Microbes and Infectious Diseases 2024; 5(3): 1098-1112

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Scc*mec*- and *spa*-based determination of genetic diversity of methicillin-resistant *Staphylococcus aureus* (MRSA) clinical isolates

Abdel-Aty Elsayed Alfeky[1], Mahmoud Mohamed Tawfick*[1,2], Mohamed Seif El-Din Ashour[1], Abdel-Nasser Abbass El-Moghazy[1]

1- Department of Microbiology and Immunology, Faculty of Pharmacy (For Boys), Al-Azhar University, Cairo, Egypt.

2- Department of Microbiology and Immunology, Faculty of Pharmacy, Heliopolis University, Cairo, Egypt.

ARTICLE INFO

Article history:
Received 9 May 2024
Received in revised form 6 June 2024
Accepted 17 June 2024

Keywords:
Methicillin resistance
Staphylococcus aureus
Spa
SCC*mec*
Genotyping
Molecular diversity

ABSTRACT

Background and Aim: Methicillin-resistant *Staphylococcus aureus* (MRSA) causes infectious diseases that are clinically problematic. Methicillin resistance is encoded by the *mecA* gene on the mobile staphylococcal cassette chromosome *mec* (SCC*mec*), with SCC*mec* types I to V being the most prevalent. Staphylococcal protein A gene (*spa*) typing is a genotyping method designed for *S. aureus*. This study aimed to use SCC*mec* and *spa* genotyping-based methods to identify the genetic diversity of clinical MRSA isolates. **Methods:** Fifty MDR-MRSA isolates from tertiary hospitals in Egypt were subjected to molecular-based typing using multiplex PCR to identify SCC*mec* types I to V and detect *spa* types via *spa* DNA sequence analysis. **Results:** The predominant SCC*mec* types were SCC*mec* V (34%) and SCC*mec* IV (32%). The *spa* sequence analysis revealed 13 definite *spa* types, with t084 (24.2%) being the most prevalent, followed by t688 (15.1%). Based on both genotyping methods, 20 diverse MRSA clones were identified, with the most predominant MRSA clones being CC15-SCC*mec*V-t084 and CC15-SCC*mec*IV-t084 (12.1%). **Conclusion:** MRSA bacteria in Egyptian hospitals are genetically different, indicating that the strains have diverse origins. Thus, regular surveillance of MRSA diversity is required to detect emergent clones for precise epidemiological evaluation and improved procedures to prevent the spread of this superbug.

Introduction

The resistance of *Staphylococcus aureus* to methicillin is due to the production of the low-affinity penicillin-binding protein PBP2a, which is encoded by the *mecA* gene positioned on the mobile genetic element SCC*mec*, thus leading to resistance to β-lactam drugs [1]. Therefore, methicillin-resistant *S. aureus* (MRSA) has always been an obstacle to antimicrobial therapy and has surpassed the number of drugs developed in the last 60 years [2]. As early as 1961, the first clinical MRSA infection was reported following the use of methicillin. Since then, MRSA has been spreading and causing life-threatening conditions worldwide, particularly hospital-associated infections [3]. In recent decades, due to horizontal DNA transfer, MRSA strains have undergone genetic restructuring by integrating foreign DNA genomic regions and through mutations. Accordingly, numerous MRSA clones have evolved and spread globally while

DOI: 10.21608/MID.2024.288254.1934

* *Corresponding author:* Mahmoud Mohamed Tawfick
E-mail address: *mahmoud_tawfick@azhar.edu.eg*

responding to increasing antibiotic selection pressure [4].

Currently, the distinction between hospital-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA) infections remains ambiguous, owing mainly to the spread of MRSA strains between communities and hospitals [5]. However, both types of infections can be discriminated based on the epidemiological and genotypic characteristics of the MRSA strains [6]. Hence, molecular-based typing is an essential approach for assessing the genetic relatedness and diversity of MRSA strains, their distribution among different clones, and, consequently, their mode of transmission. Indeed, this molecular typing scheme has been essential for developing infection control protocols to prevent the incidence and/or spread of prevalent superbug clones in healthcare facilities and communities [5,7].

Different molecular techniques are presently used for typing MRSA, including SCC*mec* typing, *spa* typing, multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE) [8]. However, typing methods, such as MLST and *spa*, are considerable for indicating the evolutionary relationship and recognizing the epidemiological dynamics of MRSA compared to other approaches [9]. The *spa* typing method relies on the presence of a variable number of tandem repeats within the polymorphic repeat region X of the protein A gene. Remarkably, the usefulness of *spa* sequence-based and SCC*mec*-based typing for accurately identifying MRSA clones has been proven [5]. Accordingly, the objective of the present study was to utilize *spa* and SCC*mec* typing methods to determine the molecular diversity of MDR-MRSA isolates from tertiary hospitals in Egypt.

Materials and Methods

MRSA isolates included in this study

Fifty MDR-MRSA isolates from two Egyptian tertiary hospitals, Al-Sayed Galal University Hospital (23/50, 46%) and Al-Demerdash University Hospital (27/50, 54%), were investigated in this study. The sample collection and identification of *S. aureus* isolates were performed in the included hospitals by dedicated members for the regular medical care of patients. Our previous publications described the isolation and identification, antimicrobial susceptibility and virulence profile of *S. aureus* bacteria, and methicillin resistance identification by detecting the *mecA* gene and further information about included MRSA isolates in this study [10,11].

The determination of whether the MDR-MRSA was from HA-MRSA or CA-MRSA infection was based on the US Centers for Disease Control and Prevention (CDC) (Atlanta, GA, USA) criteria for the definition of both types [12]. Therefore, isolates were considered HA-MRSA infections if patients had surgery or were on hemodialysis, were residing for long-term care, had a permanent catheter or percutaneous device, and had been hospitalized > 48 hours before MRSA culture and isolation. If the patient did not meet these conditions or the culture was obtained within 48 hours of admission, the infection was categorized as CA-MRSA [12]. Among the 50 studied isolates, 64% (32/50) were HA-MRSA, while 36% (18/50) were CA-MRSA. The distribution of the tested MRSA isolates from different clinical specimens and units/wards in the study hospitals is shown in **table (1)**.

DNA extraction and PCR oligonucleotide primers

According to the manufacturer's guidelines, chromosomal DNA was isolated from the MDR-MRSA isolates using the commercially available purification kit Gene Jet Genomic DNA (Thermo Scientific, USA). DNA extracts were stored in aliquots of 150 µL at –20°C. The oligonucleotide primers used for PCR were produced by Willowfort, UK. The lyophilized primers for the studied genes were reconstituted in nuclease-free water, and the concentration of each primer was adjusted to 10 pmole/µL. PCR primer sequences for detecting SCC*mec* types (SCC*mec* I, SCC*mec* II, SCC*mec* III, SCC*mec* IV, SCC*mec* V) and the *spa* gene are listed in **table (2)**.

Multiplex PCR-based SCC*mec* typing

Multiplex PCR was used to detect the SCC*mec* type according to the methods of **Maina et al.** [13] and **McClure-Warnier et al.** [15]. The reaction mixture contained 12.5 µL of Cosmo PCR Red Master Mix (Willowfort, UK), 1 µL of template DNA, and 1 µL of each forward and reverse primer, and the volume was adjusted to 50 µL with nuclease-free water. The PCR program was as follows: initial denaturation at 94°C (5 min); 35 cycles of denaturation at 94°C (45 sec), annealing at 48°C (1 min), and extension at 72°C (1 min); and a final extension at 72°C (10 min).

