our study (18, 23). No significant differences were observed between the prevalence of CA-MRSA and HA-MRSA strains in our study. CA-MRSA outbreaks and cases have been reported in various countries in the Asia-Pacific region, indicating that these strains are circulating in the region (24). High levels of resistance to azithromycin, cefoxitin, and tetracycline were found in both HA-MRSA and CA-MRSA isolates.

Based on the results of this study, linezolid, gentamicin, and tigecycline are the most effective antibiotics against MSSA and MRSA strains acquired from both community and hospital settings. Linezolid is effective against more than 98% of *Staphylococcus* infections, with resistance detected in only 0.05% of *S. aureus* infections. In many studies conducted in Iran, resistance to linezolid was either not reported or was very low (25). Studies analyzing the resistance of Staphylococcus isolates to linezolid in various countries found that the United States, Canada, and European countries had higher levels of resistance, while African and Asian countries reported the lowest (0.1%) resistance among MRSA strains (26).

The 5% linezolid resistance observed in our study is significant, although resistance was higher in hospital strains than in community strains. Consistent with our findings, studies from Egypt and Iran reported 5% linezolid resistance in MRSA isolates (26). A recent study conducted in Pakistan found that 35% of MRSA strains were resistant to linezolid (27). The presence of linezolid resistance in hospital isolates is higher than in the community, as this antibiotic is primarily prescribed in hospitals, but the emergence of resistance in community isolates is notable and concerning.

Tigecycline demonstrated favorable efficacy, following linezolid and gentamicin, against MRSA and MSSA strains in both hospital and community settings. The geographical variation in resistance to tigecycline compared to linezolid suggests different levels of antibiotic usage across regions. Despite recommendations for treating skin and soft tissue infections, recent MRSA infection treatment guidelines have not yet included tigecycline. Previous studies suggest that there are no significant differences between tigecycline and other newer medications, positioning tigecycline as a secondary or tertiary treatment option for MRSA-related infections (28, 29). Our results align with previous studies conducted in Iran, where we found SCCmec type III to be the predominant type among both hospital-acquired and CA MRSA isolates (18, 30-32).

The prevalence of SCCmec type III and type 3 *ccr* among MRSA strains from various sources, including hospitals, the environment, and animals, suggests the hospital origin of MRSA isolates in Iran (18). Additionally, our study detected other SCCmec types such as I, IV, and II, aligning with findings from other studies in Iran. A study from western Iran did not detect SCCmec V (33). However, some studies from Iran have reported the presence of *S. aureus* strains with SCCmec V (18, 30, 34). In our study, SCCmec type IV was found in both hospital-acquired (HA) and CA isolates. The detection of one isolate with SCCmec IV in a hospital suggests the potential dissemination of MRSA strains from the community to hospitals. A study from Japan indicated

that the prevalence of SCCmec IV isolates, which are primarily CA MRSA, has increased in Japanese hospitals (35). The prevalence of *pvl* gene-carrying isolates varies, but it has been reported with a higher incidence in community-associated MRSA strains. In our study, the prevalence of *pvl* gene-carrying isolates was 1.9%, which is lower than in most Iranian studies, where the prevalence ranged from 22.7% to 52.9% (36).

CA-MRSA appears to be associated with increased transmission and hospitalization, as well as skin and soft tissue infections such as furuncles, cellulitis, and skin abscesses. Rarely, it can lead to severe diseases such as necrotizing pneumonia (36). In our study, the PVL-positive strain isolated from the community was obtained from a urine sample, and PVL-positive HA strains were isolated from synovial fluid and bronchoalveolar lavage fluid. Consistent with our findings, a study from Iran reported that among 26.3% of PVL-positive strains were HA-MRSA that presumably moved to the community (37). Additionally, a recent study from two hospitals in Greece conducted between 2020 and 2022 found that 19.15% of the isolates were PVL-positive (38). The presence of PVL-positive strains in hospital isolates indicates the transfer of CA-MRSA from the community to the hospital. A systematic surveillance program is needed to identify common PVL-positive clones in the community.

