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MOLECULAR IDENTIFICATION AND GENOTYPING OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) IN DIFFERENT CLINICAL SAMPLES

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ABSTRACT:

Methicillin-resistant Staphylococcus aureus (MRSA) is a serious human pathogen that can spread in healthcare facilities and among the general public. This study was aimed to evaluate the prevalence and diversity of SCCmec types of this superbug among hospitalized patients. This study involved phenotypic identification and molecular confirmation of S. aureus based on the nuc gene, molecular detection of MRSA, SCCmec typing, and virulence factor profiling of MRSA clinical isolates obtained from hospitalized patients in Duhok province. Out of the 310 enrolled patients, 33 isolates (10.64%) were identified and confirmed as Staphylococcus aureus, of which 51.5% were identified as MRSA based on phenotypic and molecular targeting of the mecA gene. There were no discernible variations between the prevalence rates of this pathogen in different clinical sources, sexes, or age groups (p-values: 0.71, 0.39, and 0.15 respectively). The isolates had elevated rates of resistance to most antibiotic classes. They were classified as extensive drug-resistant (30.3%), multidrug-resistant (57.5%), and non-multidrug-resistant (12.1%). Additionally, SCCmec typing of MRSA by multiplex PCR identified three different SCCmec types and subtypes, including SCCmec type II (35.5%), followed by 17.64% of SCCmec type IV subtype d (IVd), and SCCmec type III (11.76%). However, 35.3% of the MRSA isolates were found to be non-typeable. Molecular profiling of major virulence factors and toxin genes revealed that 57.5% of the isolates were positive for the exfolitative toxin (ETA), 45.4% of the isolates carried TSST-1 (Toxic Shock Syndrome Toxin-1), the PVL (Panton-Valentine Leukocidin) cytotoxin was identified in 15% of the isolates, and 18.1% of the identified S. aureus isolates were positive for the ACME (arginine catabolic mobile element). The findings of the current investigation pointed out the circulating of highly virulent and extensively resistant MRSA strains among hospitalized patients, which may require active surveillance and better control policies.

KEYWORDS: S. aureus, MRSA, mecA, SCCmec, Virulence factors.

1. INTRODUCTION

The Gram-positive Staphylococcus aureus (S. aureus), is a coagulase-positive bacterium that able to survive in both anaerobic and aerobic environments (Zhang et al., 2023). Significant human pathogen S. aureus can spread in healthcare facilities as well as the general public (Asghar, 2014). This pathogen causes purulent inflammations in the throat, urinary tract, and surgical site infections by producing a variety of extracellular secretions, including enterotoxins, plasma coagulase, and multi-haemolytic toxins (Tong et al., 2015).

Millions of lives have been saved and patients' life expectancies have increased since the discovery of antibiotics. Nevertheless, the emergence of multi-drug-resistant microorganisms and the lack of efficacy of antibiotics have made drug-resistant microbes a threat (Larsen et al., 2022). Among these, Staphylococcus aureus strains that are resistant to methicillin (MRSA) stand out as a particularly potent contemporary pathogen. MRSA is a particular kind of S. aureus that is resistant to a broad class of β-lactam antimicrobial agents, which include penicillins and cephalosporins (Pillai et al., 2012).

This pathogen plays a significant role in the development of diseases such as endocarditis, bacteremia, infections of the skin and soft tissues, and infections of the bones and joints, including those that occur in hospitals (Hussein et al., 2017; Abduljabar and Naqid, 2023). Genetic variation and the progressive emergence of epidemic strains are characteristics of the MRSA epidemiology (Turner et al., 2019). The severe infection symptoms and treatment difficulties were caused by MRSA's high pathogenicity, virulence, and increased drug resistance (Ippolito et al., 2010). MRSA isolates have become more prevalent in hospital settings since it was first identified in 1960s, leading to increased infection and mortality rates (Chambers et al., 2009). Even though MRSA's incidence has recently declined in some areas, its consistently high rates of morbidity and mortality make it a serious clinical threat. Treatment that works is still difficult and needs to evaluate new antibiotics as well as ancillary services like source control, and infectious disease consultation (Turner et al., 2019).

The existence of the mecA gene and its homolog, the mecC gene, which is only 70% similar to the mecA gene, aids in MRSA resistance. They encode transpeptidase enzymes like PBP2a and PBP2c which help to confer resistance on MRSA. PBP2a exhibits decreased affinity for β-lactam antimicrobial agents such as cefoxitin, methicillin, nafcillin, and oxacillin (Larsen et al., 2022; Uehara Y., 2022).

The mecA gene is carried by the staphylococcal chromosomal cassettes mec (SCCmec), an external transposable genetic element embedded into the MRSA chromosome that facilitate swift evolution and adjustment in response to antibiotic selection pressure. The SCCmec, which ranging in size from 21 to 67 kb, are integrated into the methicillin-resistant S. aureus chromosome at a specific site called attBscc situated close to the origin of replication of S. aureus chromosome (Othman et al., 2014; Maree et al., 2022).

