Original Article

Investigating the Antibiotic Resistance Pattern of MRSA in Cancer Patients

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ABSTRACT

The emergence of methicillin-resistant Staphylococcus aureus (MRSA) represents a significant public health concern among long-term hospitalized patients, particularly those with weakened immune systems, such as cancer patients. This is primarily due to MRSA's ability to resist antimicrobial agents and drugs. The objective of this study is to ascertain the antibiotic resistance pattern of MRSA in cancer patients admitted to hospitals in the southwest region of Iran. The samples obtained from the patients were cultivated on blood agar and EMB medium. Subsequently, the positive samples containing S. aureus were identified through the application of a phenotypic method. Subsequently, the cefoxitin antibiogram was employed for the isolation of MRSA. Furthermore, the isolates were subjected to testing for simultaneous drug resistance against 12 different antibiotics. To detect the presence of the mec gene, a molecular method was employed, namely the polymerase chain reaction (PCR) technique, and electrophoresis of the obtained products was conducted. Of the 41 S. aureus samples identified, 33 were found to be methicillin-resistant S. aureus (MRSA). Of the 33 MRSA isolates, the presence of the mec gene was confirmed, and they exhibited simultaneous drug resistance. Individuals with cancer, who frequently have indwelling catheters and receive a variety of drugs and blood products, are at an elevated risk of contamination with this bacterium due to its presence on their skin and the hands of healthcare providers. The indiscriminate use of drugs and the subsequent rise in drug resistance can contribute to prolonged hospitalization and even death among these individuals. Given that Ahvaz hospitals, particularly Bagai Hospital, serve as primary treatment centers for patients with incurable and cancerous conditions in southwestern Iran, it is of significant value and importance to investigate the resistance patterns observed in patients undergoing chemotherapy and post-transplantation.

Keywords: *Staphylococcus Aureus*, Methicillin, Linezolid, Antibiotic Resistance, MRSA.

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1. Introduction

Staphylococcus aureus is acknowledged as a principal etiological agent for both hospital-acquired and community-acquired infections (1, 2). Its substantial pathogenicity, coupled with its resistance to antimicrobial agents, has elevated it to a preeminent position among global health concerns. S. aureus is responsible for a range of purulent infections and food poisoning in humans (2). This bacterium is a primary cause of surgical wound infection in hospitalized patients and infections associated with medical equipment (5, 6). Staphylococcus aureus is primarily colonized on the surface of the skin and mucous membranes and is capable of surviving in all tissues of the body. This group of microorganisms is responsible for a variety of infections in humans. Over the past three decades, methicillin-resistant S. aureus (MRSA) has emerged as a significant pathogen in both hospital and community-acquired infections. Despite the advancement of technology and drug treatment, MRSA remains a significant factor in the pathogenesis of infections, which are considered difficult to treat (10). Staphylococcus aureus is a gram-positive cocci, facultative anaerobe, and opportunistic bacterium that is one of the most prevalent hospital and community-acquired infections. Its ability to adapt to diverse environmental conditions and increasing resistance to antimicrobial drugs make it a significant concern in the field of medicine. The clinical manifestations of this bacterium are highly variable and include bacteremia, pneumonia, osteomyelitis, skin scaling syndrome, toxic shock syndrome, endocarditis, and others in humans (11, 12). Prior to the advent of penicillin, approximately 80% of infections caused by Staphylococcus aureus resulted in mortality. In the 1940s, the mortality rate associated with this pathogen underwent a notable decline with the advent of penicillin. However, the inappropriate and excessive use of antibiotics in 1942 resulted in the emergence of the inaugural penicillin-resistant strain, initially within hospital settings and subsequently within the wider community. Currently, over 90% of S. aureus strains are resistant to penicillin. This resistance is attributed to the acquisition of a plasmid that carries the gene encoding the penicillin-hydrolyzing enzyme. In response to this resistance, researchers introduced methicillin, a semi-synthetic antibiotic that is resistant to this enzyme, for clinical use. In 1961, shortly after the introduction of methicillin in England, methicillin-resistant S. aureus (MRSA) strains developed resistance to methicillin due to the acquisition of the mecA gene. Consequently, MRSA strains rapidly disseminated throughout various regions of the world and became one of the most significant hospital-associated pathogens (13, 14). The mecA gene is located on a shared chromosome in proximity to the genes encoding protein A (spa) and the biosynthetic protein of purines. The gene encodes a 78 kDa protein, designated PBP2 (15). In contrast to other proteins that bind to penicillin in the bacterial wall, this protein displays a diminished affinity for methicillin binding. PBP proteins are essential for the assembly of the bacterial cell wall. Consequently, the presence of this novel protein remains unaffected by the antibiotic, thereby enabling the bacterium to continue its life cycle unhindered. In susceptible bacteria that lack the mecA gene, methicillin exhibits a higher affinity for the PBP protein in the cell wall. This interaction results in the lysis of the bacterial cell wall, which ultimately leads to bacterial death. The mecA gene is located on a mobile genomic island known as the staphylococcal chromosomal cassette (SCCmec). SCCmec elements represent a discrete group of genomic islands that contain the mec gene complex (16). Strains of S. aureus that possess this gene demonstrate resistance to a multitude of other antibiotics, thereby exhibiting multidrug resistance. The objective of this study on S. aureus is to address the current concern regarding the prevalence of S. aureus (MRSA) and its resistance to antimicrobial and medicinal agents. In the context of long-term hospitalization, including among patients with compromised immune systems such as those undergoing cancer treatment, it is of paramount importance to ascertain the antibiotic resistance pattern of MRSA among individuals receiving treatment at Baqai Hospital of Ahvaz. This hospital is a prominent regional center for transplantation and chemotherapy. By determining the resistance pattern, it may be possible to reduce mortality rates and shorten hospital stays for these patients.