In this study, it was found that hospital isolates contained all four *agr* groups (*agr I*, *agr II*, *agr III*, and *agr IV*), while CA strains lacked the *agr* IV group. Consistent with many other studies, *agr* group I was the predominant group in hospital-acquired infection (HAI) isolates. However, *agr* II was the predominant *agr* group in CA strains (39). Interestingly, no significant difference was observed in the frequency of *agr* groups between HA and CA strains. This suggests that the distribution of *agr* groups may not be a distinguishing factor between hospital-acquired and CA strains. The discovery of five new typing sequences in *S. aureus* isolates is an interesting finding. These new sequences were reported for the first time and recorded in the PubMLST database, indicating the genetic diversity and circulation of different bacterial clones in and outside hospitals. These unique STs did not belong to any clonal complex of *S. aureus*, underscoring the need for further studies to understand their origins and potential implications in both hospital and community settings.

The evidence indicating the predominance of the ST239 hospital-acquired MRSA clone in many Asian countries, including Iran, is noteworthy. In the study by Bourbour et al., a significant proportion of MRSA strains isolated from inpatients in a teaching hospital in Tehran belonged to ST239 (50%), with ST30 detected in 30% of isolates (40). In studies conducted in hospitals in Isfahan and Tehran, Iran, it was found that 47% and 72% of clinical MRSA strains belonged to ST239, respectively (41, 42). This highlights the significant prevalence of the ST239 hospital-acquired MRSA clone in these regions, emphasizing the importance of understanding and addressing this particular strain in healthcare settings.

This data underscores the importance of continuous monitoring and focused efforts to control and prevent the spread of MRSA in these regions. Further research is needed to understand the epidemiology of MRSA strains, including the newly identified STs in this study. This will aid in developing effective strategies to control and prevent the spread of these bacteria in healthcare settings and the community. The limitation of financial resources in the study prevented the extensive use of MLST to determine the STs for a larger number of strains. This constraint may have impacted the comprehensive understanding of the genetic diversity and distribution of bacterial clones in hospital and community settings.

5.1. Conclusions

In conclusion, the dissemination of HA-MRSA isolates to the community represents a significant public health concern. This underscores the importance of implementing effective processes to control the spread of isolates from hospitals to communities and vice versa. It is crucial to establish and maintain robust infection control measures, surveillance systems, and communication channels between healthcare facilities and community health organizations to prevent the transmission of MRSA and other antibiotic-resistant bacteria. A proactive approach is essential for protecting public health and reducing the impact of antibiotic-resistant infections in both healthcare and community settings. Identifying the sources of infection in both hospital and community settings is vital for the effective prevention and control of antibiotic-resistant bacteria such as MRSA. Understanding the origins and pathways of transmission can guide the development of targeted interventions and control measures.

Footnotes

Authors' Contribution: L.S. and A.K. conceived and designed the experiments. T.D. and V.N. performed the experiments and analyzed the data. L.S. and A.K. prepared figures and tables and wrote the manuscript. A.K. and L.S. critically reviewed the manuscript. All authors have read and approved the final manuscript.

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

Data Availability: The dataset presented in the study is available on request from the corresponding author during submission or after publication.