The oxacillin screening test, oxacillin and/or cefoxitin disk diffusion method, and the oxacillin minimum inhibitory concentration test are a few of the traditional phenotypic techniques for MRSA detection. However, false negative and positive results for MRSA identification are linked to the relatively low sensitivity (87.1-93.5%) and specificity (83.5-89.3) of these conventional methods (Pillai, et al., 2012). Therefore, a quick and accurate molecular diagnostic tool, like a DNA-based assay, is needed to identify true MRSA strains from

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clinical specimens. In this regard, the "gold standard" method for diagnosing MRSA continues to be molecular identification and genotyping, which is based on the detection and typing of the mecA gene. (Anand et al., 2009; Pillai, et al., 2012).

Although some investigations have been performed in Duhok province, Kurdistan region, Iraq using both conventional tests and mecA-based molecular detection of MRSA among the general population and healthcare workers (Othman et al., 2014; Hussein et al., 2017; Hussein et al., 2019; Rasheed and Hussein, 2020; Yassin, 2022; Hami and Ibrahim, 2023; Abduljabar and Naqid, 2023).

Nevertheless, scant information exists regarding the frequency of SCCmec types in this region, and there is still a lack of data on their prevalence among hospitalized patients. Furthermore, precise and comprehensive data depicting the genotypic characteristics of SCCmec types of this superbug within our hospitals are still unavailable. Therefore, this study was designed to identify the prevalence of MRSA strains at the molecular level and to characterize the genotypic profiles of SCCmec types, aiming to provide more precise and accurate data in this context. Furthermore, in this study, hospital-acquired MRSA isolates have been screened for the production of the Panton-Valentine Leukocidin (PVL) toxin. PVL serves as a molecular marker for community-acquired strains associated with deep skin infection, soft tissue infection, necrotizing pneumonia, and tissue necrosis (Makgotlho et al., 2009), in addition to other significant virulence factors.

2. MATERIAL AND METHODS

2.1 Study design

This cross-sectional and analytical study was proposed to identify Methicillin-resistant Staphylococcus aureus (MRSA) at the molecular level, genotypic characterization of the SCCmec types, and virulence factor profiling of MRSA isolates among hospitalized patients in Duhok province, Kurdistan Region-Iraq. The current investigation was carried out between February and July 2022.

2.2 Inclusion and exclusion criteria

All inpatients who were hospitalized for active infections, including respiratory tract infections, urinary tract infections, and nasal carriage, satisfied the inclusion criteria. On the other hand, individuals who were hospitalized for less than twenty-four hours, lack of informed consent, the luck of medical records, and numerous samples from the same patient were all disqualified from participating in this study.

2.3 Sample collection and processing

In total, 310 samples were collected from diverse sources, including urine (140), nasal swabs (80), and sputum (90). Patients at Azadi Teaching Hospital and Duhok Emergency Hospital in Duhok, as well as Golan Hospital in Sumel, provided the samples for this study. The study encompassed individuals of all ages (1-83 years old) and both genders, with 129 males and 181 females. Prior to sampling, the nutrient broth was prepared, autoclaved for 15 minutes, and placed in a sterilized test tube. Nasal samples from the anterior nostrils were collected using sterilized cotton swabs (Darcon Swabs, China) moistened with sterile normal saline, which were then directly placed in the tube containing the nutrient broth. The tube was then transferred to the laboratory and incubated at 37°C for 24 hours. A sterile screw-top containers were used to collect sputum and urine samples, which were then immediately cultured on Mannitol Salt Agar (MSA) and incubated aerobically for 24 hours at 37°C.

2.4 Conventional identification

Each sample was cultivated and incubated for 24 hours at 37°C using Mannitol Salt Agar. The bacterial colony that was suspected of exhibiting S. aureus characteristics on the selective media was recovered and identified through morphological analysis using gram stain, biochemical tests like catalase and coagulase tests, and its hemolysis activity on blood agar.

2.5 Antibiotic Resistant Profiling

On Muller Hinton agar plates, freshly prepared S. aureus cultures were tested for antibiotic susceptibility using Kirby-Bauer's disc diffusion method (Tarek et al., 2024). Once the antibiotic discs were placed over the test organism culture, the plates were incubated for a full day at 37˚C. After incubation, the diameter of the zone of inhibition was measured. In accordance with the Laboratory and Clinical Standards Institute (CLSI, 2021) the results were categorized as either "Resistant" or "Sensitive" to antibiotics. Sensitivity testing was performed using ten different antibiotic discs (Bioanalyse/Turkey) belonging to eight various classes, namely Penicillin (10µg), Oxacillin (10µg), Erythromycin (15µg), Vancomycin (30µg), Doxycycline(10µg), Tetracycline(10µg), Gentamycin(10µg), Ciprofloxacin (5µg), Clindamycin(10µg), Cephalothin (30µg). Methicillin-resistant bacteria were denoted as such when they exhibited resistance to the antibiotic oxacillin (Othman et al., 2014). Bacterial isolates classified as XDR (extensive-drug-resistant) when they were susceptible to only one or two antibiotic classes. Whereas, isolates defined as MDR (Multidrug-resistant) bacteria when they demonstrated resistance to one agent in three or more antibiotic categories (Magiorakos et al., 2012; Tarek et al., 2024).

