Original Article

Study on the Prevalence of Methicillin-Resistant *Staphylococcus Aureus* Infection, Antibiotic Resistance Pattern, Biofilms Genes, and Antibiotic Resistance Genes from Clinical Samples

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ABSTRACT

The global health situation is caused by Methicillin-Resistant *Staphylococcus aureus* (MRSA) strains, which exhibit resistance to the majority of antibiotics. The emergence and spread of antibiotic resistance make the treatment of these infections more complicated and intricate. The objective of this study was to investigate the *mec*A, *bla*Z, *cna*, and *fnb*A genes and the pattern of antibiotic resistance in *S. aureus* isolates obtained from different clinical samples. In this study, 78 strains of *S. aureus* were collected from different a variety of clinical specimens. The antibiotic susceptibility of the isolates was determined via the disk agar diffusion method. The prevalence of the *mec*A, *bla*Z, *cna*, and *fnb*A genes and the antimicrobial resistance patterns exhibited by the isolates against 10 conventional antibiotic disks were evaluated in these isolates. The data were analyzed using the SPSS statistical software version 25. Of the 78 samples collected, 63 samples were found to contain the *mec*A gene representing a prevalence of (62.2%). A total 63 *S. aureus* isolates were examined, of which is present in 60 (95.2%) exhibited the *bla*Z gene and 51 (81%) exhibited the fnbA gene. The frequency of the *cna* gene was observed in 42 (66.7%) samples. Additionally, a significant correlation was identified between the *cna* and *fnb*A genes and gentamicin and tetracycline antibiotic resistance with ($P<0.05$). The antibiotic resistance pattern revealed that all the isolates exhibited resistance to oxacillin (100%), penicillin (95.2%), and demonstrated the least resistance to vancomycin (3.2%), and Trimethoprim-sulfamethoxazole (17.5%). In comparison to other studies conducted in Iran, our findings indicate an average prevalence of MRSA. However, the level of resistance to commonly used antibiotics in these isolates was considerable. In this situation, it is recommended to monitor antibiotic resistance in these hospitals and medical centers.

Keywords: *Staphylococcus Aureus*, MRSA, Antibiotic Resistance, Biofilm

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1. Introduction

It is estimated that between 20% and 25% of the healthy adult population are colonized by *S. aureus*, a Gram-positive human commensal on a regular basis. Additionally, up to 60% of individuals may experience sporadic colonization (1). It causes a wide range of infections, including bacteremia, septicemia, and infections of the skin, soft tissue, bone, and pneumonia. In rehabilitated cases, HIV cases, and people with underlying conditions like diabetes, these infections can lead to further complications and mortality. The disease is transmitted through direct contact and/ or objects. (2). The emergence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) occurred shortly after the introduction of methicillin, a new antibiotic, in 1960. Methicillin was employed as a therapeutic agent for *S. aureus strains exhibiting resistance* to penicillin. This resistance was attributed to the penicillinase product called beta-lactamases, which inactivate penicillin. The global observation of *S. aureus* methicillin resistance indicates that it is resistant to all penicillinase-resistant penicillins and cephalosporins. (3,4). MRSA strains have the capacity to produce a specific protein, designated as penicillin-bindingprotein (PBP), which exhibits reduced affinity for certain classes of penicillin. The PBP gene designated as *mec*A, can be isolated and utilized for the storage of the information. The Methicillin resistance gene (*mec*A) is transferred by a small genetic part called the staphylococcal cassette chromosome mec (SCCmec) (5). Penicillin resistance occurs in certain strains of S. aureus primarily due to the production of a protein called beta-lactamase, which is encoded by a specific gene called *bla*Z. The genes *bla*Z, *bla*I, and *bla*R1 are located in a single locus (6). Four distinct variants of the *bla*Z product have been identified through serotyping and the observation of variations in hydrolysis of selected-lactam substrates (A, B, C, D). The A, C, and D types are often observed on plasmids. In contrast, the B variant is located on the chromosome. However, in contrast to the chromosome-based *mec*A gene, the *bla*Z gene is carried by a plasmid (7). The capacity of bacteria to be identified and eliminated by the innate immune system is impeded by the formation of biofilms (8). The initial stage in the formation of a biofilm by S. aureus is the adhesion of the bacteria to different surfaces and subsequent growth on host tissues. *S. aureus* possesses a multitude of surface-bound molecules that facilitate its adhesion to other molecules. These substances, also designated microbial surface components, are capable of recognizing and attaching to different types of protein substrate. Notable examples of these proteins include fibronectin-binding proteins A and B, clumping factors A and B, collagen-binding protein, bone sialoprotein binding protein, and fibrinogen binding protein. (9,10). FnBPA adhesions stick to receptors on cells in the blood vessels, which can precipitate cardiac complications heart problems and infections in cardiac devices by activating platelets. This represents a crucial phase in the development of blood clots and their attachment to artificial parts inside the body (11). The collagen adhesin (*cna*) gene is responsible for the production of the cna protein, which facilitates the attachment of *S. aureus* to collagen tissues and cartilage. Studies have demonstrated that antibiotic resistance does not impede the capacity of MRSA strains to form biofilms (12). The objective of the present study is to examine the prevalence of biofilm genes (*cna* and *fnb*A) and antimicrobial resistance genes (*mec*A and *bla*Z) in addition to theantimicrobial resistance patterns of MRSA in Karaj, Iran.

