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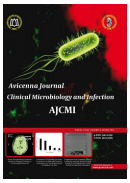

Original Article

The Molecular Investigation of the *mecA* Gene and Antibiotic Susceptibility Pattern of *Staphylococcus aureus* and *Staphylococcus epidermidis* Isolated from Patients with Immune System Disorders at Omid Hospital, Isfahan, Iran

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Abstract

Background: At present, antibiotic-resistant staphylococci, especially methicillin-resistant strains, are prevalent agents of infections in medical centers and hospitals. The objective of the present investigation was to discern and trace the methicillin resistance gene harbored in two bacterial strains, namely *Staphylococcus aureus* and *Staphylococcus epidermidis*, obtained from clinical specimens gathered from patients exhibiting immune system deficiency at Omid hospital located in Isfahan.

Methods: The present investigation was conducted utilizing a descriptive cross-sectional approach. Initially, a total of 70 clinical isolates comprising 35 isolates of *S. aureus* and 35 isolates of *S. epidermidis* were obtained from patients who were diagnosed with immunodeficiency and admitted to Omid Hospital located in Isfahan, Iran, from January 2017 to April 2018. After the characterization of the isolates via morphological and biochemical assessments, subsequent evaluation of their antibiotic sensitivity was performed through the utilization of disk diffusion and Epsilometer test (E-test). Then, the identification of the isolates was conducted using the colony PCR method incorporating primers (MCF, MCR, GAIF, and GAIR) and elucidated through molecular analysis.

Results: In this study, all isolates of *S. aureus* were resistant to cefoxitin and the MIC of this antibiotic was confirmed using E-test. However, of 35 *S. epidermidis* isolates, 30 isolates (85.7%) were resistant to oxacillin and 5 isolates (14.3%) were sensitive to oxacillin. According to the molecular findings, out of 35 isolates of methicillin-resistant *S. aureus*, 4 isolates (11.4%) had the *mecA* gene, and out of 35 isolates of *S. epidermidis*, 10 isolates (28.5%) had the *mecA* gene.

Conclusion: The present study revealed that precise detection of methicillin resistance in the aforementioned bacterial strains necessitates the employment of both phenotypic and genotypic methods. The frequency of the *mecA* gene in methicillin-resistant *S. aureus* (MRSA) was found to be declining. The incidence of methicillin-resistant *S. epidermidis* (MRSE) is on the rise.

Keywords: Methicillin-resistant *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus epidermidis*, *mecA* gene, PCR, E-test, Disc diffusion

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Introduction

Staphylococcus aureus and *Staphylococcus epidermidis* are widely recognized as ubiquitous and pathogenic bacteria implicated in hospital-acquired infections on a global scale (1-3). *S. aureus* has been observed to colonize on the skin, particularly when damaged, as well as in various other areas including the perineum, vagina, armpit, navel of neonates, and oropharynx (4, 5). This gram-positive bacterium represents a significant causative agent in the domain of healthcare-associated infections (6). Methicillin-resistant *S. aureus* (MRSA) is a strain of *S. aureus* that is resistant to methicillin and other beta-lactam antibiotics. Methicillin-resistant *S. aureus* (MRSA) constitutes a grave concern in the context of hospital-acquired infections, owing to its opportunistic nature, and exacerbates the complexity of treating infections caused by this bacterium (7,8). One of the contributory factors for the resistance of the bacteria to methicillin is the indiscriminate administration of antibiotics, particularly beta-lactam antibiotics. This may be attributed to the inadequate and uninformed