***Spa* typing**

The investigated isolates were subjected to PCR amplification using *spa* gene-specific primers and DNA sequencing of the amplified PCR products **(Table 2)**. The investigated MDR-MRSA isolates representing the most common SCC*mec* genotypes (n = 33) were subjected to PCR amplification of the polymorphic repeat region (X region) of the *spa* gene as previously described. The PCR mixture was set up in a total volume of 20 µL containing 10 µL of Cosmo PCR Red Master Mix (Willowfort, UK), 1 µL of template DNA, 1 µL of each forward and reverse primer and 7 µL of nuclease-free water. The PCR program involved initial denaturation (94°C, 5 min), followed by 30 cycles of denaturation (94°C, 30 sec), annealing (52°C, 30 sec) and extension (72°C, 1 min), and a final extension (72°C, 10 min) [14,16].

The *spa* PCR amplicons were purified with a DNA Purification Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. The purified PCR products were confirmed by agarose gel electrophoresis. The purified *spa* PCR products were sequenced using the Sanger technique by Macrogen (Seoul, South Korea). *Spa* typing was conducted following the method described by **Harmsen et al.** [14]. The *spa* sequences were assembled, and the *spa* types were identified by Ridom SeqSphere+ Software version 7.8.0. (7/2021) (Ridom GmbH, Münster, Germany) depending on repeat succession.

Clustering of the extracted *spa* types was conducted by *spa* typing minimum spanning tree (MST) based on the BURP (Based Upon Repeat Pattern) algorithm implemented in Ridom SeqSphere+. The BURP algorithm characterizes the long-term evolution of MRSA bacteria based on *spa* polymorphisms and the relationships between different *spa* types [17]. The data about the extracted spa types, their geographical spread, relative global frequency, and the predicted ST and MLST clonal complexes (MLSTCCs) were collected based on the Ridom SpaServer database available at http://spa.ridom.de/spatypes.shtml and the study of Monecke *et al*. [18].

Detection of PCR products by TBE (Tris-borate-EDTA) agarose gel electrophoresis

Amplified PCR products were detected by agarose gel electrophoresis according to previously described procedures [19]. PCR products were visualized through TBE agarose gel electrophoresis using molecular biology grade agarose (GIBCO, USA) containing ethidium bromide (Alliance Bio, USA) in 1× TBE buffer (Thermo Scientific, USA). DNA fragments were electrophoresed (at 100 V and 70 mA for 40 minutes) in an electrophoresis apparatus (Cole Parmer, Germany). For DNA visualization, the gel was placed on a UV transilluminator (Biometra, Germany) and photographed directly at the end of electrophoresis. The 100-bp and 1-Kb DNA Mwt markers (Gene Ruler, Thermo Scientific, USA) were used where appropriate for sizing DNA fragments.

Distribution of SCC*mec* types among MRSA study isolates

In the present study, all MRSA isolates were SCC*mec* typeable, and the investigated SCC*mec* types from I to V were detected. The results of multiplex PCR-based typing are shown in **figure (1)**. The results of the PCR assay revealed that the most predominant SCC*mec* types were SCC*mec* V (34%, 17/50) and SCC*mec* IV (32%, 16/50), followed by SCC*mec* II (22%, 11/50). SCC*mec* I and SCC*mec* III were less frequent types; each was detected in 3/50 (6%) of the investigated MRSA isolates, as shown in **figure (2)**.

Concerning the clinical sample source of the isolates and the SCC*mec* types of the MRSA isolates, the most predominant types, SCC*mec* V and IV, were detected in high percentages from wounds rather than from other sources. The next most prevalent type, SCC*mec* II, mainly existed in MRSA isolates from blood specimens. A higher percentage of SCC*mec* V was detected among MRSA isolates from wound swabs (8/17, 47%), followed by isolates from blood and intravenous catheters (2/17, 11.8% each). SCC*mec* type IV was predominantly detected in MRSA isolated from wound samples (6/16, 37.5%), followed by sputum (4/16, 25%) and blood (3/16, 18.7%). SCC*mec* type II was frequently detected in MRSA isolates from blood (7/11, 63.6%) and eye swabs (2/11, 18.2%). SCC*mec* type III was detected in MRSA isolates from wound swabs and blood and abscess specimens (1/3, 33.3%). SCC*mec* type I was mainly detected in MRSA isolates from abscess (2/3, 66.7%) and urine (1/3, 33.3%) **(Table 3)**.

Concerning the hospitals included in this study, the most predominant SCC*mec* types in Al-Demerdash University Hospital were SCC*mec* IV and SCC*mec* V (33.3% each, 9/27), followed by SCC*mec* II (26%, 7/27) and SCC*mec* I (7.4%, 2/27). The most predominant SCC*mec* type in Al-Sayed Galal University Hospital was SCC*mec* V (34.8%,

8/23), followed by SCC*mec* IV (30.4%, 7/23), SCC*mec* II (17.4%, 4/23), SCC*mec* III (13.1%, 3/23) and SCC*mec* I (4.3%, 1/23), as presented in **table (3)**.

Prevalence of SCC*mec* types in HA-MRSA and CA-MRSA isolates

Concerning the type of MRSA infection, HA-MRSA isolates (64%, 32/50) included all SCC*mec* types (I–V). Among these isolates, the most prevalent SCC*mec* types were SCC*mec* II, SCC*mec* IV and SCC*mec* V (28.1%, 9/32 each), followed by SCC*mec* III (9.4%, 3/32) and SCC*mec* I (6.2%, 2/32). The CA-MRSA isolates (36%, 18/50) comprised four SCC*mec* types, with the most frequent being SCC*mec* V (44.4%, 8/18), followed by SCC*mec* IV (38.9%, 7/18), SCC*mec* II (11.1%, 2/18) and SCC*mec* I (5.6%, 1/18). SCC*mec* III was not detected among CA-MRSA isolates **(Table 4)**.

Compared with the hospitals of study, at Al-Sayed Galal Hospital, four SCC*mec* types were detected among the HA-MRSA isolates (n = 23), where the most prevalent type was SCC*mec* IV (6/17, 35.3%), followed by SCC*mec* V (29.4%, 5/17) and SCC*mec* III and SCC*mec* II (17.6%, 3/17 each). Four SCC*mec* types were detected among CA-MRSA isolates from the same hospital (n = 23), with percentages of 50% (3/6) for SCC*mec* V and 16.7% (1/6) for SCC*mec* IV, SCC*mec* II and SCC*mec* I **(Table 4)**. On the other hand, at Al-Demerdash Hospital, for the HA-MRSA isolates (n = 15), SCC*mec* II (40%, 6/15) was the most prevalent type, followed by SCC*mec* V (26.7%, 4/15), SCC*mec* IV (20%, 3/15) and SCC*mec* I (13.3%, 2/15), with the absence of SCC*mec* III, while three SCC*mec* types were detected among the CA-MRSA isolates (n = 12), with high frequencies of SCC*mec* IV (50%, 6/12) and SCC*mec* V (41.7%, 5/12) **(Table 4**).

The *spa*-based typing of MRSA isolates

In this study, *spa* typing revealed 13 different *spa* types among the 33 MDR-MRSA isolates examined, representing the most common types in SCC*mec* typing [SCC*mec* V (17/33, 51.5%) and SCC*mec* IV (16/33, 48.5%)]. Remarkably, all investigated isolates were typeable according to *spa* analysis. The *spa* type with the highest percentage of isolates was t084 (24.2%, 8/33), followed by t688 (15.1%, 5/33), t127 (12.1%, 4/33), t037 (9.1%, 3/33), t132, t238, t903 and t1991 (6%, 2/33). The other *spa* types were less frequent, with t355, t2253, t3643, t5111 and 5227 representing 3% (1/33) of each type **(Table 5)**.