2.6 MOLECULAR DETECTION AND IDENTIFICATION

2.6.1 Genomic extraction of DNA

Fresh 24-hour-old bacterial colonies were used to extract DNA using the AddPrep Bacterial Genomic DNA Extraction Kit (Code: 10027, addbio, Korea) and the supplier's protocol for Gram-positive bacteria. The NanoDropTM 2000/2000c Spectrophotometer (Thermo Fisher ScientificTM/Catalog number: ND2000CLAPTOP) was used to qualify and quantify the extracted DNA samples. Before using the DNA samples, they were frozen in the freezer at -20°C until use.

2.6.2 Species-specific PCR for S. aureus

Using species-specific oligonucleotide primer pairs for the nuc gene (Table 1), PCR assays have been used to confirm all phenotypically identified S. aureus isolates at the molecular level as previously described (Othman et al., 2014). The PCR reaction contained 12.5µl of master mix (add bio, Korea), 1µl of forward primer (10µmol/µl), 1µl of reverse primer (10µmol/µl), 2µl of DNA, and 8.5µl of distilled water, for a total volume of 25µl. For amplification reactions, Applied Biosystem (USA) Thermal cycler was employed. The PCR initial denaturation was set for 3 minutes at 94°C, 30 cycles of denaturation for 1 minutes at 92°C, annealing for 1minutes at 55°C, extension for 45 seconds at 72°C, and final extension step for 5 minutes at 72°C.

Table 1: Oligonucleotide primers used in the present study for Molecular detection of S. aureus and MRSA.

Target	Primers	Sequences (5′-3′)	Product size	Specificity/ gene	Reference
Molecular detection of S. aureus and MRSA	nuc-F	GCGATTGATGGTGATACGGTT	280bp	nuc gene	Othman et al., 2014
	nuc-R	AGCCAAGCCTTGACGAACTAAAGC			
	mecA-F	TGGCTATCGTGTCACAATCG	310bp	mecA gene	Havaei et al., 2015
	mecA-R	CTGGAACTTGTTGAGCAGAG			

2.6.3 mecA-PCR assay

As previously mentioned by Havaei et al. (2015), the mecA specific primer pair (Table 1) was used in the mecA-PCR assay. For a total volume of 25µl, the PCR reaction used 12.5µl of master mix (add bio, Korea), 1µl of each of the forward and reverse primers (10µmol/µl), 2µl of DNA, and 8.5µl of distilled water. The PCR amplification conditions were 3 minutes at 94°C as the initial denaturation, 30 cycles of denaturation for 45 seconds at 92°C, annealing for 45 seconds at 55°C, extension for 30 seconds at 72°C, and final extension for five minutes at 72°C.

2.6.4 SCCmec Typing-Assay

A multiplex PCR assay was carried out for all the mecA-positive isolates using eight pairs of unique and specific primers (Table 2) for SCCmec typing (Makgotlho et al., 2009). The primer mixture, which was made up of eight sets of primers, were mixed in a 10x ratio. To accomplish this, 0.5 ml sterile Eppendorf tubes were filled with 1µl of each primer (100µm/µl), and sterile ddH_2O was added to prepare a 50µl primer mixture. The PCR reaction contained 12.5µl of master mix (add bio, Korea), 5µl of the primer mix, 2µl of DNA, and 5.5µl of distilled water, for a total volume of 25µl. Utilizing an Applied Biosystem (USA) Thermal cycler, the multiplex PCR amplification process involved 5 minutes of initial denaturation at 94°C, ten cycles of denaturation for 30 seconds at 94°C, annealing for 90 seconds at 60°C, and 90 seconds of extension at 72°C. Thereafter, twenty-five cycles of 45 seconds at 94°C for denaturation, 45 seconds at 55°C for annealing, 90 seconds at 72°C for extension, and 10 minutes at 72°C for the final extension step.

Table 2: Oligonucleotide primers used in the present study for SCCmec typing MRSA.

Target	Primers	Sequences (5′-3′)	Product size	Specificity/ gene	Reference
Genotyping of MRSA SCCmec (Multiplex PCR)	Type I-F	GCTTTAAAGAGTGTCGTTACAGG	613bp	SCCmec I	Makgotlho et al. 2009
	Type I-R	GTTCTCTCATAGTATGACGTCC			
	Type II-F	CGTTGAAGATGATGAAGCG	398bp	SCCmec II	
	Type II-R	CGAAATCAATGGTTAATGGACC			
	Type III-F	CCATATTGTGTACGATGCG	280bp	SCCmec III	
	Type III-R	CCTTAGTTGTCGTAACAGATCG			
	Type IVa-F	GCCTTATTCGAAGAAACCG	776bp	SCCmec IVa	
	Type IVa-R	CTACTCTTCTGAAAAGCGTCG			
	Type IVb-F	TCTGGAATTACTTCAGCTGC	493bp	SCCmec IVb	
	Type IVb-R	AAACAATATTGCTCTCCCTC			
	Type IVc-F	ACAATATTTGTATTATCGGAGAGC	200bp	SCCmec IVc	
	Type IVc-R	TTGGTATGAGGTATTGCTGG			
	Type IVd-F	CTCAAAATACGGACCCCAATACA	881bp	SCCmec IVd	
	Type IVd-R	TGCTCCAGTAATTGCTAAAG			
	Type V-F	GAACATTGTTACTTAAATGAGCG	325bp	SCCmec V	
	Type V-R	TGAAAGTTGTACCCTTGACACC			