prescription practices of physicians or the non-performance of an antibiogram (9,10). The prevalence of methicillin resistance among *S. epidermidis* isolated from hospital samples ranges from 75% to 90% (11). Usually, the carriers are the reservoir of antibiotic resistance genes and cause commensal *S. epidermidis* to become a pathogen. Most antibiotic resistance genes are coded by plasmids and can be transferred from methicillin-resistant strains to sensitive strains (12). Generally, these plasmids carry antibiotic resistance genes. According to findings obtained from the investigation conducted by researchers, it was revealed that the occurrence of MRSA strains was reported in European medical facilities from 1961 to 1963, a few years after their introduction (13,14). The prevalence rates of this phenomenon have been reported to exceed 70% in Asian nations, including China, Korea, and Taiwan while surpassing 50% in North America and Iran and attaining 20% in Europe (15). Methicillin is a semi-synthetic penicillinase-resistant penicillin. Methicillin resistance is one of the most important and common resistance patterns among *S. aureus* strains that is caused by the presence of the *mecA* gene, which is chromosomally coded (16,17). The development of resistance to antimicrobial agents in MRSA strains has been attributed to the production of a distinctive binding protein referred to as Penicillin Binding Protein 2a (PBP2a). This protein has a substantially weakened affinity towards β-lactam antibiotics, thus prompting the development of bacterial strains that are more resistant to these therapeutic agents (18-20). PBP2a is encoded by the *mecA* gene which is located on a large mobile genetic element called staphylococcal cassette chromosome *mec* (*SCCmec*) and is present in the chromosome of resistant strains (21-24). According to the reports of the World Health Organization, patients who are infected with MRSA are hospitalized for a longer period of time than those who are infected with methicillin sensitive *S. aureus* (MSSA). Therefore, in addition to the cost of treatment, the infection can progress to bacteremia or endocarditis (25,26). Complications of infection such as kidney and liver failure are also more prevalent among MRSA patients than among patients infected with MSSA (27,28) and it has even been observed that the mortality rate is significantly higher among patients infected with MRSA, especially those with immune system defects, cancer patients, transplant recipients, AIDS patients, elderly, infants, pregnant women, diabetics, and so on, than among MSSA patients (29-33). Timely diagnosis and isolation of these patients can prevent the spread of MRSA and MSSA strains in the hospital environment and medical staff. Based on statistical analysis, it has been determined that over 70% of *S. epidermidis* strains isolated from hospitals have been found to exhibit resistance to methicillin. Furthermore, the majority of these strains have demonstrated a level of multidrug resistance, which makes their treatment difficult and expensive (34-36). The objective of this study was to analyze the *mecA* gene and antibiotic susceptibility pattern in *S. aureus* and *S. epidermidis* strains isolated from patients with immune system disorders at Omid Hospital in Isfahan, Iran. The disk diffusion and E-test methods were employed to isolate the gene, followed by the colony PCR method for identification of the *mecA* gene.

Materials and Methods

Clinical Isolates

A total of 70 isolates of MRSA and MRSE isolated from clinical samples of patients with immune system deficiency in Omid hospital in Isfahan were evaluated. These isolates were from different clinical samples, including wounds, sputum, urine, blood, trachea, and so on. Immunodeficiency patients (cancer patients, transplant patients, AIDS patients, the elderly, infants, pregnant women, diabetic patients, etc) were collected and standard tests were performed to isolate and identify methicillin-resistant isolates, which included preparation of Gram staining slides, culture in mannitol salt agar, DNAse agar and coagulase and catalase tests as well as antibiogram against cefoxitin, oxacillin, vancomycin, novobiocin, bacitracin antibiotics, and cefoxitin, oxacillin, and vancomycin E-tests (37).

Disc Diffusion Method

The disk release test was performed using disks (Mast, England) containing cefoxitin (30 µg), bacitracin (0.04 µg), vancomycin (30 µg), and novobiocin (5 µg). Two strains of *S. aureus* (ATCC 33591 and ATCC 25923) and two strains of *S. epidermidis* (ATCC 29887 and ATCC 12228) were used for positive and negative control. The antibiotic sensitivity test was performed using the Kirby-Bayer disk diffusion method on Mueller Hinton agar (MHA) medium, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (38).

Determination of MIC Using E-test Method

The E-test was performed on each isolate individually to verify the results of the disk diffusion method. For this test, a bacterial suspension equal to 0.5 McFarland standard was prepared and cultured on MHA medium using sterile swap in four directions. Then, each E-test strip was placed separately on the plate. In this study, E-test belonging to (Mast, England), cefoxitin, vancomycin, and oxacillin were used for the isolates. Two strains of *S. aureus* (ATCC 33591 and ATCC 25923) and two strains *S. epidermidis* (ATCC 29887 and ATCC 12228) were used again for positive and negative control (37,39).