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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. Mun YS, Hwang YJ. Novel spa and multi-locus sequence types (MLST) of Staphylococcus aureus samples isolated from clinical specimens in Korean. *Antibiotics (Basel)*. 2019;**8**(4). [PubMed ID: 31671880]. [PubMed Central ID: PMC6963223]. https://doi.org/10.3390/antibiotics8040202.
3. Afroz S, Kobayashi N, Nagashima S, Alam MM, Hossain AB, Rahman MA, et al. Genetic characterization of Staphylococcus aureus isolates carrying Panton-Valentine leukocidin genes in Bangladesh. *Jpn J Infect Dis*. 2008;**61**(5):393-6. [PubMed ID: 18806351].
4. Darboe S, Dobreniecki S, Jarju S, Jallow M, Mohammed NI, Wathuo M, et al. Prevalence of Panton-Valentine leukocidin (PVL) and antimicrobial resistance in community-acquired clinical Staphylococcus aureus in an urban Gambian hospital: a 11-year period retrospective pilot study. *Front Cell Infect Microbiol*. 2019;**9**:170. [PubMed ID: 31192162]. [PubMed Central ID: PMC6540874]. https://doi.org/10.3389/fcimb.2019.00170.
5. Traber KE, Lee E, Benson S, Corrigan R, Cantera M, Shopsin B, et al. agr function in clinical Staphylococcus aureus isolates. *Microbiology (Reading)*. 2008;**154**(Pt 8):2265-74. [PubMed ID: 18667559]. [PubMed Central ID: PMC4904715]. https://doi.org/10.1099/mic.0.2007/011874-0.
6. Sharma A, Lee S, Park YS. Molecular typing tools for identifying and characterizing lactic acid bacteria: a review. *Food Sci Biotechnol*. 2020;**29**(10):1301-18. [PubMed ID: 32995049]. [PubMed Central ID: PMC7492335]. https://doi.org/10.1007/s10068-020-00802-x.
7. Hong X, Zhou S, Dai X, Xie D, Cai Y, Zhao G, et al. Molecular typing and characterization of Staphylococcus aureus isolates from burn wound infections in Fujian, China. *Front Microbiol*. 2023;**14**:1236497. [PubMed ID: 37799609]. [PubMed Central ID: PMC10547878]. https://doi.org/10.3389/fmicb.2023.1236497.
8. Mahon C, Lehman D, Manuselis G. 5th Edidtion, editor. *Textbook of Diagnostic Microbiology*. Missouri: Elsevier; 2014.
9. Clinical and Laboratory Standards Institute. *M100: Performance Standards for Antimicrobial Susceptibility Testing*. **41**. Clinical and Laboratory Standards Institute; 2021.
10. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect*. 2012;**18**(3):268-81. [PubMed ID: 21793988]. https://doi.org/10.1111/j.1469-0691.2011.03570.x.
11. Gholizadeh P, Aghazadeh M, Ghotaslou R, Ahangarzadeh Rezaee M, Pirzadeh T, Kose S, et al. CRISPR-cas system in the acquisition of virulence genes in dental-root canal and hospital-acquired isolates of Enterococcus faecalis. *Virulence*. 2020;**11**(1):1257-67. [PubMed ID: 32930628]. [PubMed Central ID: PMC7549939]. https://doi.org/10.1080/21505594.2020.1809329.
12. Mahmoudi H, Pourhajibagher M, Chiniforush N, Soltanian AR, Alikhani MY, Bahador A. Biofilm formation and antibiotic resistance in meticillin-resistant and meticillin-sensitive Staphylococcus aureus isolated from burns. *J Wound Care*. 2019;**28**(2):66-73. [PubMed ID: 30767648]. https://doi.org/10.12968/jowc.2019.28.2.66.
13. Goudarzi M, Fazeli M, Goudarzi H, Azad M, Seyedjavadi SS. Spa typing of Staphylococcus aureus strains isolated from clinical specimens of patients with nosocomial infections in Tehran, Iran. *Jundishapur J Microbiol*. 2016;**9**(7). https://doi.org/10.5812/jjm.35685.
14. Zhang K, McClure JA, Elsayed S, Louie T, Conly JM. Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome mec types I to V in methicillin-resistant Staphylococcus aureus. *J Clin Microbiol*. 2005;**43**(10):5026-33. [PubMed ID: 16207957]. [PubMed Central ID: PMC1248471]. https://doi.org/10.1128/JCM.43.10.5026-5033.2005.
15. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of Staphylococcus aureus. *J Clin Microbiol*. 2000;**38**(3):1008-15. [PubMed ID: 10698988]. [PubMed Central ID: PMC86325]. https://doi.org/10.1128/JCM.38.3.1008-1015.2000.
16. Dadashi M, Nasiri MJ, Fallah F, Owlia P, Hajikhani B, Emaneini M, et al. Methicillin-resistant Staphylococcus aureus (MRSA) in Iran: A systematic review and meta-analysis. *J Glob Antimicrob Resist*. 2018;**12**:96-103. [PubMed ID: 28941791]. https://doi.org/10.1016/j.jgar.2017.09.006.
17. Azzam A, Khaled H, Mosa M, Refaey N, AlSaifi M, Elsisi S, et al. Epidemiology of clinically isolated methicillin-resistant Staphylococcus aureus (MRSA) and its susceptibility to linezolid and vancomycin in Egypt: a systematic review with meta-analysis. *BMC Infect Dis*. 2023;**23**(1):263. [PubMed ID: 37101125]. [PubMed Central ID: PMC10134521]. https://doi.org/10.1186/s12879-023-08202-2.
18. Rahimi F, Shokoohizadeh L. Characterization of methicillin resistant Staphylococcus aureus strains among inpatients and outpatients in

a referral hospital in Tehran, Iran. *Microb Pathog*. 2016;**97**:89-93. [PubMed ID: 27265678]. https://doi.org/10.1016/j.micpath.2016.06.006.