2. Materials and Methods

In this study, 78 strains of *S. aureus* were collected over a six-month period from clinical samples including urine and wounds from two laboratories in Karaj. The samples were transported using the Tryptic Soy Broth (TSB) transport medium was used to transfer these samples and delivered to the laboratory within a maximum of two hours. The samples were cultured on blood agar and mannitol salt agar from (Ibresco, Iran) were used to culture these samples in plates at 37°C for 24-48 hours. The *S. aureus* strain was then isolated by standard microbiological methods,including: catalase, coagulase, mannitol fermentation and DNase tests.

2.1. Detection of *S. Aureus* Specific *Nuc* Gene

The DNA was extracted from the samples using the boiling method. All isolated strains were subjected to analysis via the polymerase chain reaction(PCR) method. Specifically, *nuc* primers (F: 5 AGCGATTGATGGTGATACGG-3 and R: 5-ATACGCTAAGCCACGTCCAT-3) were employed for the identification of *S. aureus* strains (13). The PCR was conducted in a total volume of 25 µl ,comprising 14 µl master amplicon (YTA, Tehran, Iran), 1 pmol of each forward and reverse primer,and 8 µl of distilled water containing 2 µl of template DNA. The following PCR parameters were employed for the 35 -cycle PCR: an initial denaturation at 94°C for 2 minutes, denaturation at 94°C for 1 minute, annealing at 55°C for 0.5 minutes, extension at 72°C for 2 minutes and a final extension at 72°C for 5 minutes (13). The PCR results (226 bp) were visualized using 1.5% agarose gel electrophoresis (YTA, Tehran, Iran).

2.2. Antimicrobial Susceptibility of *S. Aureus* Isolates

To confirm the precise identity of the MRSA isolates, the presence of the*mec*A gene *was* verified through the use of by amplification PCR. Subsequently, the investigation was conducted to investigate the susceptibility patterns of the following 10 antimicrobial agents, representing a diverse range of antimicrobial classes. The susceptibility of the samples to the discs of Tetracycline (30 µg), Oxacillin (1 µg), Doxycycline (30 µg), Erythromycin (15 µg), Trimethoprim/sulfamethoxazole (25 µg), Amikacin (15 µg), Penicillin (10 µg), Vancomycin (30 µg), Ciprofloxacin (5 µg) and Gentamicin (10 µg) (Padtan Teb, Iran). The antibiotic susceptibility patterns of *S. aureus* isolates were determined by the Kirby-Bauer method, and the results

were interpreted in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (14).

2.3. DNA Extraction

The DNA was extracted from the samples using the boiling method. A loopful of bacterial colonies was suspended in 300 µl of sterile distilled water and then heated for 20 minutes. The resulting liquid part was then used as a DNA sample in the PCR mixture after being spun it in a centrifuge for 15 minutes at a fast speed of 13,000 rpm (16).