2.6.5 Molecular typing of S. aureus virulence factors

The present investigation involved the molecular typing and profiling of major virulence factors targeting the virulence and toxin genes of all the identified S. aureus isolates. These factors included the cytotoxin gene (lukS/F-PV) for PVL (panton valentine leukocidin), the tst gene for TSST-1 (toxic shock syndrome toxin-1), the eta gene for ETA (exfoliative toxin), and the arcA gene for a surrogate marker ACME (arginine catabolic mobile element), in the arc gene cluster (Hadyeh et al., 2019). The PCR reaction, per each targeted gene, a total volume of 25µl, made up of 12.5µl of master mix (add bio, Korea), 1µl each of the forward and reverse primers (10µmol/µl), 2µl of DNA, and 8.5µl of distilled water.

2.6.5.1 PVL toxin gene (lukS/F-PV)

Luk-PVL-F and Luk-PVL-R primer pairs (Table 3) were used to screen the identified S. aureus isolates for the presence of the lukS/F-PV gene by PCR. The PCR amplification procedure involved 5 minutes of initial denaturation at 95°C, 30 seconds of annealing at 55°C, 30 seconds of extension at 72°C, and 5 minutes of final extension at 72°C.

Table 3**:** Oligonucleotide primers used in the present study for Molecular profiling of major virulence factors and toxin genes of S. aureus isolates.

Target	Primers	Sequences (5′-3′)	Product size	Specificity/ gene	Reference
S. aureus Virulence Factors and Toxin Genes	Luk-PVL-F	ATCATTAGGTAAAATGTCTGGACATGATCCA	433bp	PVL cytotoxin gene (lukS/F-PV)	Hadyeh et al., 2019
	Luk-PVL-R	GCATCAAGTGTATTGGATAGCAAAAGC			
	GTSSTR-F	ACCCCTGTTCCCTTATCATC	350bp	TSST-1 (tst gene)	
	GTSSTR-R	TTTTCAGTATTTGTAACGCC			
	GETAR-F	GCAGGTGTTGATTTAGCATT	93bp	Exfoliative toxin ETA (eta gene)	
	GETAR-R	AGATGTCCCTATTTTTGCTG			
	arcA-F	GAGCCAGAAGTACGCGAG	724bp	ACME-arcA (ACME I in the arc gene cluster)	
	arcA-R	CACGTAACTTGCTAGAACGAG			

2.6.5.2 TSST-1 toxin gene (tst gene)

The S. aureus isolates were subjected to PCR using GTSSTR-F and GTSSTR-R primer pair (Table 3) for detection of tst gene encoding TSST-1 toxin. The PCR program was run as 5min. of initial denaturation at 95°C, 35 cycles of denaturation at 95°C for 2 minutes, 45 seconds of annealing at 54°C, 45 seconds of extension at 72°C, and 5 minutes of final extension at 72°C.

2.6.5.3 Exfoliative toxin (eta) gene

The isolates of S. aureus were subjected to PCR assay using GETAR-F and GETAR-R primer pair (Table 3) for detection of eta gene encoding Exfoliative toxin. The PCR conditions were as follows: 5 minutes of initial denaturation at 95°C; 35 cycles of denaturation at 95°C for 2 minutes; 45 seconds of annealing at 54°C; 1 minute of extension at 72°C; and 10 minutes of final extension at 72°C.

2.6.5.4 Arginine Catabolic Mobile Element (ACME) arcA gene

PCR detection of the arcA gene was performed implementing a primer pair arcA-F and arcA-R (Table 3). The PCR conditions included 5 minutes of initial denaturation at 95°C, 30 seconds of annealing at 55°C, 45 seconds of extension at 72°C, and 10minutes of final extension at 72°C.

2.7 Data analysis

The prevalence rates were shown as percentages and the p-value < 0.05 was designated as the significance level. The groups were compared using the chi-square test.

3. RESULTS

3.1 Isolation and Prevalence

Inthecurrentstudy,basedonthe phenotypic characterizations, including colony characteristics on Mannitol Salt agar, Microscopic examinations, and Biochemical tests, 33 out of 310 patients (10.64%) were found to be positive for Staphylococcus aureus. Furthermore, the highest prevalence rate of 12.14% (17/140) was observed among urine specimens. Followed by 10% (9/90) in sputum samples. In contrast, the lowest prevalence rate of 8.75% (7/80) was found among nasal swab samples. However, no significant differences were found among them (p-value = 0.71). In addition, it was found that the prevalence rate of this bacterium varies between males 12.4% (16/129) and females 9.39% (17/181); however, no significant differences were observed between them (p-value = 0.39). Also, it was observed that the highest prevalence rate was recorded among the age group 15-45 years old 12.72% (21/165), whereas the lowest prevalence rate was noticed among the age group ≤14, which were 4.87% (2/41). However, no statistically significant variations were observed among these different age groups and the rate of infections (p-value = 0.15), as detailed in Table 4

Table 4: The distribution of specimens and positive cases according to the sample sources, genders, and age groups.