19. Liu Y, Xu Z, Yang Z, Sun J, Ma L. Characterization of community-associated Staphylococcus aureus from skin and soft-tissue infections: a multicenter study in China. *Emerg Microbes Infect*. 2016;**5**(12). e127. [PubMed ID: 27999423]. [PubMed Central ID: PMC5180372]. https://doi.org/10.1038/emi.2016.128.
20. Tajik S, Najar-Peerayeh S, Bakhshi B, Golmohammadi R. Molecular characterization of community-associated methicillin-resistant Staphylococcus aureus in Iranian burn patients. *Iran J Pathol*. 2019;**14**(4):284-9. [PubMed ID: 31754357]. [PubMed Central ID: PMC6824769]. https://doi.org/10.30699/ijp.2019.94189.1917.
21. Hoseini Alfatemi SM, Motamedifar M, Hadi N, Sedigh Ebrahim Saraie H. Analysis of virulence genes among methicillin resistant Staphylococcus aureus (MRSA) strains. *Jundishapur J Microbiol*. 2014;**7**(6). e10741. [PubMed ID: 25371805]. [PubMed Central ID: PMC4217665]. https://doi.org/10.5812/jjm.10741.
22. Sedighi I, Moez HJ, Alikhani MY. Nasal carriage of methicillin resistant Staphylococcus aureus and their antibiotic susceptibility patterns in children attending day-care centers. *Acta Microbiol Immunol Hung*. 2011;**58**(3):227-34. [PubMed ID: 21983324]. https://doi.org/10.1556/AMicr.58.2011.3.6.
23. Armin S, Karimi A, Pourmoghaddas Z, Azimi L, Fallah F, Tahbaz SV. Evaluation of methicillin-resistant Staphylococcus virulence genes and antibiotics susceptibility in Iranian population. *J Res Med Sci*. 2022;**27**:36. [PubMed ID: 35968212]. [PubMed Central ID: PMC9374147]. https://doi.org/10.4103/jrms.JRMS_543_19.
24. Wong JW, Ip M, Tang A, Wei VW, Wong SY, Riley S, et al. Prevalence and risk factors of community-associated methicillin-resistant Staphylococcus aureus carriage in Asia-Pacific region from 2000 to 2016: a systematic review and meta-analysis. *Clin Epidemiol*. 2018;**10**:1489-501. [PubMed ID: 30349396]. [PubMed Central ID: PMC6190640]. https://doi.org/10.2147/CLEP.S160595.
25. Shirvani F, Sannai Dashti A, Seifi K. Staphylococcus and Linezolid Resistance in Iran. *Arch Pediatr Infect Dis*. 2018;**In Press**(In Press). https://doi.org/10.5812/pedinfect.12236.
26. Shariati A, Dadashi M, Chegini Z, van Belkum A, Mirzaii M, Khoramrooz SS, et al. The global prevalence of Daptomycin, Tigecycline, Quinupristin/Dalfopristin, and Linezolid-resistant Staphylococcus aureus and coagulase-negative staphylococci strains: a systematic review and meta-analysis. *Antimicrob Resist Infect Control*. 2020;**9**(1):56. [PubMed ID: 32321574]. [PubMed Central ID: PMC7178749]. https://doi.org/10.1186/s13756-020-00714-9.
27. Wali M, Shah MS, Rehman TU, Wali H, Hussain M, Zaman L, et al. Detection of linezolid resistance cfr gene among MRSA isolates. *J Infect Public Health*. 2022;**15**(10):1142-6. [PubMed ID: 36155853]. https://doi.org/10.1016/j.jiph.2022.09.002.
28. Cai Y, Wang R, Liang B, Bai N, Liu Y. Systematic review and meta-analysis of the effectiveness and safety of tigecycline for treatment of infectious disease. *Antimicrob Agents Chemother*. 2011;**55**(3):1162-72. [PubMed ID: 21173186]. [PubMed Central ID: PMC3067123]. https://doi.org/10.1128/AAC.01402-10.
29. Rodvold KA, Gotfried MH, Cwik M, Korth-Bradley JM, Dukart G, Ellis-Grosse EJ. Serum, tissue and body fluid concentrations of tigecycline after a single 100 mg dose. *J Antimicrob Chemother*. 2006;**58**(6):1221-9. [PubMed ID: 17012300]. https://doi.org/10.1093/jac/dkl403.
30. Goudarzi M, Navidinia M, Dadashi M, Hashemi A, Pouriran R. First report of methicillin-resistant Staphylococcus aureus carrying the mecC gene in human samples from Iran: prevalence and molecular characteristics. *New Microbes New Infect*. 2021;**39**:100832. [PubMed ID: 33425366]. [PubMed Central ID: PMC7777544]. https://doi.org/10.1016/j.nmni.2020.100832.