Colony-PCR and Identification of its Products

Colony PCR is a method of DNA fragment amplification by PCR, which is done using a microorganism colony without the need for DNA extraction. The first step of unwinding DNA double helix into two single strands of DNA for the first primer is done at 94 °C for 300 seconds, followed by 40 cycles of amplification, including the

denaturation step at 94 °C for 15 seconds, the annealing step at 55 °C for 15 seconds, and the extension step at 72 °C for 20 seconds, and the final elongation step at 72 °C for 300 seconds. The first step of unwinding DNA double helix into two single-stranded DNA molecules for the second primer is done at 94 °C for 300 seconds, followed by 40 amplification cycles, including the denaturation step at 94 °C for 30 seconds, the annealing step at 55 °C for 30 seconds, and the extension step at 72 °C for 60 seconds, and the final elongation step at 72 °C for 300 seconds. It should be noted that the positive control had the *mecA* gene and the negative control did not have this gene. Moreover, sterile distilled water can be used for negative control (40,41). The primer sequences used in the present investigation are delineated in Table 1.

Statistical Analysis

In order to statistically analyze the data obtained from this research, Chi-square test was used in SPSS (version 17) at the confidence level of $P \leq 0.05$.

Results

In general, in this study, out of 70 isolates of *Staphylococcus*, including 35 isolates of *S. aureus* and 35 isolates of *S. epidermidis*, were resistant to methicillin. They were isolated from surgery, ICU, operating room, neurology, and gastroenterology departments of Omid hospital in Isfahan. Out of 35 *S. aureus* isolates, 21 samples belonged to women (60%) and 14 samples belonged to men (40%). Of 35 isolates of *S. epidermidis*, 20 samples belonged to women (57.2%) and 15 samples belonged to men (42.8%). The clinical samples were collected from patients with immune system deficiency (diabetics, AIDS patients, transplant recipients, elderly, newborns, pregnant women, etc.). The highest frequency of *S. aureus* strain was observed in the trachea of 17 samples (48.5%) and the lowest frequency was observed in infectious eye discharge (1 sample, 2.8%) and urine (1 sample, 2.8%). The highest frequency of *S. epidermidis* strain was observed in the blood of 24 samples (68.5%), and the lowest frequency was observed in the catheter of 1 sample (2.8%) and the cerebrospinal fluid of 1 sample (2.8%)

The Results of Identification of Isolates Using Phenotypic and Biochemical Tests

In this study, the identified isolates, including *S. epidermidis* and *S. aureus*, were observed as gram-positive cocci and as single or double or irregular grape-shaped clusters using gram staining. After identifying the morphology and arrangement of bacteria with the help of gram staining, the identification of these bacteria was achieved through the application of targeted biochemical assays.

Table 1. The Primers Employed in this Study for Detecting the *mecA* Gene

Primers	(3´) Primer Sequence (5´)	Product (bp)
GaiF	AAAATCGATGGTAAAGGTTGGC	307
GaiR	AGTTCTGCAGTACCGGATTTGC	
MCF	TGGCTATCGTGTCACAATCG	500
MCR	CTGGAACTTGTTGAGCAGAG	

Results of the Antibiotic Sensitivity Test by Disk Diffusion Method

The results of the antibiotic sensitivity test of clinical isolates of *S. aureus* and *S. epidermidis* are presented in Table 2 and Figure 1. In this research, out of a total of 35 clinical isolates of *S. aureus*, all 35 isolates (100%) were resistant to both cefoxitin and bacitracin, and 100% of the isolates were sensitive to vancomycin and novobiocin. Out of a total of 35 clinical isolates of *S. epidermidis*, 4 isolates (11.4%) were sensitive to cefoxitin and 31 isolates (88.5%) were resistant to cefoxitin. All 35 isolates (100%) were resistant to bacitracin and 10 isolates (28.5%) were sensitive to vancomycin. Additionally, 25 isolates (71.4%) were resistant to vancomycin and all 35 isolates (100%) were sensitive to novobiocin.