Sample Source	No. of samples	S. aureus N% (No. of +ve)	p-value
Urine	140	12.14% (17/140)	0.71
Sputum	90	10% (9/90)	
Nasal swab	80	8.75 %(7/80)	
Total	**310**	**10.64% (33/310)**	
Gender	**No. of samples**	**S. aureus N% (No. of +ve)**	**p-value**
Female	181	9.39% (17/33)	0.39
Male	129	12.4% (16/33)	
Total	**310**	**10.64% (33/310)**	
Age groups	**No. of samples**	**S. aureus N% (No. of +ve)**	**p-value**
≤14	41	4.87% (2/41)	0.15
(15-45)	165	12.72% (21/165)	
>45	104	9.61% (10/104)	
Total	**310**	**10.64% (33/310)**	

3.2 Antibiotic Susceptibility Profiles

The results of antibiotic susceptibility profiles, against ten different antibiotic discs (Figure 1), revealed that the highest resistant (100%) was shown against Penicillin, whereas the lowest resistant rate (15.2%) was against Gentamycin. Furthermore, the identified isolates showed high resistant rates (94%, 90.9%, 78.7%, and 72.7%) against each of Cephalothin, Tetracycline, Doxycycline, Clindamycin, respectively. In contrast, relatively lower resistance rates were observed against each of Erythromycin (57.5%), Vancomycin (45.5%), and Ciproflaxcin (33%). Moreover, regarding the phenotypic detection of MRSA based on Oxacillin disc on Muller-Hinton agar (CLSI, 2021), 16 isolates (48.5%) were preliminary identified as methicillin-resistant S. aureus. In addition, based on the resistant patterns of the identified isolates against different antibiotic classes used in this investigation, these isolates were categorized into extensively drug resistant (XDR), multidrug resistant (MDR), and non-multidrug resistant (non-MDR), which had ratios of 30.3%, 57.5%, and 12.1%, respectively (Table 5).

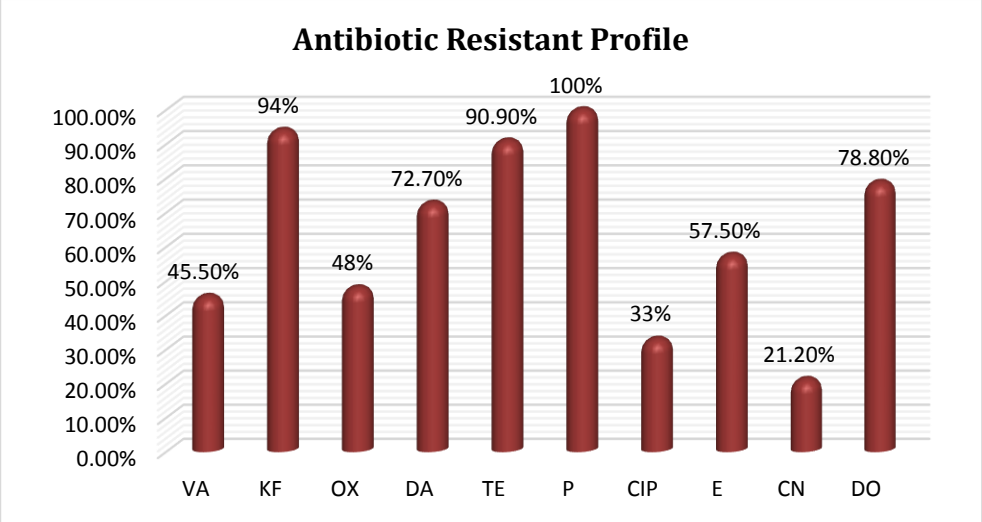

Figure 1: Char representation of the antibiotic resistant profiles of the identified S. aureus isolates.

3.3 Molecular Identification of MRSA

All the 33 identified S. aureus isolates were confirmed at the molecular level targeting nuc gene and they were subjected to mecA-specific PCR assay for molecular detection of mecA gene. It was found that 51.5% (17/33) of the identified isolates were mecA positive and confirmed to be MRSA (Table 5). These results were relatively consistent with those of phenotypic detection of MRSA, taking into consideration the use of Oxacillin disc 10µg instead of the recommended disc dosage of 1µg due to the unavailability of Oxacillin 1µg.

3.4 Genotyping of SCCmec

The mecA-positive isolates (MRSA) were subjected to a multiplex PCR assay using eight sets of primers for genotyping and subtyping of Staphylococcal Cassette Chromosome mec (SCCmec) and determining the prevalence of each SCCmec type and subtype. The results revealed that three different SCCmec types and subtypes were identified, including SCCmec type II, SCCmec type III, and SCCmec type IV, subtype d (IVd) as shown in (Figure 2). The prevalence of the identified SCCmectypes and subtypes was varied. It was found that 35.3% (6/17) of MRSA isolates belonged to SCCmec type II, which was the most prevalent SCCmec type, followed by 17.64% (3/17) of SCCmec type IV subtype d, and 11.76% (2/17) of SCCmec type III. However, 35.3% (6/17) were found to be non-typeable, as presented in Table5.