2.4. Detection of Genes

PCR and electrophoresis techniques were employed to determine the presence of *mec*A, *bla*Z, *cna*, and *fnb*A genes. A multiplex PCR was conducted on the in two *cna* and *fnb*A genes: The resulting PCR products were visualized with 3% agarose gel (YTA, Tehran, Iran) for the amplified genes,which contained Safe Stain (YTA, Tehran, Iran), The molecular approach was optimized using *S.aureus* ATCC 25923 as the control strain. The primer sequences and PCR conditions utilized for the detection of genes are presented in Table 1. The amplification conditions for the *mec*A gene were as follows: denaturation for two minutes at 94°C, followed by 40 cycles of 94°C for two minutes, 57°C for 60 seconds, 72°C for 2 minutes, and finally extension at 72°C (10). The amplification conditions for the *bla*Z gene were as follows: the process commenced with heating at 94°C for 5 minutes, followed by 35 cycles of heating at 94°C for 30 seconds, cooling at 55°C for 30 seconds, and heating again at 72°C for 30 seconds. Subsequently, a final heating step at 72°C for 10 minutes (11) is performed. The amplification conditions for the *fnb*A and *cna* genes were as follows:an initial denaturation was performed, wherein the temperature was first set at 95°C for two minutes. Subsequently 50 cycles were conducted,wherein the temperature was initially set at 95°C for 30 seconds, then at 55°C for 1 minute, and finally at 72°C for 1 minute with 1 minute for the final step (12,14).

2.5. Statistical Analysis

The results were analyzed using the Pearson's chi-square test or Fisher's exact test was used to analyze the results and a value of $P<0.05$ was considered statistically significant (SPSS 25.0, SPSS Inc. Chicago, IL, USA).

3. Results

3.1. Antibiotic Susceptibility

Of the 63 clinical samples in which the presence of the *mec*A gene was confirmed, 40 (63.5%) samples were urine samples and 23 (36.5%) were wound samples. The overall resistance of MRSA isolates to antimicrobial agents was 100% for oxacillin; 95.2% for penicillin; 31.7% for ciprofloxacin, tetracycline and doxycycline. The resistance rates for erythromycin; amikacin; gentamicin; trimethoprim-sulfamethoxazole; and vancomycin were 30.2%, 27%, 23.8%, 17.5%, and 3.2%, respectively. The overall antibiotic susceptibility pattern of strains to antimicrobial agents is illustrated in Table2. Figure 1 illustrates the prevalence of antibiotic resistance in clinical samples.

Table 1. Primers sequences as per standard reference

Gene	Primer Sequences (5′ to 3′)	Product Size (bp)	annealing	Reference
*mec*A	F: TGCTATCCACCCTCAAACAGG R: AACGTTGTAACCACCCCAAGA	268	57°C	10
*bla*Z	F: AAGAGATTTGCC TATGCTTC R: GCTTGACCACTT TTATCAGC	518	55°C	11
*fnb*A	F: CATAAATTGGGAGCAGCATCA R: ATCAGCAGCTGAATTCCCATT	128	55 °C	12
cna	F: AAAGCGTTGCCTAGTGGAGA R: AGTGCCTTCCCAAACCTTTT	192	55 °C	13

Table 2. The pattern of antibacterial susceptibility for *S. aureus* isolates.

Antibiotic	Sensitive (%)	Intermediate (%)	Resistance (%)
Oxacillin	---	---	63 (100)
Penicillin	3 (4.8)	---	60 (95.2)
Ciprofloxacin	35 (55.6)	8 (12.7)	20 (31.7)
Tetracycline	34 (54)	9 (14.3)	20 (31.7)
Doxycycline	36 (57.1)	7 (11.1)	20 (31.7)
Erythromycin	37 (58.7)	7 (11.1)	19 (30.2)
Vancomycin	62 (96.8)	---	2 (3.2)
Amikacin	35 (55.6)	11 (17.5)	17 (27)
Gentamicin	48 (76.2)	---	15 (23.8)
SXT*	48 (76.2)	4 (6.3)	11 (17.5)