Based on the *spa* phylogeny, the examined isolates belonged to nine clades: the first included *spa* types t084 and t5111, the second comprised *spa* types t127 and t2253, the third included *spa* types t037 and t238, the fourth involved *spa* type t688, the fifth comprised *spa* types t1991 and t5227, the sixth included *spa* type t132, the seventh involved *spa* type t903, the eighth comprised *spa* type t355, and the ninth clade included *spa* type t3643. The phylogenetic tree of *spa* types of individual MDR-MRSA isolates was generated by the neighbour-joining method implemented in Ridom SeqSphere+ Software **(Figure 3)**.

Concerning clonal characterization, the predicted MLSTCCs for the identified *spa* types in this study revealed that there was clonal diversity, with seven MLSTCCs identified in the investigated MRSA isolates, namely, CC15 (t084, 24.2%), CC5 (t688, 15.1%), CC1 (t127, 12.1%), CC8 (t037, 9.1%), CC913 (t1991, 6%), ST46 (t132, 6%) and ST152 (t355, 3%). MLSTCCs for the other *spa* types, including t238, t3643, t903, t5227, t5111 and t2253, have not yet been assigned **(Table 5)**.

The minimum spanning tree (MST) clustered the resulting *spa* types according to the genetic distance between different *spa* types into five MST clusters [*spa* clonal complexes (*Spa*CCs)] and one singleton, where the MST cluster distance threshold equals nine, and the minimum MST cluster isolate count equals two. These MST clusters comprised MST Cluster 1 (7 MST nodes, 14 MRSA isolates, maximum distance of 18), which included seven *spa* types (t127, t2253, t5227, t1991, t355, t037 and t238). MST Cluster 2 (two MST nodes, 9 MRSA isolates, maximum distance of 1) included *spa* types t084 and t5111, while MST Cluster 3 (one MST node, 5 MRSA isolates, maximum distance 0) included *spa* type t688. The MST Cluster 4 (one MST node, 2 MRSA isolates, maximum distance 0) included *spa* type t132, and MST Cluster 5 (one MST node, 2 MRSA isolates, maximum distance 0) included *spa* type t903. The BURP algorithm revealed one singleton, which included *spa* type t3643 (one MST node, one MRSA isolate). The Ridom SeqSphere+ minimum spanning tree was also constructed based on the BURP genetic distances between *spa* types, as shown in **figure (4)**.

MRSA clones based on combined SCC*mec* and *spa* typing

The genetic grouping of 33 MRSA isolates, based on the combination of *spa* and SCC*mec* types, revealed 20 identified MRSA clones. The most common *spa*-SCC*mec* MRSA clones were the t084-SCC*mec*V and t084-SCC*mec*IV clones (12.1%, 4/33 each). This was closely followed by each of the t688-SCC*mec*V and t127-SCC*mec*V clones (9.1%, 3/33) and each of the t037-SCC*mec*V, t688-SCC*mec*IV and t903-SCC*mec*IV clones (6%, 2/33 each). The other less frequent clones were t127-SCC*mec*IV, t037-SCC*mec*IV, t238-SCC*mec*V, t238-SCC*mec*IV, t1991-SCC*mec*V, t1991-SCC*mec*IV, t132-SCC*mec*V, t132-SCC*mec*IV, t355-SCC*mec*V, t3643-SCC*mec*V, t5227-SCC*mec*IV, t5111-SCC*mec*IV, and t2253-SCC*mec*IV (3%, 1/33 for each clone) **(Table 5)**.

Based on the global description of MRSA clones obtained by combining their MLSTCCs with SCC*mec*-*spa*, 20 different MRSA clones were observed among the investigated isolates. The most predominant MRSA clones were CC15-SCC*mec*V-t084 (12.1%, 4/33) and CC15-SCC*mec*IV-t084 (12.1%, 4/33). This was followed by CC5-SCC*mec*V-t688 and CC1-SCC*mec*V-t127 (9.1%, 3/33), and CC8-SCC*mec*V-t037, CC5-SCC*mec*IV-t688 and SCC*mec*IV-t903 (6.1%, 2/33). Other MRSA clones were less frequently distributed, including CC1-SCC*mec*IV-t127, CC8-SCC*mec*IV-t037, ST46-SCC*mec*V-t132, ST46-SCC*mec*IV-t132, ST152-SCC*mec*V-t355, CC913-SCC*mec*V-t1991, CC913-SCC*mec*IV-t1991, SCC*mec*V-t238, SCC*mec*IV-t238, SCC*mec*IV-t5227, SCC*mec*IV-t5111, SCC*mec*IV-t2253 and SCC*mec*V-t3643 (3%, 1/33)

.

Table 1. MRSA isolates, clinical specimens and units/wards in the study hospitals.

Specimens	MRSA isolates No. (%)	Type of MRSA infection		Hospitals		Wards/Units of isolation	
		HA-MRSA No. (%)	CA-MRSA No. (%)	SUH (%*)	DUH (%*)	Type	No. (%**)
Wound swabs	15 (30%)	6 (40%)	9 (60%)	5 (10%)	10 (20%)	Outpatients	9 (60%)
						Surgery	4 (26.7%)
						ICU	2 (13.3%)
Blood	13 (26%)	13 (100%)	–	7 (14%)	6 (12%)	ICU	12 (92.3%)
						Surgery	1 (7.7%)
Sputum	5 (10%)	3 (60%)	2 (40%)	3 (6%)	2 (4%)	ICU	3 (60%)
						Chest	2 (40%)
Urine	4 (8%)	–	4 (100%)	1 (2%)	3 (6%)	Outpatients	3 (75%)
						Urology	1 (25%)
Abscess	4 (8%)	4 (100%)		2 (4%)	2 (4%)	Surgery	4 (100%)
Eye swabs	3 (6%)	–	3 (100%)	2 (4%)	1 (2%)	Ophthalmology	2 (66.7%)
						Outpatients	1 (33.3%)
IV catheters	3 (6%)	3 (100%)	–	2 (4%)	1 (2%)	ICU	3 (100%)
Urinary catheters	1 (2%)	1 (100%)	–	–	1 (2%)	ICU	1 (100%)
Endotracheal aspirates	2 (4%)	2 (100%)	–	1 (2%)	1 (2%)	ICU	2 (100%)
Total	50 (100%)	32 (64%)	18 (36%)	23 (46%)	27 (54%)	–	50 (100%)

*****Percentage was correlated to the total number of MDR-MRSA isolates (n = 50)
**Percentage was correlated to the total number of the corresponding type of clinical source
SUH: AL-Sayed Galal University Hospital; DUH: Al-Demerdash University Hospital

Table 2. The PCR oligonucleotide primers and amplicon size.

Target gene	Primer sequence (5′→3′)	Amplicon size (bp)	Reference
SCC*mec* I	*Fw*: GCTTTAAAGAGTGTCGTTACAGG *Rv*: GTTCTCTCATAGTATGACGTCC	613	[13]
SCC*mec* II	*Fw*: CGTTGAAGATGATGAAGCG *Rv*: CGAAATCAATGGTTAATGGACC	398	
SCC*mec* III	*Fw*: CCATATTGTGTACGATGCG *Rv*: CCTTAGTTGTCGTAACAGATCG	280	
SCC*mec* IV	*Fw*: GCCTTATTCGAAGAAACCG *Rv*: CTACTCTTCTGAAAAGCGTCG	776	
SCC*mec* V	*Fw*: GAACATTGTTACTTAAATGAGCG *Rv*: TGAAAGTTGTACCCTTGACACC	325	
spa	1113_ *Fw*: TAAAGACGATCCTTCGGTGAGC 1514_ *Rv*: CAGCAGTAGTGCCGTTTGCTT	Variable (180–670)	[14]

Table 3. Frequency of SCC*mec* types among MRSA isolates from different clinical sources