2. Materials and Methods

2.1. Sample Collection

2.1.1. Isolation of *S. Aureus*

In this study, a cross-sectional analytical approach was employed to examine samples of blood cultures, fluids, and catheters obtained from patients. Upon arrival at the microbiology laboratory, the samples were cultured on blood agar and EMB media. Subsequently, the cultures were incubated at 37 °C for a period of 24 hours. The presence of grayish-white colonies was indicative of a positive culture result. Subsequently, the colonies were subjected to phenotypic tests, including catalase, mannitol salt agar, DNase, and tubular coagulase tests, to confirm the presence of S. aureus. Once the identity of S. aureus was confirmed, the samples were stored in TSB medium with glycerol at -70°C for a period of six months. A total of 41 S. aureus isolates were obtained and subsequently preserved for further analysis. The research was conducted using samples of S. aureus collected from cancer patients who were hospitalized in various departments of Beqai Hospital and other hospitals in Ahvaz. The study period spanned from the beginning of April to the end of September 2023. The samples for this research were selected in accordance with the recommendations of specialist physicians (Figure 1).

2.2. Isolation of *S. aureus* Resistant to Methicillin

Once the requisite sample size had been obtained through the disk diffusion agar method, in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines for cefotaxime antibiotics (30 micrograms),

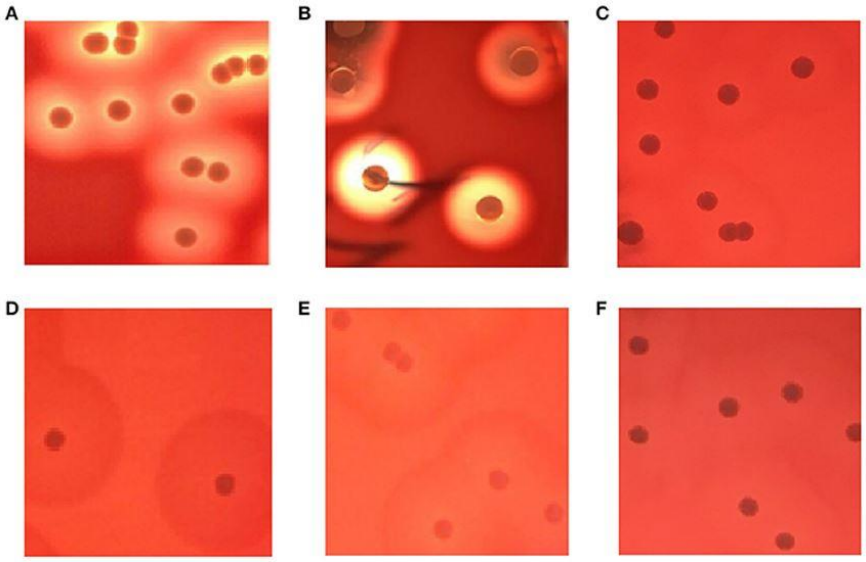

Figure 1. *S.aureus* colony on blood agar medium. **(A)** control strain *S.aureus* ATCC25923; **(B)** SCHP; **(C)** SIHP(growing in the microaerophilic condition); **(D)** SIHP(growing in the aerobic condition); **(E)** SIHP(growing in the anaerobic condition); **(F)** SIHP after 10 passages(17). This figure is distributed under the terms of the Creative Commons Attribution License (CC BY).

the methicillin-resistant S. aureus strain was successfully isolated. Subsequently, the antibiogram method was conducted on a Hilton Muller agar medium. A sterile swab or loop was employed to transfer a turbidity equivalent to the McFarland standard turbidity (1.5 x 10^8 bacterial cells per ml) onto the medium. Subsequently, sterile tweezers were employed to position the antibiogram disks on the agar surface. Subsequently, the plates were incubated at 37 °C for 24 hours. The formation of halos around the disks was observed and classified as sensitive, semi-sensitive, or resistant according to the criteria established by the Kirby-Bauer method.