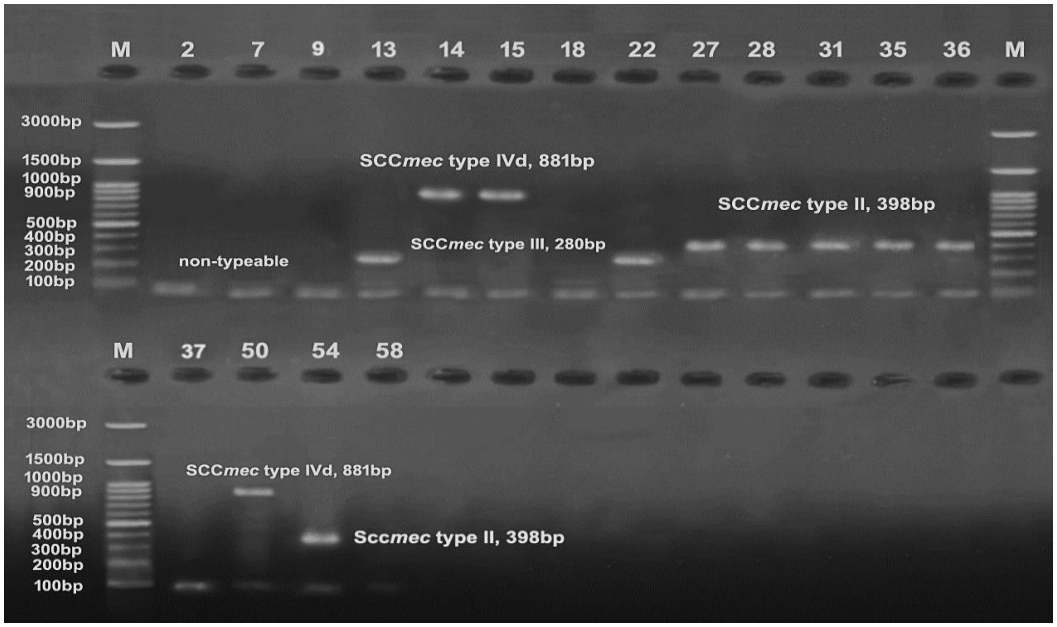

Figure2: Agarose gel electrophoresis for the multiplex PCR results of MRSA SCCmec typing and subtyping. Lane M represent molecular weight markers. Lanes 2 to 58 represent MRSA isolates typed.

3.5 Virulence factor profiling

The current study also targeted the molecular profiling of major virulence factors and toxin genes of the identified S. aureus isolates, including MRSA strains. The isolates were screened by PCR assays for molecular detection of major virulence factors and toxin genes that play important roles in survival, transmission, invasion, cytotoxicity, and bacterial pathogenicity. It was observed that 57.5% (19/33) of the isolates were positive for the exfolitative toxin A (ETA) encoded by the eta gene. Also, it was found that 45.4% (15/33) of the isolates carried Toxic Shock Syndrome Toxin-1 (TSST-1) encoded by the tst gene. Additionally, the Panton-Valentine Leukocidin (PVL) cytotoxin encoded by the lukS/F-PV gene was identified in 15.1% (5/33) of S. aureus isolates. Moreover, it was noted that 18.1% (6/33) of the identified S. aureus isolates were positive for the arginine catabolic mobile element (ACME) in the arc gene cluster (arcA gene), as shown in Table 5.

The detailed profiles of the identified S. aureus isolates in terms of the prevalence rates of MRSA, SCCmec typing, virulence factor and toxin genes, as well as the antibiotic-resistant patterns are presented in Table 5.

Table 5: Represents isolate number, profile, and prevalence rates of MRSA, SCCmec type, virulence factors, and the antibiotic pattern of the identified S. aureus isolates.

Isolate No.	MRSA (mecA)	SCCmec Type	eta	tst	lukS/F-PV	arcA	Antibiotic Pattern
1							XDR
2	+	Non-typeable	+				XDR
5				+			MDR
7	+	Non-typeable					MDR
8							MDR
9	+	Non-typeable	+	+			MDR
10							MDR
11			+	+			MDR
12				+			MDR
13	+	Type III	+	+			MDR
14	+	TypeIVd					XDR
15	+	TypeIVd	+				XDR
16			+	+			MDR
18	+	Non-typeable					XDR
22	+	TypeIII	+	+		+	MDR
27	+	TypeII					MDR

28	+	TypeII						Non-MDR
31	+	Non-typeable			+	+		MDR
34		TypeII				+	+	XDR
35	+	TypeII				+	+	XDR
36	+	TypeII			+	+	+	MDR
37	+	Non-typeable					+	XDR
40					+			MDR
41					+	+	+	XDR
43						+		Non-MDR
46					+	+	+	MDR
48						+		Non-MDR
49					+			Non-MDR
50	+	TypeIVd			+	+		MDR
54	+	TypeII			+		+	XDR
55								MDR
57					+			MDR
58	+	Non-typeable			+			MDR
Prevalence rates	51.5%	TypeII (35.3%) TypeIII (11.76%) TypeIVd (17.64%) Non-typeable (35.3%)	57.5 %	45.4 %	15.1 %	18.1 %	Non-MDR (12.1%) MDR (57.5%) XDR (30.3%)	

* The (+) symbol represents the presence of the corresponding gene.