Group	SCC*mec* type	MRSA isolates in SCC*mec* type No. (%)*	Source specimen	MRSA isolates from source specimen No. (%)**	Hospitals	
					SUH No. (%)*	DUH No. (%)*
A	SCC*mec* V	17 (34%)	Wound	8 (47%)	3 (17.6%)	5 (29.4%)
			Blood	2 (11.8%)	2 (11.8%)	–
			Sputum	1 (5.9%)	–	1 (5.9%)
			Urine	1 (5.9%)	–	1 (5.9%)
			Abscess	1 (5.9%)	1 (5.9%)	–
			Eye swab	1 (5.9%)	1 (5.9%)	–
			IV catheter	2 (11.1%)	1 (5.9%)	1 (5.9%)
			Urinary catheter	1 (5.9%)	–	1 (5.9%)
B	SCC*mec* IV	16 (32%)	Wound	6 (37.5%)	1 (6.25%)	5 (31.25%)
			Blood	3 (18.75%)	2 (12.5%)	1 (6.25%)
			Sputum	4 (25%)	3(18.75%)	1 (6.25%)
			Urine	2 (12.5%)	–	2 (12.5%)
			ETAs	1 (6.25%)	1 (6.25%)	–
C	SCC*mec* II	11 (22%)	Blood	7 (63.6%)	2 (18.2%)	5 (45.5%)
			ETAs	1 (9.1%)	–	1 (9.1%)
			Eye swab	2 (18.2%)	1 (9.1%)	1 (9.1%)
			IV catheter	1 (9.1%)	1 (9.1%)	–
D	SCC*mec* III	3 (6%)	Wound	1 (33.3%)	1 (33.3%)	–
			Blood	1 (33.3%)	1 (33.3%)	–
			Abscess	1 (33.3%)	1 (33.3%)	–
E	SCC*mec* I	3 (6%)	Abscess	2 (66.7%)	–	2 (66.7%)
			Urine	1 (33.3%)	1 (33.3%)	–
Total		50 (100%)		50 (100%)	23 (46%)	27 (54%)

* Percentage calculated to the number of MDR-MRSA isolates (n = 50)
** Percentage calculated to the number of isolates in each group
ETAs: endotracheal aspirates
SUH: AL-Sayed Galal University Hospital; DUH: Al-Demerdash University Hospital

Table 4. The frequencies of SCC*mec* types in HA-MRSA and CA-MRSA regarding the clinical source and the study hospitals.

Clinical specimens	HA-MRSA			CA-MRSA		
	Total No. of isolates n = 32	Hospital		Total No. of isolates n = 18	Hospital	
		SUH n = 17	DUH n = 15		SUH n = 6	DUH n = 12
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Wounds	6 (18.8)	2 (11.8)	4 (26.6)	9 (50)	3 (50)	6 (50)
Blood	13 (40.6)	7 (41.2)	6 (40)	–	–	–
Sputum	3 (9.4)	3 (17.6)	–	2 (11.1)	–	2 (16.7)
Urine	–	–	–	4 (18%)	1 (16.7)	3 (25)
Abscess	4 (12.5)	2 (11.8)	2 (13.3)	–	–	–
Eye swabs	–	–	–	3 (16.7)	2 (33.3)	1 (8.3)
IV catheters	3 (9.4)	2 (11.8)	1 (6.7)	–	–	–
Urinary catheters	1 (3.1)	–	1 (6.7)	–	–	–
ETAs	2 (6.2)	1 (5.8)	1 (6.7)	–	–	–
Total	32 (64)	17 (53.1)	15 (46.9)	18 (36)	6 (33.3)	12 (66.7)
SCC*mec* types in each hospital	**SCC*mec* type**	**No. (%)**	**No. (%)**	**SCC*mec* type**	**No. (%)**	**No. (%)**
	SCC*mec* V	5 (29.4)	4 (26.7)	SCC*mec* V	3 (50)	5 (41.7)
	SCC*mec* IV	6 (35.3)	3 (20)	SCC*mec* IV	1 (16.7)	6 (50)
	SCC*mec* III	3 (17.6)	–	SCC*mec* III	–	–
	SCC*mec* II	3 (17.6)	6 (40)	SCC*mec* II	1 (16.7)	1 (8.3)
	SCC*mec* I	–	2 (13.3)	SCC*mec* I	1 (16.7)	–
SCC*mec* types collectively in both hospitals	**SCC*mec* type**	**No. (%*)**		**SCC*mec* type**	**No. (%**)**	
	SCC*mec* V	9 (28.1%)		SCC*mec* V	8 (44.4%)	
	SCC*mec* IV	9 (28.1%)		SCC*mec* IV	7 (38.9%)	
	SCC*mec* III	3 (9.4%)		SCC*mec* III	–	
	SCC*mec* II	9 (28.1%)		SCC*mec* II	2 (11.1%)	
	SCC*mec* I	2 (6.2%)		SCC*mec* I	1 (5.6%)	

***** Percentage calculated to the number of HA-MRSA (n = 32 isolates).
** Percentage calculated to the number of CA-MRSA (n = 18 isolates).
SUH: Al-Sayed Galal University Hospital; DUH: Al-Demerdash University Hospital; ETAs: endotracheal aspirates.

Table 5. The frequencies of *spa* types of the tested MRSA isolates from different clinical samples.

Group	*spa* type*	MLST CCs	Isolate No.	Clinical Source	Type of infection	SCC*mec* type	*spa*-SCC*mec* type (n = 20)	*spa* type repeats Succession*
1	t084	CC15	25	Wound	CA	IV	t084-SCC*mec* IV	07-23-12-34-34-12-12-23-02-12-23
			27	Blood	HA			
			100	Blood	HA			
			166	Urine	CA			
			50	Urine	CA	V	t084-SCC*mec* V	
			96	Sputum	CA			
			106	Wound	CA			
			170	Blood	HA			
2	t688	CC5	76	Abscess	HA	V	t688-SCC*mec* V	26-23-17-34-17-16
			158	Wound	CA			
			159	Wound	CA			
			109	ETAs	HA	IV	t688-SCC*mec* IV	
			153	Wound	HA			
3	t127	CC1	24	Wound	HA	V	t127-SCC*mec* V	07-23-21-16-34-33-13
			145	Wound	CA			
			168	Blood	HA			
			74	Blood	HA	IV	t127-SCC*mec* IV	
4	t037	CC8	1	Wound	CA	V	t037-SCC*mec* V	15-12-16-02-25-17-24
			71	Wound	HA			
			36	Sputum	HA	IV	t037-SCC*mec* IV	
5	t238	–	165	U. catheter	HA	V	t238-SCC*mec* V	15-21-12-16-02-16-02-25-17-24-24
			161	Sputum	HA	IV	t238-SCC*mec* IV	
6	t1991	CC913	54	Sputum	HA	IV	t1991-SCC*mec*IV	08-17
			61	Wound	HA	V	t1991-SCC*mec* V	
7	t132	ST46	23	IV catheter	HA	V	t132-SCC*mec* V	09-34-16-34
			146	Wound	CA	IV	t132-SCC*mec* IV	
8	t903	–	85	Urine	CA	IV	t903-SCC*mec* IV	26-22-19-17-17-20-17-12
			122	Wound	CA			
9	t355	ST152	43	Eye swab	CA	V	t355-SCC*mec* V	07-56-12-17-16-16-33-31-57-12
10	t3643	–	117	IV catheter	HA	V	t3643-SCC*mec* V	04-17-34-17-32-23-24-24-24
11	t5227	–	164	Wound	CA	IV	t5227-SCC*mec*IV	07-56-21-12
12	t5111	–	44	Sputum	CA	IV	t5111-SCC*mec*IV	186-23-12-34-34-12-12-23-02-12-23
13	t2253	–	12	Wound	HA	IV	t2253-SCC*mec*IV	07-12-21-17-34-34-33-34

* *Spa* typing based on Ridom SeqSphere+ software; MLSTCCs: multilocus sequence typing clonal complexes; A minus sign (−) indicates the absence of data from Ridom *spa* Server database. ETAs: endotracheal aspirates.