2.3. Methicillin-Resistant *S. aureus* Antibiotic Sensitivity and Resistance Test

Following the acquisition of 33 MRSA isolates, all isolates were cultured on Muller's medium to ascertain their resistance to the following antibiotics: ciprofloxacin, tetracycline, gentamicin, linezolid (30 micrograms), clindamycin (2 micrograms), erythromycin (15 micrograms), and ampicillin, which were sourced from Patan Teb Company. The methodology employed was consistent with the aforementioned standard antibiogram procedure. Half of the McFarland standard was removed, cultured, and the resulting antibiograms were placed on the surface of the medium. Subsequently, the samples were incubated for 24 hours at 37 °C to observe halo formation. The susceptibility results were interpreted in accordance with the Kirby-Bauer method, whereby isolates were classified as sensitive, semi-sensitive, or resistant. Antimicrobial susceptibility testing was conducted using the Kirby-Bauer disk diffusion method, in accordance with the guidelines established by the Clinical and Laboratory Standards Institute (CLSI) in 2023. Isolates exhibiting resistance to three or more antibiotic classes were classified as multidrug-resistant.

2.4. Detection of mecA by PCR Technique

2.4.1. DNA Extraction

A parstous kit was utilized to extract the DNA of methicillin-resistant S. aureus bacteria. The polymerase chain reaction (PCR) was conducted using a 20-microliter volume. The materials and compounds utilized for the PCR reaction are presented in Table 1 for reference. To perform the polymerase chain reaction (PCR) reaction, the aforementioned compounds were added to a 2-ml microtube. The reaction was conducted using a thermocycler, with 35 cycles of the polymerase chain reaction (PCR) employed for gene amplification. The following order was observed for the reaction steps:

1. Initial denaturation: temperature of 35 °C Celsius for 5 minutes.
2. Denaturation: temperature of 94°C for 45 seconds.
3. Connection of primers: temperature of 58 °C for 58 seconds.
4. Prolongation: temperature of 72 °C for 45 seconds.
5. Final elongation: 72°C for 5 minutes.

In addition to the samples subjected to analysis, a negative control devoid of cDNA was employed in each series of polymerase chain reaction (PCR) reactions. The primers utilized for the amplification of the Mec-A gene included a pair of specific primers, as detailed below:

Forward: 5-AAAATCGATGGTAAAGGTTGG-3
Reverse: 5-AGTTCTGGAGTACCGGATTTGC-3

The products of 33 MRSA isolates that were amplified by PCR technique were taken to agarose electrophoresis to search for the mec gene.