*Trimethoprim-sulfamethoxazole

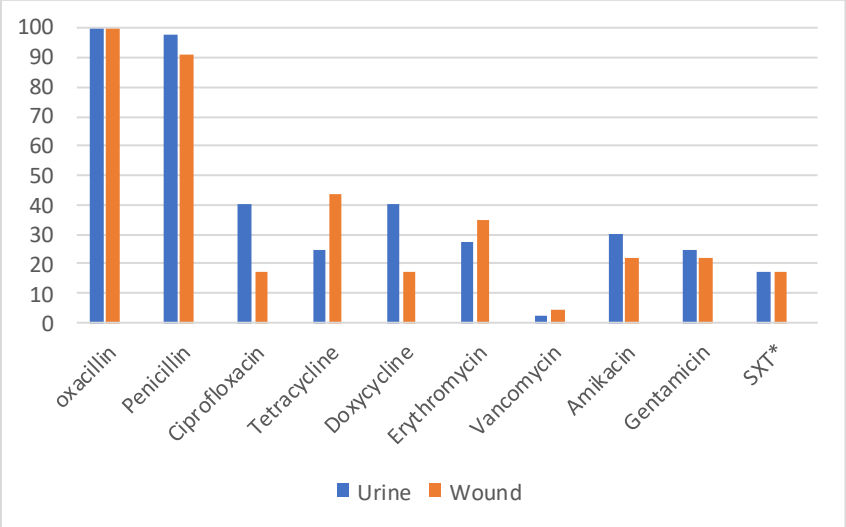

Figure1. Percentage of antibiotic resistance in clinical samples

3.2. Gene pattern characterization

In this study, the presence of antibiotic resistance and biofilm -related genes was evaluated in *S. aureus* isolates by PCR method. All isolates were subjected to investigation with regard to the presence of the *cna, fnbA, mecA and blaZ* genes. The prevalence of the *cna*, *fnb*A, *mec*A and *bla*Z genes was determined to be as 66.7%, 81%, 46% and 95.2% respectively. The result of PCR for the *cna, fnbA, mecA and blaZ* genes from *S. aureus* isolates are presented in Figure 2.

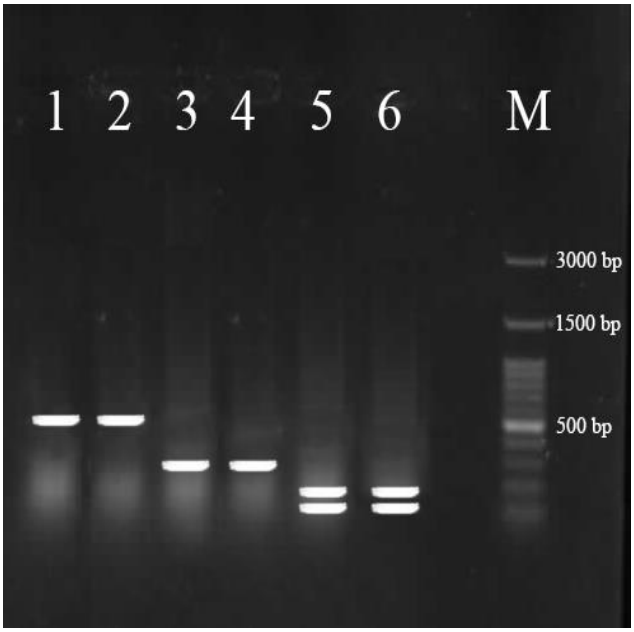

Figure2. Amplification of *mec*A, *fnb*A, *can and bla*Z genes from *S. aureus* isolates. Lane M, DNA marker (100 bp); Lane1 and 2, *bla*Z (518 bp); Lane 3 and 4, *fnbA* (268 bp); Lane 5 and 6, *fnb*A and *cna* (128 and 192 bp).