Figure 1. Agarose gel electrophoresis of multiplex PCR assay identifying SCC*mec* types from I – V and *mecA* gene in MDR-MRSA isolates. Lane M: 100-bp ladder size marker and other lanes are SCC*mec* type and *mecA* results of MDR-MRSA isolates

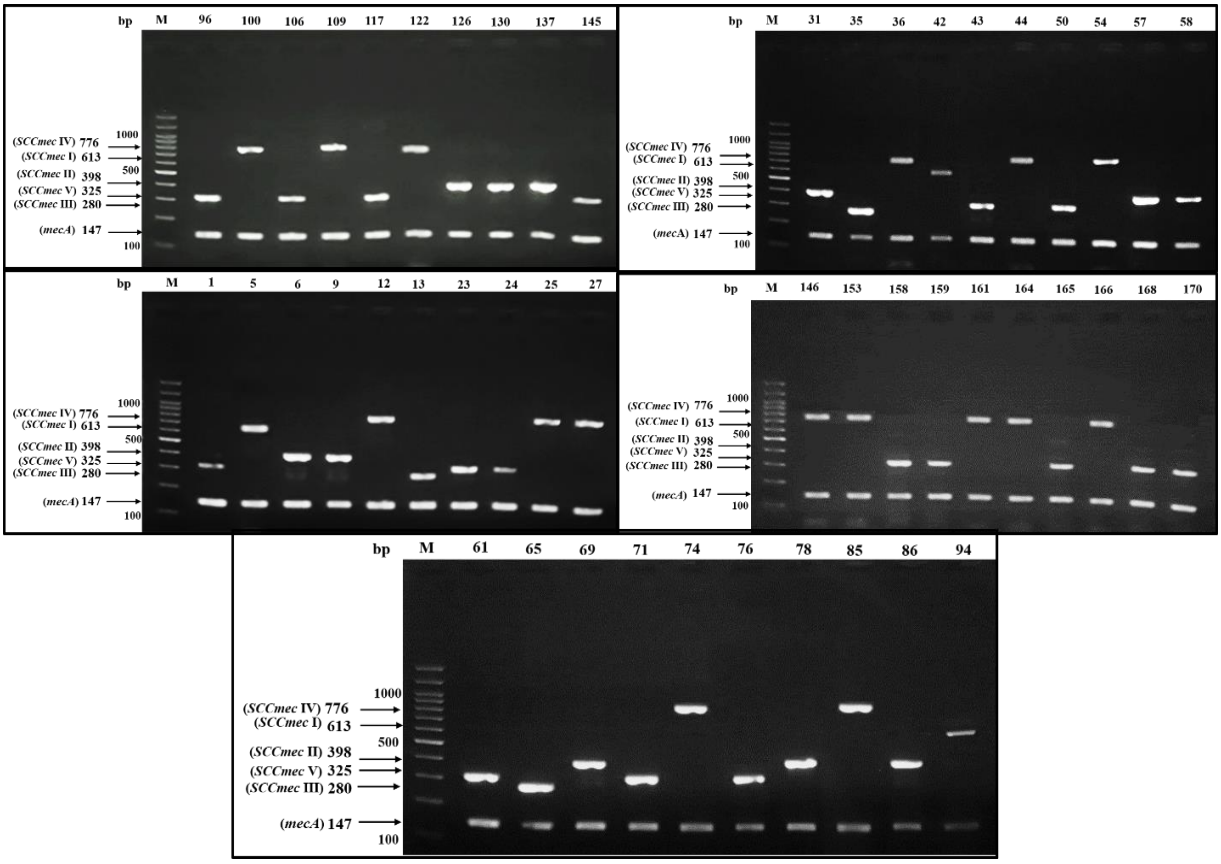


Figure 2. The frequency of SCC*mec* types of MDR-MRSA isolates.

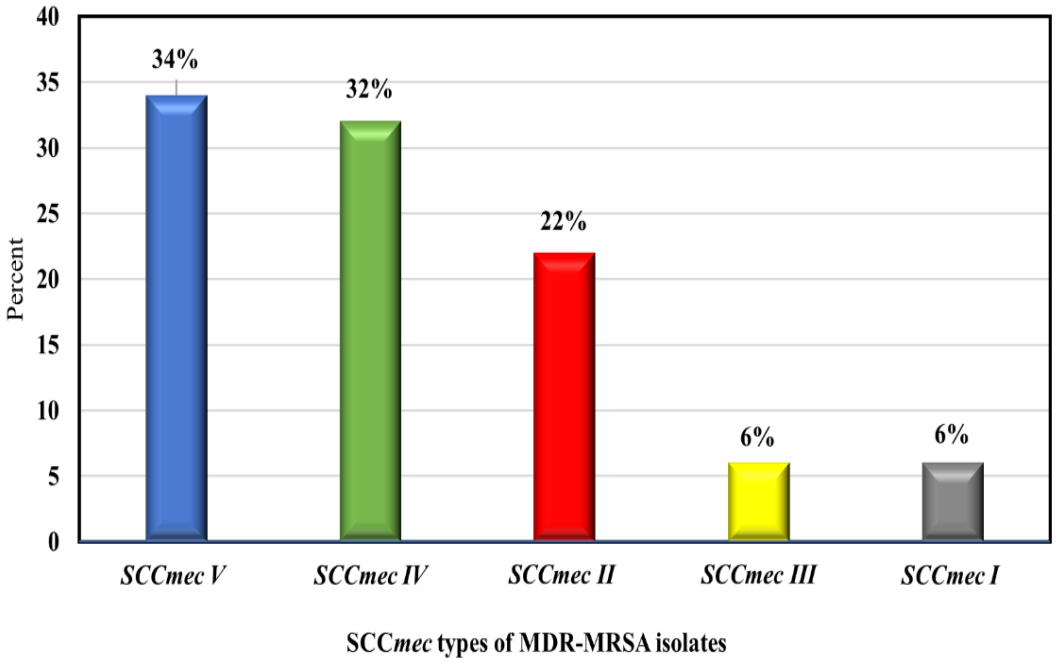


Figure 3. Phylogenetic tree of *spa* types of individual MDR-MRSA isolates generated with Ridom SeqSphere+ Software Neighbor-Joining method; within each column, the individual MDR-MRSA isolate No., and its specific *spa* type. Clustering distance: 24