31. Rasheed NA, Hussein NR. Methicillin-resistant Staphylococcus aureus carriage rate and molecular characterization of the staphylococcal cassette chromosome mec among Syrian refugees in Iraq. *Int J Infect Dis*. 2020;**91**:218-22. [PubMed ID: 31843670]. https://doi.org/10.1016/j.ijid.2019.12.006.
32. Moosavian M, Shahin M, Navidifar T, Torabipour M. Typing of staphylococcal cassette chromosome mec encoding methicillin resistance in Staphylococcus aureus isolates in Ahvaz, Iran. *New Microbes New Infect*. 2018;**21**:90-4. [PubMed ID: 29379604]. [PubMed Central ID: PMC5773478]. https://doi.org/10.1016/j.nmni.2017.11.006.
33. Dini M, Shokoohizadeh L, Jalilian FA, Moradi A, Arabestani MR. Genotyping and characterization of prophage patterns in clinical isolates of Staphylococcus aureus. *BMC Res Notes*. 2019;**12**(1):669. [PubMed ID: 31639052]. [PubMed Central ID: PMC6805666]. https://doi.org/10.1186/s13104-019-4711-4.
34. Ghanbari F, Saberianpour S, Zarkesh-Esfahani F, Ghanbari N, Taraghian A, Khademi F. Staphylococcal Cassette Chromosome mec (SCCmec) Typing of methicillin- resistant Staphylococcus aureus strains isolated from community and hospital-acquired infections. *Avicenna J Clin Microbiol Infect*. 2017;**4**(2):42244. https://doi.org/10.5812/ajcmi.42244.
35. Nakaminami H, Takadama S, Ito A, Hasegawa M, Jono C, Noguchi M, et al. Characterization of SCCmec type IV methicillin-resistant Staphylococcus aureus clones increased in Japanese hospitals. *J Med Microbiol*. 2018;**67**(6):769-74. [PubMed ID: 29676727]. https://doi.org/10.1099/jmm.0.000735.
36. Elston JW, Barlow GD. Community-associated MRSA in the United Kingdom. *J Infect*. 2009;**59**(3):149-55. [PubMed ID: 19619897]. https://doi.org/10.1016/j.jinf.2009.07.001.
37. Tajik S, Najar-Peerayeh S, Bakhshi B. Hospital clones of Panton-Valentine leukocidin-positive and methicillin-resistant Staphylococcus aureus circulating in the Tehran community. *J Glob Antimicrob Resist*. 2020;**22**:177-81. [PubMed ID: 31874221]. https://doi.org/10.1016/j.jgar.2019.12.010.
38. Vittorakis E, Vica ML, Zervaki CO, Vittorakis E, Maraki S, Mavromanolaki VE, et al. Examining the prevalence and antibiotic susceptibility of S. aureus strains in hospitals: An analysis of the pvl gene and its co-occurrence with other virulence factors. *Microorganisms*. 2023;**11**(4). [PubMed ID: 37110264]. [PubMed Central ID: PMC10140963]. https://doi.org/10.3390/microorganisms11040841.
39. 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.
40. Bourbour S, Beigverdi R, Beheshti M, Jabalameli F, Emaneini M. Identification of major sequence types among aminoglycoside resistant Staphylococcus aureus and Staphylococcus epidermidis strains isolated from clinical samples. *Iran J Microbiol*. 2022;**14**(3):305-12. [PubMed ID: 37124846]. [PubMed Central ID: PMC10132341]. https://doi.org/10.18502/ijm.v14i3.9760.
41. Havaei SA, Vidovic S, Tahmineh N, Mohammad K, Mohsen K, Starnino S, et al. Epidemic methicillin-susceptible Staphylococcus aureus lineages are the main cause of infections at an Iranian university hospital. *J Clin Microbiol*. 2011;**49**(11):3990-3. [PubMed ID: 21940478].

[PubMed Central ID: PMC3209102]. https://doi.org/10.1128/JCM.05445-11.

42. Goudarzi M, Bahramian M, Satarzadeh Tabrizi M, Udo EE, Figueiredo AM, Fazeli M, et al. Genetic diversity of methicillin resistant Staphylococcus aureus strains isolated from burn patients in Iran: ST239-SCCmec III/t037 emerges as the major clone. *Microb Pathog*. 2017;**105**:1-7. [PubMed ID: 28179118]. https://doi.org/10.1016/j.micpath.2017.02.004.