The frequency of the *bla*Z gene was observed in urine and wound samples at 97.5% and 91.3%, respectively. No significant relationship was observed between the frequency of this gene and the clinical samples (P>0.05). The frequency of the *mec*A gene was observed in urine and wound samples at 50% and 39.1%, respectively. No significant relationship was found between the frequency of this gene and clinical samples (P>0.05). Additionally, the frequency of the *fnb*A gene was observed in urine and wounds at 80% and 82.6%, respectively. The frequency of the *cna* gene was observed to be present in urine and wounds at the rate of 67% and 69%, respectively. A significant relationship was observed between the frequency of this gene and wound samples (P>0.05). In isolates with the *fnb*A, 81% also exhibited the *cna* gene, demonstrating a significant relationship between these frequencies. The *bla*Z gene was present in 95.2% of the samples, indicating a significant relationship between the presence of this gene and the production of beta-lactamase enzyme in *S. aureus* isolates (P<0.05). A significant correlation was observed between gentamicin and tetracycline antibiotic resistance with *cna* and the *fnb*A genes (P<0.05).

4. Discussion

S. aureus is a common bacterium that colonizes the human skin and mucous membranes. However, *S. aureus* is also a major causative agent of hospitaland community-associated infection that can result in life- threatening disease (17). The control of antibiotic-resistant *S. aureus* strains have been dependent on three factors: ensuring proper hand hygiene among healthcare workers, restriction of antibiotic use, and prompt identification and isolation of infected patients (18,19). In this study, 63 isolates of MRSA were examined. Of these, 60 (95.2%) exhibited the blaZ genes, while 51 (81%) demonstrated the fnbA gene. .Additionally, the

frequency of the *cna* gene was observed in 42 isolates (66.7%). The results of the antibiotic resistance pattern showed that all isolates were resistant to penicillin, while the lowest resistance was observed with vancomycin. In the study by Gomes et al. (20) in Brazil, 56 strains of *S. aureus* resistant to methicillin were isolated from blood cultures. Of these, 86% of the samples were positive for the presence of the *blaZ* gene, and 84% of the isolates had the *mecA* gene. In Egypt, Amr and Al Gammal (21) reported that of 114 *S. aureus* strains, 90 (78.9%) were MRSA, and 10 strains (8.8%) were resistant to vancomycin. Of the MRSA strains, 88 out of 90 carried the *mecD* gene, and all 10 vancomycin-resistant isolates were positive for both the *mecA* and *vanA* genes.Kim and Lee (22) conducted a study in Korea in which oral saliva samples were collected from a total of 112 patients with dental diseases, including 80 outpatients in dental hospitals and 32 patients in dental clinics. Among these, 37 *S. aureus* strains were positive for the *blaZ* gene: 27 strains from hospital patients and 10 from clinic patients.In India, Naseer and Jayaraj (23) reported that out of 360 *S. aureus* strains, only 7 (1.9%) were vancomycin-resistant and positive for the presence of the *mecA* gene.In a study conducted by Mohammadi et al. (24) at a burn center in Tehran, of 83 *S. aureus* isolates, 74.7% were positive for the *cna* gene, and 42.1% had the *fnbA* gene. Khasawneh et al. (25) reported that out of 57 *S. aureus* isolates, 22 were MRSA. The prevalence of the *cna* and *fnbA* genes in MRSA isolates was 40.9% and 81.8%, respectively. Similar studies indicate that the prevalence of resistant *S. aureus* is highly dependent on geographical region, biological patterns, and regional antibiotic usage, contributing to variations in study outcomes. Therefore, monitoring shifts in antibiotic resistance patterns over time may greatly aid in treating *S. aureus* infections. To prevent drug resistance, performing an antibiogram test prior to antibiotic use is recommended PCR testing on penicillin-resistant strains may be necessary to detect beta-lactamase-producing organisms, allowing for more accurate reporting. It is also recommended to limit non-prescription antibiotic use in unnecessary cases to reduce antibiotic resistance levels.

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Authors' Contribution
A comprehensive literature review and research, conceptualization, methodology, supervision, project administration, writing-reviewing and editing, methodology, investigation, studies analysis: Z.H., A.S.B., F.B., Writing original draft preparation, writing-reviewing and editing, and methodology: B.J.L., P.M.G investigation. Validation and Reviewing: M.H.K., A.S.B.

Ethics
All experimental procedures were carried out with the utmost respect for the principles of ethical research, ensuring the welfare and safety of the participants.

Conflict of Interest
The authors declare that they have no conflicts of interest to disclose.

Data Availability
The data that support the findings of this study are available on request from the corresponding author.

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