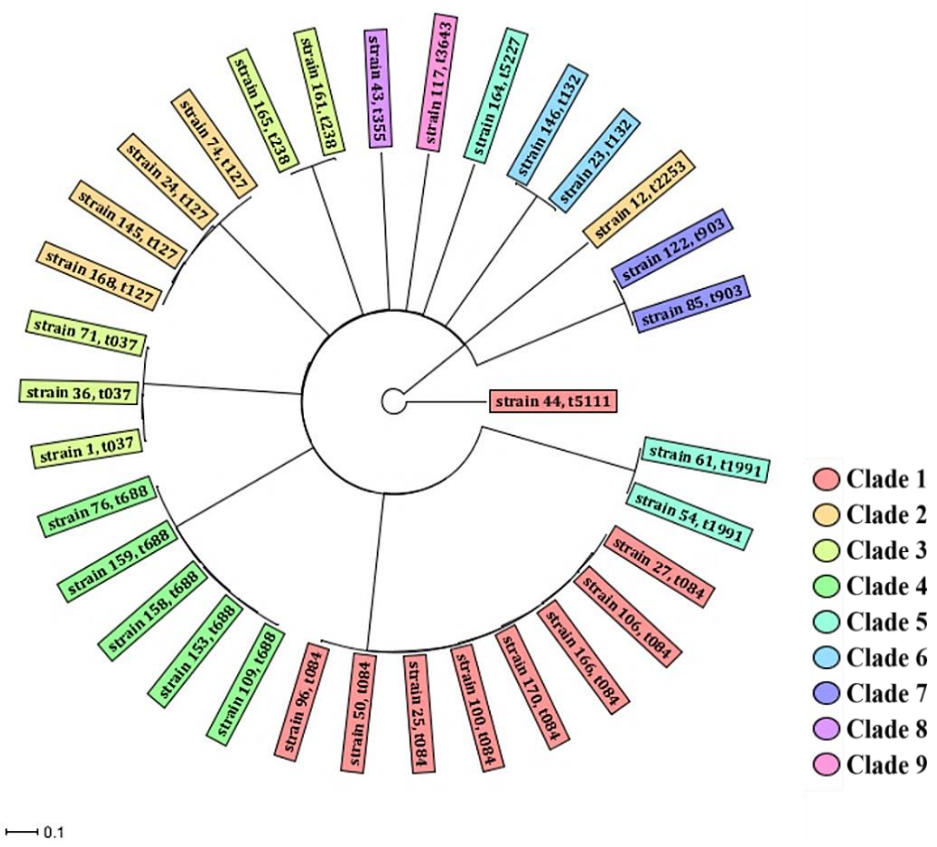
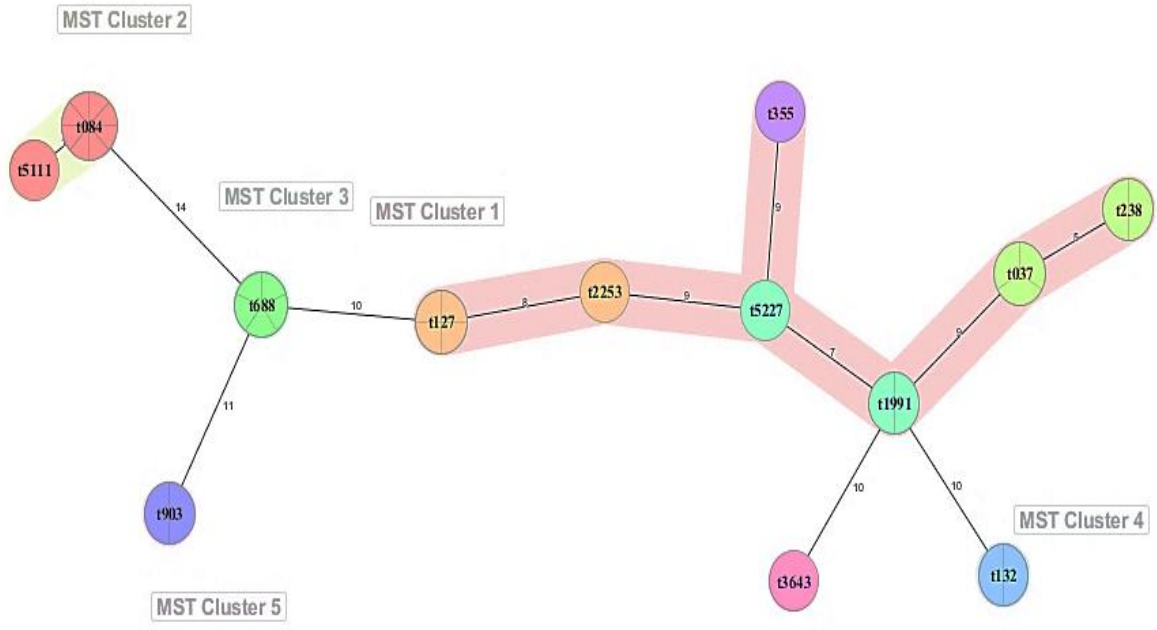


Figure 4. Minimum spanning tree demonstrating the diversity of identified *spa* types (constructed by the *spa* typing MST based upon BURP algorithm implemented in Ridom SeqSphere+ Software). The number on the edges between the nodes indicates the distance between two *spa* types, which is calculated based on the BURP algorithm.



Discussion

Detecting MRSA strain outbreaks and identifying transferred clones are necessary for the molecular epidemiological evaluation of MRSA infections and adequate infection control measures. Furthermore, identifying circulating MRSA clones is critical for preventing the transmission of common clones inside hospitals, the community, or from the community to hospitals and back. This study utilized SCC*mec* and *spa* genotyping-based methods to characterize MRSA isolates from Egyptian hospitals and studied their genetic relatedness and diversity. It is believed that SCC*mec* IV and SCC*mec* V are likely more mobile than other SCC*mec* types [20]. This assumption could be supported by the high rates of SCC*mec* IV and SCC*mec* V recorded in the current study. The present study's findings agree with those of a previous study from Egypt, which reported that SCC*mec* V and *SCCmec* IV are more frequent among MRSA isolates from Egyptian hospitals [7]. In addition, the findings of this study are consistent with many studies from different countries, which reported that MRSA isolates commonly belong to the SCC*mec* IV and SCC*mec* V types, while other types are less frequent [5,21-26]. A previous study from Pakistan indicated that the most common type was SCC*mec* IV (43%), followed by SCC*mec* II (25%) and SCC*mec* III (22%), but SCC*mec* V accounted for only 10% of the isolates [27]. In contrast, the results of the present study are inconsistent with those of a previous study from Egypt, which reported that 57%, 22%, and 11% of MRSA isolates harboured SCC*mec* III, SCC*mec* IV and SCC*mec* V, respectively [9]. The variation in the frequency of diverse SCC*mec* types could be attributed to the horizontal transmission of the mobile genetic element among strains in the community or healthcare settings affected by the social status difference of communities in different developing and developed countries or the infection control measures implemented in various hospitals.

This study explored the prevalence of SCC*mec* types among MRSA isolates concerning the clinical sample source of the isolates. The most predominant types, SCC*mec* V and IV, were detected in high percentages among MRSA isolates from wound swabs rather than other sources. The next most prevalent type, SCC*mec* II, mainly existed in MRSA blood isolates. This result is consistent with previous studies that reported similar findings [23,24]. Concerning the hospitals in this study, the most predominant SCC*mec* type in Al-Demerdash University Hospital was SCC*mec* IV. However, the most predominant SCC*mec* type in Al-Sayed Galal University Hospital was SCC*mec* V. This finding indicates the variable distribution of SCC*mec*-harbouring MRSA isolates among healthcare settings in the same country or even in the same geographic region.

SCC*mec* I, II and III are related to HA-MRSA strains, while SCC*mec* types IV and V are common among CA-MRSA strains [5]. However, as noted in the current study, HA-MRSA isolates harboured all SCC*mec* types, and CA-MRSA isolates carried four of the five SCC*mec* types. This finding is consistent with the findings reported by **Hadyeh et al.** [20]. In addition, this finding is supported by the fact that the relationship between SCC*mec* type and community- or hospital-onset infection is less clear, possibly due to the transfer of MRSA strains from healthcare settings to the community and vice versa [9]. Several MRSA clones have emerged worldwide and in the Middle East region [28]. The predominance of the SCC*mec* IV and SCC*mec* V types in the current study agrees with studies on CA-MRSA and HA-MRSA isolates conducted in neighbouring geographical areas such as Jordan [29] and Gaza [30]. Therefore, in Egypt and possibly neighbouring countries, the population structure of MRSA in the community is starting to mirror that found in the healthcare settings, making the boundaries between these two groups of infections unclear.