4. DISCUSSIONS

Methicillin-resistant Staphylococcus aureus's (MRSA) ability to cause serious and potentially fatal infections make it a major health risk in developing countries. It can infect both patients and healthcare staffs and have a detrimental impact on the standard of care given in hospitals (Heudorf et al., 2016; Hnaihen et al., 2023). MRSA's resistance to numerous antibiotics, which make it hard to treat, has earned it the moniker "superbug." (Foster TJ., 2004; Lo et al., 2022). The occurrence of MRSA is widespread in many countries and its prevalence differs across various geographical regions and among different institutions. There was a gap in knowledge regarding the prevalence and diversity of SCCmec types of this pathogen among hospitalized patients in our hospitals, therefore this study aimed to provide a precise and accurate data regarding the SCCmec types and their antibiogram as well as virulence factor profiles.

Overall, among the 310 hospitalized patients tested for the presence of Staphylococcus aureus, the prevalence of this bacterium were 10.64%, of which 51.5% were found to be MRSA. This prevalence rate of S. aureus was relatively comparable with that reported by Hami and Ibrahim (2023) who reported 12.6% of this bacterium in clinical samples (urine). Also, the prevalence rate of MRSA was in line with that obtained by Hussein et al, (2019), who reported the prevalence rate of 50.4% of MRSA among the Hospital Staff. However, Hami and Ibrahim (2023) found a much higher prevalence rate of MRSA (75.7%) in Zakho General Hospital. In contrast, the prevalence of MRSA in the present study was almost tripled than that reported by Yassin, (2022) in a cross-sectional study conducted in Duhok city, who reported 18% of MRSA among medical college students. This inconsistency and fluctuations in the prevalence rate are most probably due to variations in sample size, subject type, and sample source, as each of these studies targeted a specific type and source of samples for their studies, as well as differences in age, sex, hygienic level. Besides, people should be cautious that, in addition to variations in antibiotic resistance, differences in the virulence and toxin profiles of the isolates play significant roles in bacterial responses, survival, and adaptations to different environments, which ultimately play a key role in bacterial infection and significantly contribute to its pathogenicity (Malachowa et al., 2010). Moreover, the current study reported variations in the distribution and the prevalence of S. aureus according to the sample sources (urine, sputum, and nasal swabs), patient's sex (male and female) as well as among different age groups of patients enrolled in this study. However, no statistically significant correlations were observed between these groups and bacterial prevalence and infection rates. This is primarily because S. aureus has a high level of virulence on the one hand and significant genetic flexibility on the other, which allows it to adapt to different environmental circumstances and infect nearly any source at any age.

Furthermore, the high prevalence rate of multidrug resistance (57.5%) and extensively drug resistance (30.3%) of S. aureus isolates, including MRSA. These rates together accounted for 87.8% of antibiotic resistance among hospitalized patients, which is regrettable and worrisome. This is because they are the leading cause of hospital acquired infections and can transmit to the community, which eventually hard to eradicate. The overall resistant rates, against different antibiotics used in this study, were relatively compatible with those reported by Abdulqader and Saadi (2019) in Erbil city, who reported a relatively high resistant rates (20-60%) of S. aureus isolates including MRSA to the majority of the antibiotics used. However, the multidrug resistance rate (57.5%) reported in the current study was almost doubled comparing with the multidrug resistance of S. aureus isolates (33.3%) reported by AL-Salihi et al, (2023) in Kirkuk city, Iraq. The prevalence of MRSA is still used to gauge the proper use of antibiotics as well as effective infection control and prevention techniques. It is believed that improper use of antibiotics is associated with a high prevalence of resistance to antimicrobials, including methicillin and other antimicrobials (Uehara Y, 2022).