Table1. Materials used for the PCR

chemical mixture	volume (microliters)
Master mix PCR2x (amplicom)	10
10micromol forward primer	0.5
10µmol reverse primer	0.5
DNA (100 nanograms)	3
Distilled water	6

3. Results

A total of 41 isolates of S. aureus were identified and analyzed from the samples received at the microbiology laboratory of Beqai and other hospitals in Ahvaz during the initial six-month period of 2023. Subsequently, the samples were cultured on blood agar and EMB, and the positive samples underwent a battery of tests, including coagulase, MSA, catalase, and DNase, to ascertain their identity as S. aureus. Of the 41 isolates, 17 (41.5%) were obtained from hospitalized women, while the remaining 24 (58.5%) were from hospitalized men. The majority of these isolates, specifically 35 (85.4%), were derived from blood culture samples. Furthermore, four isolates (9.75%) were derived from blood collection catheters, while two (4.9%) originated from liquid samples. Among the age groups, the youngest patient with a positive catheter sample was a four-year-old girl with a diagnosis of cancer. Conversely, the oldest patient with a positive blood culture was a 67-year-old male. It is noteworthy that the age group of men over 45 years old exhibited the highest resistance among the various age groups (Table 2 and Figure 2). Of the 41 isolates of S. aureus obtained through the phenotypic method of an antibiogram, in which cefoxitin antibiotic resistance was investigated, 33 isolates (80.5%) were reported to be resistant to methicillin (Figure 3). A total of 33 methicillin-resistant Staphylococcus aureus (MRSA) isolates were tested for susceptibility to a range of antibiotics. The highest level of resistance was observed in response to ampicillin, with all 33 isolates (100%) exhibiting resistance. Subsequently, erythromycin exhibited the highest resistance, with 27 isolates (81.8%) demonstrating resistance. Gentamicin demonstrated the second-highest resistance, with 26 isolates (78.8%) displaying resistance. Trimethoprim-sulfoxazole demonstrated the third-highest resistance, with 21 isolates (63.6%) displaying resistance. Clindamycin demonstrated the fourth-highest resistance, with 13 isolates (39.4%) displaying resistance. These findings are presented in Table 3. Of the 33 isolates tested against 12 antibiotics, 28 (85%) exhibited multidrug resistance, as detailed in Table 4. In the molecular method, 33 MRSA isolates were amplified in PCR technique to search for the mec gene, then the obtained products were subjected to electrophoresis, and at this stage, 33 isolates were reported to have the mec gene (Figure 4).

4. Discussion

One of the most significant obstacles in the management and prophylaxis of staphylococcal infections is the bacterium's inherent resistance to a multitude of antibiotics, including beta-lactams, aminoglycosides, and macrolides. This resistance has resulted in the proliferation of infections caused by S. aureus, as well as a number of associated complications. These issues include an increase in the number of injuries inflicted on hospitalized patients, higher treatment costs due to the necessity of expensive antibiotics, extended hospital stays for patients, elevated medical insurance costs, and, most importantly, an increased mortality rate. This situation has imposed significant limitations on the ability of medical practitioners to effectively treat infections caused by S. aureus. Consequently, individuals with weakened immune systems, particularly cancer patients with ports and catheters who regularly receive intravenous blood and drug infusions, are at the highest risk of infection with S. aureus, which can be present on their skin or on the hands of nurses (18-21). The objective of the present study was to investigate the phenomenon of drug resistance in cancer patients caused by this bacterium. A total of 41 isolates of S. aureus were obtained using the phenotypic method of antibiogram, with a specific focus on cefoxitin antibiotic resistance. Of the isolates, 33 (80.5%) were found to be resistant to methicillin. Further testing was conducted on the 33 MRSA isolates using different antibiotics, revealing that ampicillin exhibited the highest resistance rate (100%), followed by erythromycin (81.8%), gentamicin (78.8%), tetracycline (69.7%), trimethoprim-sulfamethoxazole (61.6%), and clindamycin (57.6%). Of the 33 isolates tested against 13 antibiotics, nine exhibited simultaneous resistance to five antibiotics: ampicillin, gentamicin, tetracycline, erythromycin, and clindamycin. Furthermore, seven isolates exhibited simultaneous resistance to an additional set of five antibiotics: ampicillin, erythromycin, tetracycline, ciprofloxacin, and trimethoprim-sulfamethoxazole. Furthermore, three isolates demonstrated resistance to four antibiotics: ampicillin, tetracycline,