Significantly, CA-MRSA could be present among the bacterial species of the normal flora of various body regions, including the skin, hands, groin and nasal cavity of healthy individuals and healthcare staff. Normal flora containing MRSA can cause infections when individuals become immunocompromised due to hospitalization, have an open wound or surgery, or have other diseases. Therefore, strict infection control activities must be implemented in hospitals because this interface allows the cross-infection of HA-MRSA and CA-MRSA. This perspective is reinforced by **Bartels et al.'s study**, which indicated a rapid shift to CA-MRSA, where SCC*mec* type IV was found in 86% of MRSA isolates [31], and **Djoudi et al.'s** study, which attributed this observation to increasing CA-MRSA colonization rates [32].

The *spa* typing method is a good option for MRSA typing for epidemiological purposes. It is quick and straightforward to perform and interpret

and has excellent reproducibility [9]. In this study, the *spa* typing of the 33 MDR-MRSA isolates representing the most common SCC*mec* types [SCC*mec* V (17/33, 51.5%) and SCC*mec* IV (16/33, 48.5%)] revealed 13 distinct *spa* types. The most frequent *spa* types were t084, t688, t127 and t037. The less frequent *spa* types included t132, t238, t903, t1991, t355, t2253, t3643, t5111 and 5227. These results are consistent with those of previous studies [7,26] reporting several *spa* types among MRSA isolates, with *spa* type t084 being the predominant type. However, another study revealed 29 different *spa* types, where the most prevalent types were t386, t044, t008 and t223 [20].

According to the *spa* phylogeny, the examined isolates belonged to nine *spa* clades. This finding is inconsistent with a previous study from Egypt, which reported that MRSA isolates were grouped into three different clades: clade 1 included t044, t267, t786 and t127; clade 2 included t304, t037 and t1622; and clade 3 comprised t688, t223 and t6978 [9]. The *spa* type t127 had the highest global frequency among all *spa* types detected in this study, followed by t084, t037, t355, t688, t238, t1991, t132, t903 and t2235. Other *spa* types, including t3643, t5227 and t5111, are less frequently distributed worldwide. In particular, *spa* types t688, t127 and t037 have been distributed worldwide and disseminated in Egypt, Arab countries in the Middle East and some African countries. The *spa* types t084, t132, t903, t355, t1991 and t2253 disseminated and originated from countries in the Middle East and Africa. This may explain why *spa* type t084 was more prevalent than other types. Moreover, *spa* types, including t3643, t5227 and t5111, are mainly disseminated in Europe [33].

Regarding clonal characterization, the predicted MLSTCCs for the identified *spa* types in this study revealed seven MLSTCCs identified in the investigated MRSA isolates, namely, CC15 (t084, 24.2%), CC5 (t688, 15.1%), CC1 (t127, 12.1%), CC8 (t037, 9.1%), CC913 (t1991, 6%), ST46 (t132, 6%) and ST152 (t355, 3%). This finding is consistent with the **Udo et al.** study [26] in Kuwait, which revealed that CC15 was the predominant MRSA clonal complex among the studied MRSA isolates and indicated that CC15 clones emerged at a greater percentage than other clones. The present study's findings also agree with those of a study conducted in Saudi Arabia and Egypt, which recorded six clonal complexes, namely, CC5, CC8, CC22, CC30, CC80, and CC88, while one *spa* type was not assigned [7]. However, the study by **Hadyeh et al.** [20] from Palestine reported a wide range of CCs, with 12 MLSTCCs identified. The 12 identified MLSTCCs were CC22, CC1, CC8, CC80, CC8/239, CC30, CC5, CC913, CC6, CC121, CC126 and CC15. Additionally, an **Alseqely et al.** [9] study from Egypt reported nine MLST profiles (ST-22, ST-1, ST-5, ST-6, ST-80, ST-97, ST-239, ST-241, and ST-1502) among the investigated MRSA isolates.

In the present study, based on the global description of MRSA clones by MLSTCCs-SCC*mec*-*spa*, 20 different MRSA clones were observed among the investigated MRSA isolates. This indicates that various sources of MRSA infections and dissemination sources differed. The present study showed that CC15-SCC*mec*V-t084 (12.1%) and CC15-SCC*mec*IV-t084 (12.1%) MRSA clones were the most predominant among the investigated MRSA isolates. This finding follows the study of **Udo et al.** [26], which revealed that the CC15-SCC*mec*V-t084 clone was predominant among the studied MRSA isolates. However, **Goudarzi et al.** [5] showed that ST22-SCC*mec*IV-t790 was the dominant MRSA clone, and **Alseqely et al.** [9] revealed five MRSA clones, namely, ST-239-SCC*mec*III-t304, ST-1502-SCC*mec*IV-t044, ST-4808-SCC*mec*IV-t267, ST-22-SCC*mec*IV-t223, and ST-22- SCC*mec*IV-t6978.

Conclusion

This study revealed that molecular and/or sequence-based typing methods are reliable tools for the epidemiological tracking of MRSA strains in hospital environments and can provide essential insights into controlling the spread of MRSA infections. In addition, the present study demonstrated the high prevalence of MRSA SCC*mec* V and SCC*mec* IV types, and diverse *spa* types were identified where *spa* type t084 was more predominant. Given the detected MRSA diversity, the SCC*mec* and *spa* typing techniques could be preferable for molecular epidemiological investigations of MRSA infections. The identified MRSA clones in the present study were genetically diverse or polyclonal, with the CC15-SCC*mec*V-t084 and CC15-SCC*mec*IV-t084 MRSA clones being prevalent. Future observational studies on MRSA should aim to understand its epidemiology better and detect the emergence of new MRSA strains for effective infection control measures and better healthcare management.

Conflict of interest disclosure

The authors declare no potential conflicts of interest.

Funding

The authors reported no funding associated with the work featured in this article.

References

1- **Raut S, Bajracharya K, Adhikari J, Pant SS, Adhikari B.** Prevalence of methicillin-resistant *Staphylococcus aureus* in Lumbini medical college and teaching hospital, Palpa, Western Nepal. BMC Research Notes 2017; 10(1): 1-7.

2- **Hiramatsu K, Katayama Y, Matsuo M, Sasaki T, Morimoto Y, Sekiguchi A, et al.** Multidrug-resistant Staphylococcus aureus and future chemotherapy. Journal of Infection and Chemotherapy 2014; 20(10): 593-601.

3- **Adeiza SS., Onaolapo JA, Olayinka BO.** Prevalence, risk factors, and antimicrobial susceptibility profile of methicillin-resistant Staphylococcus aureus (MRSA) obtained from nares of patients and staff of Sokoto state-owned hospitals in Nigeria. GMS Hygiene and Infection Control 2020; 15: 25.

4- **Ismail MAH, Kamarudin N, Abdul Samat MN, Raja Abdul Rahman RMF, Saimun, S., Tan TL, et al.** Methicillin-Resistant Staphylococcus aureus (MRSA) clonal replacement in a Malaysian teaching hospital: Findings from an eight-year interval molecular surveillance. Antibiotics 2021; 10(3): 320.

5- **Goudarzi M, Goudarzi H, Sá Figueiredo AM, Udo EE, Fazeli M, Asadzadeh M, et al.** Molecular characterization of methicillin-resistant *Staphylococcus aureus* strains isolated from intensive care units in Iran: ST22-SCCmec IV/t790 emerges as the major clone. PloS one 2016; 11(5): e0155529.

6- **Campanile F, Bongiorno D, Borbone S, Stefani S.** Hospital-associated methicillin-resistant Staphylococcus aureus (HA-MRSA) in Italy. Annals of clinical microbiology and antimicrobials 2009, 8: 22.

7- **Abo-Shady HM, Bakr AEA, Hashad ME, Alzohairy MA.** Staphylococcus aureus nasal carriage among outpatients attending primary health care centers: a comparative study of two cities in Saudi Arabia and Egypt. Brazilian Journal of Infectious Diseases 2015: 19: 68-76.