E-test Results

The results of this test showed that out of 35 methicillin-resistant isolates, 27 samples (77.1%) were resistant to vancomycin and 8 isolates (22.9%) were sensitive to vancomycin. All strains of MRSA exhibited complete resistance (100%) to cefoxitin as depicted by the results obtained from the E-test strips (Figure 2). The results obtained for *S. epidermidis* isolates in this study showed that out of 35 methicillin-resistant isolates, 20 samples (57.2%) were resistant to cefoxitin and 15 isolates (42.8%) were sensitive to cefoxitin. Out of 35 isolates of *S. epidermidis* that exhibited resistance to methicillin, 10 isolates (28.5%) were resistant to vancomycin and 25 isolates (71.5%) were sensitive to vancomycin. Out of 35 isolates of *S. epidermidis* that exhibited resistance to methicillin, 30 isolates (85.7%) were resistant to oxacillin and 5 samples (14.3%) were determined to be oxacillin-sensitive (Figure 3).

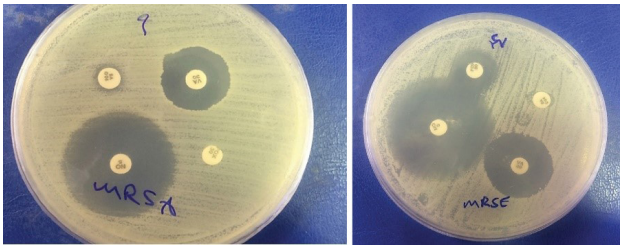

Figure 1. Antibiogram Result of One Isolate of MRSA and MRSE

Table 2. Antibiotic Sensitivity and Resistance Pattern of MRSA and MRSE Isolates According to the Type of Antibiotic by Disk Diffusion Method

Antibiotics	Concentration (µg/mL)	MRSA (n = 35)		MRSE (n = 35)	
		Resistance No. (%)	Sensitive No. (%)	Resistance No. (%)	Sensitive No. (%)
Cefoxitin	30	35 (100)	-	31 (88.5)	4 (11.4)
Bacitracin	0.04	35 (100)	-	35 (100)	-
Vancomycin	30	27 (77.1)	8 (22.9)	25 (71.4)	10 (28.5)
Novobiocin	5	-	35 (100)	-	35 (100)

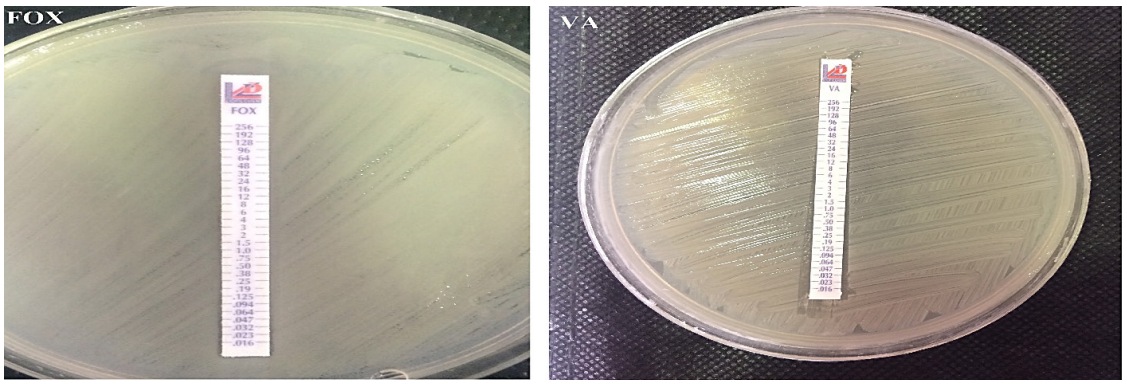

Figure 2. Determination of MIC for Cefoxitin and Vancomycin Antibiotics in Two MRSA Isolates Using E-test