Table 2. Relationship between age, gender and type of samples

Gender	**Male**				**Female**				Total
Age	**0-15**	**16-30**	**31-45**	**>45**	**0-15**	**16-30**	**31-45**	**>45**	
Blood	9.8%(4)	9.8%(4)	17.1%(7)	14.6%(6)	9.8%(4)	4.9%(2)	7.4%(3)	12.2%(5)	100%(41)
Liquids	-	-	2.4%(1)	-	-	2.4%(1)	-	-	4.9%(2)
Catheters	-	2.4%(1)	2.4%(1)	-	2.4%(1)	-	2.4%(1)	-	9.8%(4)

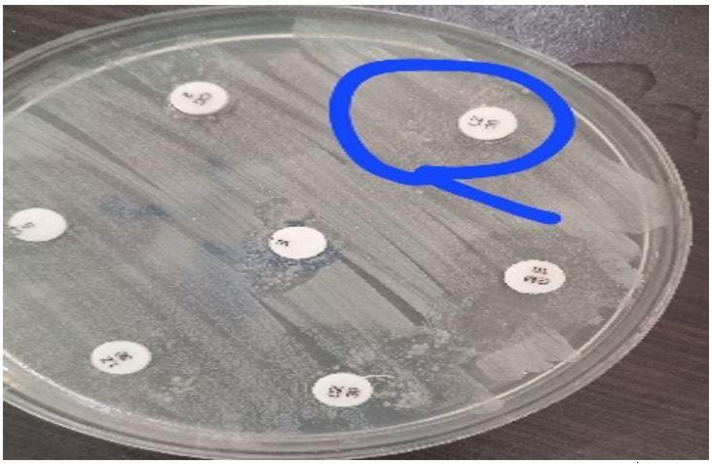

Figure 2. MRSA resistance pattern to different antibiotics

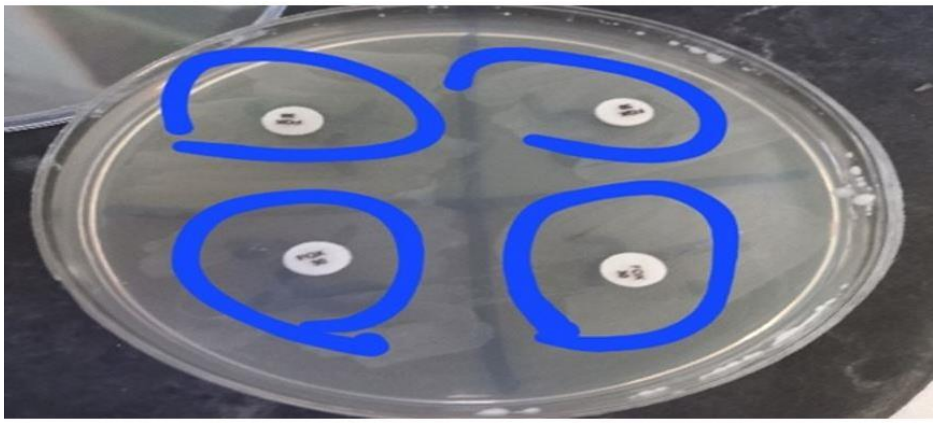

Figure 3. Determining the pattern of resistance to cefoxetine antibiotic.

Table 3. The pattern of mrsa antibiotic sensitivite and resistance to different antibiotics.

situation / Antibiotic	sensitivity	semi sensitive	Resistance
Ampicillin	-	-	33(100%)
Erythromycin	5(15.1%)	1(3%)	27(81.8%)
Gentamycin	7(21.2%)	-	26(78.8%)
Ciprofloxacin	17(51.5%)	3(9.1%)	13(39.4%)
Clindamycin	17(51.5%)	3(9.1%)	13(39.4%)
Linezolid	30(90.1%)	-	3(9.1%)
Cefazolin	13(39.4%)	5(15.1%)	15(45.5%)
Imipenem	18(54.4%)	4(12.1%)	11(33.3%)
Azithromycin	10(30.3%)	4(12.1%)	10(30.3%)
Chloramphenicol	32(97%)	-	1(3%)
Rifampicin	24(72.7%)	6(18.2%)	3(9.1%)
trimethoprim sulfoxazole	7(21.2%)	5(15.1%)	21(63.6%)

Table 4. Multi drug resistance.