8- **Alkharsah KR, Rehman S, Alnimr A, Diab A, Hawwari A, Tokajian S.** Molecular typing of MRSA isolates by spa and PFGE. Journal of King Saud University-Science 2019; 31(4): 999-1004.

9- **Alseqely M, Newton-Foot M, Khalil A, El-Nakeeb M, Whitelaw A, Abouelfetouh A.** Association between fluoroquinolone resistance and MRSA genotype in Alexandria, Egypt. Scientific reports 2021; 11(1): 4253.

10- **Alfeky AAE, Tawfick MM, Ashour MS, El-Moghazy ANA.** High Prevalence of Multidrug Resistant Methicillin-Resistant Staphylococcus aureus in Tertiary Egyptian Hospitals. The Journal of Infection in Developing Countries 2022; 16(5): 795-806.

11- **Alfeky AAE, Tawfick MM, Ashour MS, El-Moghazy ANA.** Molecular characterization of key virulence traits among multidrug-resistant methicillin-resistant Staphylococcus aureus isolates from some Egyptian hospitals. Al-Azhar Journal of Pharmaceutical Sciences 2022; 66(2): 173-192.

12- **Xie X, Bao Y, Ouyang N, Dai X, Pan K, Chen B, et al.** Molecular epidemiology and characteristic of virulence gene of community-acquired and hospital-acquired methicillin-resistant Staphylococcus aureus isolates in Sun Yat-sen Memorial hospital, Guangzhou, Southern China. BMC Infectious Diseases 2016; 16(1): 1-10.

13- **Maina EK, Kiiyukia C, Wamae CN, Waiyaki PG, Kariuki, S.** Characterization of methicillin-resistant Staphylococcus aureus from skin and soft tissue infections in patients in Nairobi, Kenya. International journal of infectious diseases 2013; 17(2): e115-e119.

14- **Harmsen D, Claus H, Witte W, Rothganger J, Claus H, Turnwald D, et al.** Typing of methicillin-resistant Staphylococcus aureus in a university hospital setting by using novel software for spa repeat determination and database management. Journal of Clinical Microbiology 2003; 41(12): 5442-5448.

15- **McClure-Warnier JA, Conly JM, Zhang K.** Multiplex PCR assay for typing of staphylococcal cassette chromosome mec types I to V in methicillin-resistant Staphylococcus aureus. Journal of visualized experiments: JoVE 2013; (79): e50779.

16- **Cuny C, Layer F, Werner GATAL, Harmsen D, Daniels-Haardt I, Jurke A, et al.** State-wide surveillance of antibiotic resistance patterns and spa types of methicillin-resistant Staphylococcus aureus from blood cultures in North Rhine-Westphalia, 2011–2013. Clinical Microbiology and Infection 2015: 21(8): 750-757.

17- **Mellmann A, Weniger T, Berssenbrügge C, Rothgänger J, Sammeth M, Stoye J, et al.** Based Upon Repeat Pattern (BURP): an algorithm to characterize the long-term evolution of Staphylococcus aureus populations based on spa polymorphisms. BMC Microbiology 2007; 7: 98.

18- **Monecke S, Coombs G, Shore AC, Coleman DC, Akpaka P, Borg M, et al.** A field guide to pandemic, epidemic and sporadic clones of methicillin-resistant Staphylococcus aureus. PloS one 2011; 6(4): e17936.

19- **Farag AM, Tawfick MM, Abozeed MY, Shaban EA, Abo-Shadi MA.** Microbiological profile of ventilator-associated pneumonia among intensive care unit patients in tertiary Egyptian hospitals. The Journal of Infection in Developing Countries 2020; 14(2): 153-161.

20- **Hadyeh E, Azmi K, Seir RA, Abdellatief I, Abdeen Z.** Molecular characterization of methicillin-resistant Staphylococcus aureus in West Bank-Palestine. Frontiers in Public Health 2019; 7: 130.

21- **Otto M.** Basis of virulence in community-associated methicillin-resistant Staphylococcus aureus. Annual review of Microbiology 2010; 64: 143-162.

22- **Caboclo RM, Cavalcante FS, Iorio NL, Schuenck RP, Olendzki AN, Felix MJ, et al.** Methicillin-resistant Staphylococcus aureus in Rio de Janeiro hospitals: Dissemination of the USA400/ST1 and USA800/ST5 SCCmec type IV and USA100/ST5 SCCmec type II lineages in a public institution and polyclonal presence in a private one. American Journal of Infection Control 2013; 41(3): e21-e26.

23- **Machuca MA, Sosa LM, González CI.** Molecular typing and virulence characteristic of methicillin-resistant Staphylococcus aureus isolates from pediatric patients in Bucaramanga, Colombia. PloS One 2013; 8(8): e73434.

24- **Wang X, Li X, Liu W, Huang W, Fu Q, Li M.** Molecular characteristic and virulence gene profiles of community-associated methicillin-resistant Staphylococcus aureus isolates from pediatric patients in Shanghai, China. Frontiers in Microbiology 2016; 7: 1818.

25- **Ahmadi E, Khojasteh M, Mortazavi SM, Khan-Mohammadi F, Kazemnia A, Beheshtipour J, et al.** Prevalence of and risk

factors for methicillin-resistant Staphylococcus aureus nasal carriage in the West of Iran: a population-based cross-sectional study. BMC Infectious Diseases 2019; 19(1): 899.

26- **Udo EE, Boswihi SS, Mathew B, Noronha B, Verghese T, Al-Jemaz A, et al.** Emergence of methicillin-resistant Staphylococcus aureus belonging to clonal complex 15 (CC15-MRSA) in Kuwait hospitals. Infection and Drug Resistance 2020; 13: 617-626.

27- **Sohail M, Latif Z.** Molecular typing of methicillin-resistant Staphylococcus aureus (MRSA) isolated from device-related infections by SCCmec and PCR-RFLP of coagulase gene. Advancements in Life Sciences 2018; 6(1): 34-40.

28- **Tokajian S.** New epidemiology of Staphylococcus aureus infections in the Middle East. Clinical Microbiology and Infection 2014; 20(7): 624-628.

29- **Al-Bakri AG, Al-Hadithi H, Kasabri V, Othman G, Kriegeskorte A, Becker K.** The epidemiology and molecular characterization of methicillin-resistant staphylococci sampled from a healthy Jordanian population. Epidemiology & Infection 2013; 141(11): 2384–2391.

30- **Al Laham N, Mediavilla JR, Chen L, Abdelateef N, Elamreen FA, Ginocchio CC, et al.** MRSA clonal complex 22 strains harboring toxic shock syndrome toxin (TSST-1) are endemic in the primary hospital in Gaza, Palestine. PLoS One 2015; 10(3): e0120008.

31- **Bartels MD, Boye K, Larsen AR, Skov R, Westh H.** Rapid increase of genetically diverse methicillin-resistant Staphylococcus aureus, Copenhagen, Denmark. Emerging infectious diseases 2007; 13(10): 1533–1540.

32- **Djoudi F, Bonura C, Benallaoua S, Touati A, Touati D, Aleo A, et al.** Panton-Valentine leukocidin positive sequence type 80 methicillin-resistant Staphylococcus aureus carrying a staphylococcal cassette chromosome mec type IVc is dominant in neonates and children in an Algiers hospital. The New Microbiologica 2013; 36(1): 49–55.

33- **Monecke S, Skakni L, Hasan R, Ruppelt A, Ghazal SS, Hakawi A, et al.** Characterization of MRSA strains isolated from patients in a hospital in Riyadh, Kingdom of Saudi Arabia. BMC Microbiology 2012; 12: 146.