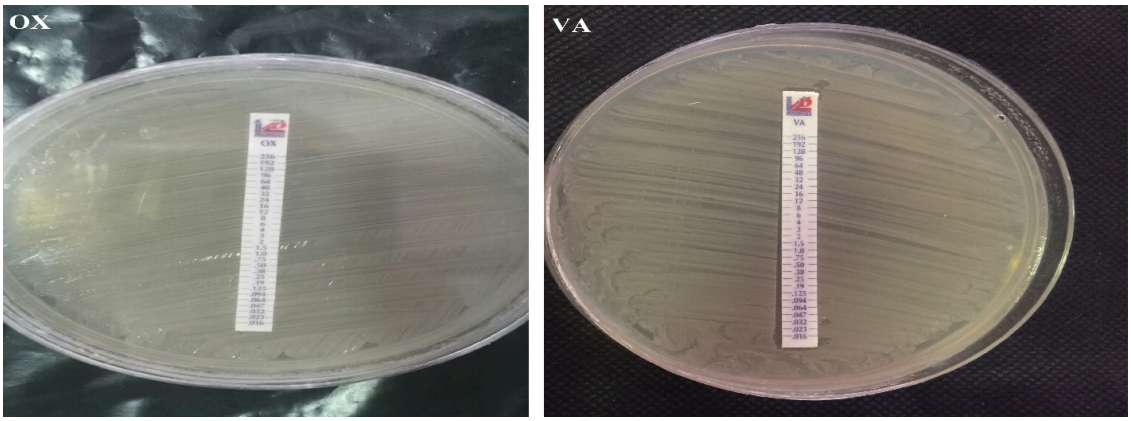

Figure 3. Determination of MIC for Oxacillin and Vancomycin Antibiotics in Two MRSE Isolates Using E-test

The Results of Colony PCR to Detect the mecA Gene in MRSAs

In this study, two primers were used for the identification of the *mecA* gene. The first primer used was only able to identify a part of the *mecA* gene that was the same in all the variants, and the second primer completed the identification of the *mecA* gene in the desired variants. After the isolation of methicillin-resistant strains by phenotypic method, the *mecA* gene was detected by PCR. Two strains of *S. aureus* (ATCC 33591 and ATCC 25923) were used for positive and negative control, respectively. The obtained results indicated that out of 35 isolates of MRSA, 4 isolates (11.4%) had the *mecA* gene and 31 isolates (88.6%) lacked the *mecA* gene (Figure 4).

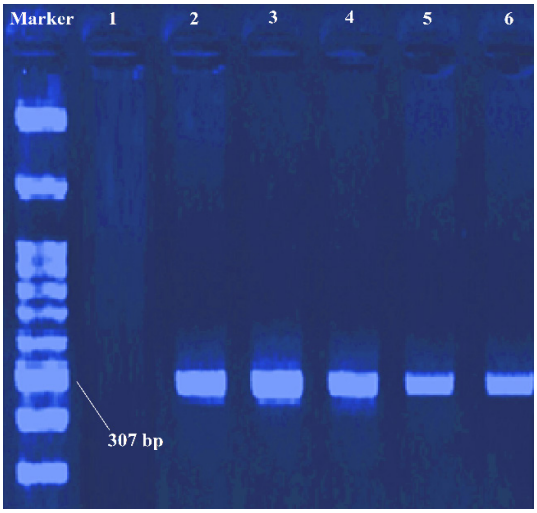

Figure 4. Results of PCR and the Presence of *mecA* Gene for Several MRSA Isolates. *Note*: Lane 2: Weighted marker 100 bp, Lanes 3-5: Clinical samples, Lane 6: Positive control, Lane 1: Negative control

Results of Colony PCR to Trace the mecA Gene in MRSEs

After the isolation of methicillin-resistant strains by phenotypic method, the *mecA* gene was detected by PCR. Two strains of *S. epidermidis* ATCC 29887 and *S. epidermidis* ATCC 12228 were used for positive and negative control, respectively. The results obtained from 35 isolates of MRSE showed that 10 isolates (28.5%) had the *mecA* gene and 25 isolates (71.4%) did not have the *mecA* gene (Figure 5).

Discussion

In recent years, *S. aureus* and *S. epidermidis* have been identified as prominent etiological agents of hospital-acquired infections in vulnerable individuals with compromised immune function (42,43). According to a global survey, an observed correlation ranging from 65% to 85% linked clinical strains to MRSE. The increasing incidence of nosocomial infections and the mounting issue of antimicrobial resistance necessitate the prompt implementation of a more expeditious diagnostic method in healthcare facilities. It is recommended that genotypic tests should be replaced with phenotypic tests (44,45). In