Multidrug Resistance	Number of isolates	Phenotypic pattern of resistance
7 antibiotics	5((15.1%)	Ampicillin,Erythromycin,Gentamycin, Ciprofloxacin,Clindamycin,Cefazolin Imipenem
5 antibiotics	4(12.1%)	Ampicillin, clindamycin, ciprofloxacin,erythromicin, Gentamicin,
5antibiotics	3(9.1%)	Ampicillin, erythromicin, clindamycin, gentamicin, Ciprofloxacin
4 antibiotics	4(12.1%)	clindamycin gentamicin, ampicillin, ciprofloxacin
4antibiotics	3(9.1%)	Ampicillin, erythromicin, clindamycin, gentamicin
3antibiotics	1(3%)	Ampicillin, erythromicin, Rifampicin
3antibiotics	1(3%)	Gentamicin, Ciprofloxacin, Eerythromicin
3 antibiotics	2(6%)	Ampicillin, erythromicin, Imipenem
3 antibiotics	3 (9.1%)	Ampicillin, erythromycin, Ciprofloxacin
3 antibiotics	2(6%)	Ampicillin, erythromicin ،gentamicin

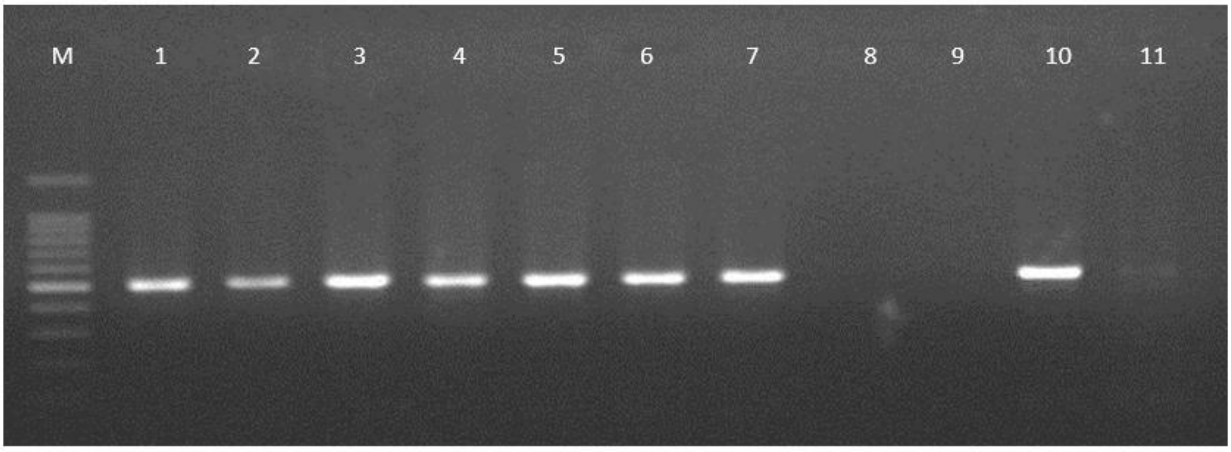

Figure 4. Electrophoresis of MRSA isolates 1 to 7 positive isolates 8, 9, and 11, negative control, 10, positive control, M (Ladder)