In addition, the current study reported three diverse SCCmec types and subtypes, of which the most prevalent one

was SCCmec type II (35.3%), followed by SCCmec type IVd (17.64%), SCCmec type III (11.76%), and 35.3% were found to be non-typeable. The overall diversity of SCCmec types in the current study was relatively in line with that previously reported by Hussein NR, (2016) with different frequencies. For instance, in this study, the SCCmec type IV subtype d was the second most common type among hospitalized patients, whilst in his investigation, Hussein NR, (2016) reported the SCCmec type IV as the less common strain among others, without mentioning its subtype. It is noteworthy to mention that, to the best of our knowledge, with the exception of a single brief report by Hussein NR, (2016) in this regard, which was conducted on healthcare workers in Duhok city, no previous reports were available in the literature targeting the prevalence and diversity of MRSA-SCCmec types and subtypes in hospitalized patients. Therefore, this study might add an important information to the literature regarding the prevalence and the diversity of MRSA SCCmec types and the prevalence of each SCCmec type and subtype among hospitalized patients, as well as the virulence and toxin profiles of each type circulating in our hospitals. Determining and identifying these strain types is crucial because these components have been used in studies on the evolution of staphylococci, as well as being used as a molecular epidemiology tool in healthcare settings (Uehara Y, 2022). In the current study, the genetic determinants of major and important virulence factors and toxins have been profiled at the molecular level for all the identified isolates, including MRSA strains. The eta gene which encode the exfolitative toxin A was identified in 57.5% of the total isolates and in 58.8% of MRSA strains. This result is in contrast with that reported by Rasheed and Hussein, (2020) in Duhok city, who found neither eta nor etb genes. This might be due to differences in the sample type, which targeted community-associated MRSA rather than clinical samples.

Virulent strains of S. aureus secrete exfoliative toxins (ETs), also known as epidermolytic toxins (proteolytic and attacking the epidermis), which cause the superficial epidermis to lose cell-cell adhesion. They have been connected to blister formation and skin peeling due to keratinocyte loss and cell-cell adhesion (Mariutti et al., 2017). Moreover, this study detected the tst gene in 45.4% of the isolates, which encode staphylococcal Toxic Shock Syndrome Toxin-1 (TSST-1). A higher prevalence rate (80.26%) of this gene was reported by Rasheed and Hussein, (2020) in the same region among CA-MRSA. The toxic shock syndrome toxin-1 (TSST-1), is an incredibly potent exotoxin produced by S. aureus called a superantigen (SAg) that can mediate the potentially fatal disease known as toxic shock syndrome (TSS). A functional benefit of this toxin to the bacteria's life cycle is to manipulate the innate and adaptive host immune system in a way that supports S. aureus life cycle (Tuffs et al., 2019). The lukS/F-PV gene encode the Panton-Valentine Leukocidin (PVL) cytotoxin was another important virulence factor identified in 15% of S. aureus isolates. A much lower prevalence rate of this gene was reported by Rasheed and Hussein, (2020) in this area among CA-MRSA. Certain MRSA strains and community-associated methicillin-susceptible S. aureus produce this virulence factor, which facilitates leukocyte lysis and tissue necrosis. Invasive infections like necrotizing pneumonia can also be brought on by PVL-associated S. aureus, which is primarily responsible for skin and soft-tissue infections (Darboe et al., 2019). The prevalence of arA gene in the arc gene cluster of the arginine catabolic mobile element (ACME) was also documented in 18.1% of the identified S. aureus isolates with a relatively higher prevalence (23.5%) among MRSA strains. A significantly higher rate of this gene (56.58%) was reported by Rasheed and Hussein, (2020) in the Iraqi Community. It is believed that this transposable genetic element, which integrates into the staphylococcal chromosome similarly to the SCCmec, aids in the spread, colonization, and persistence of this pathogen on human skin and have role in strain's virulence, as it considered as a delegate marker for virulence in bacteraemia, or strain survival (Seidl et al., 2017; O'Connor et al., 2018).

The uneven distribution of the total number of samples obtained from each source, as well as the unevenness of those samples numbers by age and gender, are, in our opinion, the specific limitations or weaknesses of the current study. These factors may have an impact on certain statistical analyses of correlations. However, with regard to the identification of S. aureus and MRSA, genotyping, and virulence factor profiling, the study employed more sophisticated and reliable molecular methodologies in addition to precise molecular strategies. In the current investigation, thorough knowledge and an extensive understanding of MRSA prevalence and genotypic variation are provided across several infection sites by the inclusion of a wide variety of clinical samples, including urine, sputum, and nasal swabs. Additionally, the study's main findings were intriguing and filled in a knowledge gap, specifically pertaining to hospitalized patients' genotyping of SCCmec of MRSA, which was not previously covered in our institutions. When taken as a whole, these will greatly expand and enrich the body of current literature by introducing new relevant knowledge and further valuable information. Knowing the molecular traits of MRSA in various clinical samples is essential for developing effective infection management and treatment plans, as it is a major pathogen in healthcare settings.

CONCLUSIONS

This study identified an alarming prevalence rate of multidrug resistance and extensive drug resistance among S. aureus isolates, including MRSA, in hospitalized patients. Therefore, improper use and overuse of antibiotics should be prevented. The current study identified diverse SCCmec types and subtypes with variable rates. Also, the findings of the current investigation pointed out the circulating of highly virulent and extensively resistant MRSA strains among hospitalized patients, which may require active surveillance and better control policies.

Ethical Approval

The Research Ethics Committee, a joint commission between the University of Duhok and the Directorate of General Health in Duhok, granted approval for this study. The permission letter reference number (21122021-12-12) issued on 21st December 2021 (Appendix). The different clinical specimens included in the current investigation were collected with the consent of the patients.

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Conflicts of interest

There is no conflict of interest disclosed by the authors.

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