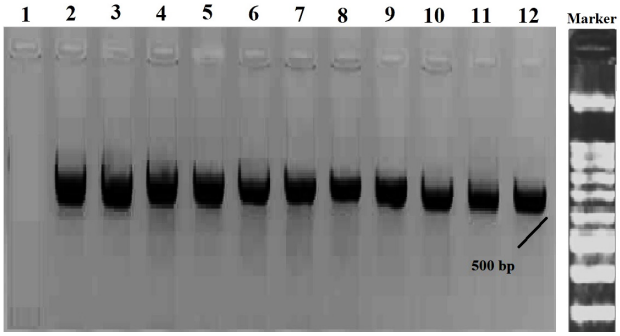

Figure 5. Results of PCR and the Presence of *mecA* Gene for Several MRSE Isolates. *Note*: Well 2: Weighted marker 100 bp, Wells 3-11: Clinical samples, Well 12: Positive control, Well 1: Negative control

this research, 35 isolates of *S. aureus* and 35 isolates of *S. epidermidis* were selected from 70 clinical isolates in a period of six months. The prevalence of *S. aureus* strain exhibited significant variability among different sample types, with the trachea demonstrating the highest frequency (48.5%). The *S. epidermidis* strain with the greatest frequency was observed in the blood of 24 samples, accounting for 68.5% of the total. The current research found that cefoxitin resistance was displayed by MRSA strains, which was later confirmed through an E-test performed to measure the MIC of cefoxitin. However, it should be noted that the E-test of cefoxitin was not explicitly outlined in the CLSI guidelines for MRSE isolates. One of the important reasons that has attracted attention to *S. aureus* bacteria today is the resistance mechanisms of these bacteria given that the prevalence of MRSA strains in hospitals and medical centers is increasing (46). Pishva et al reported that the rate of resistance to methicillin in *S. epidermidis* isolates using the agar dilution method was 10.9% and the rate of resistance to oxacillin was 13.5% using the E-test (47). Another study performed by Sharma et al indicated that all the isolates were susceptible to vancomycin, linezolid, and teicoplanin in the disc diffusion test while maximum resistance was noted against penicillin (100%) and 25% of the isolates were found to be resistant to methicillin. A comparison between resistance patterns of methicillin-resistant and methicillin-sensitive strains showed that methicillin-resistant isolates had higher levels of resistance to other antibiotics (48). Based on the results obtained in our study for MRSE isolates, 28.5% of the isolates were sensitive to vancomycin using the E-test, indicating that the sensitivity to this antibiotic was reduced compared to the results of Sharma and Hejira. The results obtained in our study also showed that the analysis of MRSE strains using the E-test method determined 85.7% resistance to oxacillin, indicating a significant increase compared to the results of the study conducted by Pishva et al. The increase in oxacillin resistance is likely a result of individuals intentionally taking antibiotics, particularly antibiotics from the penicillin family such as amoxicillin, ampicillin, nafcillin, and similar ones. In a study conducted by Pishva et al in Isfahan, the resistance rate of *S. epidermidis* isolates to methicillin in Al-Zahra Hospital was reported to be 73% (47). In other studies, the prevalence of MRSA was determined to be 6.3%. Moreover, among all isolates of *S. aureus*, the prevalence of MRSA was observed to be 61.8%. The presence of *mec* genes was observed in 96.8% of MRSA isolates, with the remaining 3.2% exhibiting an absence of *mec* genes. The co-occurrence of *mecA* and *mecC* was identified in 57.1% of the MRSA isolates. The antibiotics that exhibited the highest level of resistance were penicillin and amoxicillin/clavulanic acid, followed by norfloxacin, levofloxacin, ciprofloxacin, azithromycin, erythromycin, moxifloxacin, and sulfamethoxazole/trimethoprim, with resistance rates of 91.2%, 87.1%, 83.9%, 78.6%, 77.4%, 69.8%, and 54.9%, respectively. In contrast, vancomycin and teicoplanin displayed high efficacy, with a success rate of 98.4% in combating MRSA (49). Our findings showed that out of 35 MRSA isolates, only 4 isolates (11.4%) had the *mecA* gene and out of 35 MRSE isolates, 10 isolates (28.5%) had the *mecA* gene, indicating a lower frequency of this gene in MRSE isolates compared to the studies conducted between 2016 and 2018 (48-52) but a higher frequency compared to the results of the study conducted by Rahimi in 2012 (53). Kondo et al isolated 99 strains of *S. aureus* from America, Canada, England, Ireland, and Europe and after performing microbial and PCR testing, the findings revealed that 16 of the isolates were found to have the *mecA* gene (54). In another study, researchers sampled 50 patients who underwent joint plastic surgery. These patients were examined after receiving antibiotics for a period of 24, 36, and 48 months, and their clinical samples were taken. It was determined that 38 patients had MRSE and 12 patients had MRSA (55). Prasad et al studied patients with implant-related infections. A total of 91 clinical samples were obtained and biochemical and PCR tests were performed. Of 55 (42.2%) isolates of *S. epidermidis*, 23 (41.8%) samples were multiple drug-resistant and 15.3% of them were resistant to methicillin and had the *mecA* gene (56). In another study, 26 nasal samples were collected from premature babies and antibiotic and microbial tests along with PCR were performed on the isolates. Based on the results of the PCR test, the most frequently isolated species were *S. epidermidis* (38.3%) and *S. haemolyticus* (38%), followed by other *Staphylococcus* species. It should be noted that the isolated staphylococci had multiple drug resistance and some of them had the *mecA* gene (57). In the study conducted by Du et al, a variety of microbial strains were isolated from hospitalized patients, outpatients, and hospital personnel. Subsequently, the isolates were subject to biochemical and antibiotic analyses employing two distinct methods, namely E-test and disk diffusion, along with molecular identification techniques. According to the results obtained from their research, 44.8% of isolates were MRSE and MRSE-*ST2-SCCmecIII* was the predominant clone in clinical isolates, almost resistant to all antibiotics used in the study (58). In the study of Cherifi