cefazolin, and trimethoprim-sulfamethoxazole. Ultimately, 14 isolates exhibited simultaneous resistance to three antibiotics: ampicillin, tetracycline, and gentamicin. In order to detect the presence of the mec gene, a molecular method was employed whereby the MRSA isolates were subjected to a process of polymerase chain reaction (PCR) amplification. Subsequent agarose gel electrophoresis confirmed the presence of the mec gene in the 33 isolates. These findings are consistent with reports and research conducted in Iran and other parts of the world, which indicate a significant increase in the prevalence of MRSA and the development of multiple drug resistance (20, 22-24). In a study (25), the antibiotic resistance pattern of S. aureus strains isolated from clinical samples was determined. The findings indicated that S. aureus exhibited resistance to methicillin in 75 of the 100 samples examined. The phenotypic evaluation of the antibiotic resistance pattern in methicillin-resistant S. aureus strains demonstrated that the molecular analysis confirmed the presence of the mecA gene in 68% of the isolates, which is consistent with the findings of this study. In a study conducted by Wang et al., investigating the antibiotic resistance pattern of S. aureus strains, the results indicated resistance to ampicillin (56%), gentamicin (22%), and erythromycin (54%). It is noteworthy that the observed resistance to erythromycin is consistent with the findings of the present study. The objective of Zamanian's study was to investigate the prevalence and pattern of antibiotic resistance in Staphylococcus aureus strains isolated from Imam Reza Hospital in Kermanshah. The results indicated that the majority of S. aureus cases were isolated from the emergency department (43.7%), followed by blood samples (40.1%) and urine samples (23.9%). The most prevalent antibiotic resistances were observed against erythromycin, cefoxitin, and clindamycin (27). The present study demonstrated similarities to our previous study in terms of resistance and susceptibility to cefoxitin and clindamycin antibiotics. In a study conducted by Omidi in Isfahan and Kashan, the prevalence of methicillin-resistant S. aureus strains was found to be 16.4% among the 146 clinical samples collected. Furthermore, 3.1% of the strains exhibited resistance to the antibiotic linezolid. In the molecular investigation conducted via PCR, all samples of methicillin-resistant S. aureus were found to carry the mecA gene (28). This research is in accordance with our study with regard to antibiotic resistance to linezolid. The

objective of the Ochi study was to investigate the antibiotic resistance of Staphylococcus aureus among patients undergoing orthopedic surgery. The findings indicated that 96.2% of the isolates exhibited resistance to four or more antibiotics. Among the cases, 88.5% exhibited multidrug resistance (MDR), while only 7.6% demonstrated resistance to a single antibiotic. Furthermore, 65.4% of the S. aureus isolates exhibited resistance to methicillin, while 34.6% remained methicillin-sensitive. These findings are consistent with those of the current study with regard to concurrent drug resistance (29). As indicated in the report procured from Baqai and other medical facilities in Ahvaz, it is advised that the infection control committees of these hospitals evaluate the antibiotics currently in use in order to ascertain their level of resistance. Moreover, it is recommended that these committees regularly update charts in the specialized care departments, indicating the antibiotics that should be avoided due to their high resistance. This measure is designed to prevent the prescription of such antibiotics by medical practitioners employed in those departments. Consequently, this initiative would significantly contribute to the expeditious identification of MRSA-related infections in clinical samples obtained from the hospital. Moreover, the implementation of this strategy would result in several advantages, including enhanced patient care, decreased treatment expenses, and shorter hospital stays. Additionally, it would serve as an early indicator of the emergence of resistance to linezolid, a crucial antibiotic that is currently regarded as a last-resort treatment option. The findings demonstrate a pervasive rise in antibiotic resistance, which presents a significant challenge in the management of infections caused by these resistant strains. It is therefore imperative to prevent the escalation of resistance not only to the aforementioned antibiotics, but also to those that are commonly used. This can be accomplished by refraining from issuing prescriptions for antibiotics in the absence of a valid medical indication and by limiting the use of antibiotics to those instances where they are truly necessary. The present study also demonstrates that there is no statistically significant correlation between antibiotic resistance and the prevalence of methicillin-resistant Staphylococcus aureus in the ward, sampling site, and hospital. However, when considering age and gender, it is evident that men over the age of 45 years display higher levels of resistance. Moreover, in comparison to previous studies, a higher prevalence was observed among the different sampling sites, specifically in blood samples.

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Authors' Contribution
All authors contributed equally to the study design, data collection and analysis, and manuscript preparation.

Ethics
The present research did not involve the use of human or animal subjects.

Conflict of Interest
All authors certify no conflict of interest.

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Data Availability
All the data are incorporated into the manuscript.

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