et al, 84 samples were isolated from hospitalized patients, 66 of which were *S. epidermidis* and the rest were *S. aureus*. Based on the results of the PCR test on *S. epidermidis* strains, 3 isolates had *mecA*. However, in the present study, out of 35 isolates of MRSA, only 4 isolates (11.4%) had the *mecA* gene, and out of 35 isolates of MRSE, 15 isolates (42.8%) had the *mecA* gene. The findings showed that the prevalence of the *mecA* gene among MRSA is decreasing, while the prevalence of this gene is increasing in MRSE (59). In a study performed by Noshak et al, *S. epidermidis* and *S. haemolyticus* strains were isolated from patients and healthcare workers. The detection of methicillin resistance among isolates was accomplished via the utilization of the cefoxitin disk diffusion test. Cefoxitin and cotrimoxazole demonstrated the highest resistance rates, with a value of 81.5%. Among all the MRSE and methicillin-resistant *S. haemolyticus* isolates, 66 *mecA*-positive isolates were detected (60). In further analysis, it was found that out of the 27 isolates of *S. aureus*, approximately 55.6% of the isolates exhibited MRSA. The PCR analysis involved choosing various strains of *S. aureus* for examination. It was observed that a substantial proportion (53.3%) of the MRSA isolates were found to possess the *mecA* gene. Conversely, it was noted that all MSSA isolates tested negative for the presence of the *mecA* gene (61). A study conducted by Siddiqui et al yielded similar findings, indicating that only 36.5% of the subjects exhibited *mecA* positivity. However, all MSSA isolates tested negative in PCR (62). Due to the high prevalence and clinical importance of these infections, it is necessary for the hospital staff to be aware and plan to develop methods of prevention, treatment, and successful control of these infections in treatment systems (infection control unit), especially for patients with immune system deficiency in the community and hospitals are a priority.

Conclusion

Evaluating the prevalence of isolates with virulence genes as well as investigating drug resistance in hospitals can be effective in controlling infectious diseases in people with immune system deficiencies. The present study found that the incidence of the *mecA* gene in MRSA is in decline, whereas it is on the rise for MRSE.

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Authors' Contribution

Conceptualization: Zahra Babaei.
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Formal analysis: Zahra Babaei.
Funding acquisition: Zahra Babaei.
Investigation: Zahra Babaei.
Methodology: Zahra Babaei.
Project administration: Monir Doudi, Zahra Babaei, Ladan Rahimzadeh Torabi.
Resources: Zahra Babaei.
Software: Monir Doudi, Zahra Babaei, Ladan Rahimzadeh Torabi.
Supervision: Monir Doudi, Zahra Babaei.
Validation: Monir Doudi, Zahra Babaei, Ladan Rahimzadeh Torabi.

Competing Interests

There is no conflict of interests as stated by the authors.

Ethical Approval

Not applicable.

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