Antibiotic resistance Pattern of Methicillin-resistant Staphylococcus aureus Isolated from Hospitalized Patients

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ABSTRACT

Methicillin-resistant Staphylococcus aureus (MRSA) strains circulating among populations and crossing borders constitute a major problem for health control and require a fast and simple genotypic approach. The objective of this study was to determine the prevalence, molecular types and drug resistance pattern of S. aureus isolated from Hospitalized Patients in teaching Hospitals of Ahvaz. This cross-sectional study was from April to September 2023, MRSA strains were identified by phenotypic and molecular methods. The antibiotics studied were, Cefoxitin (15 μg), Gentamicin (10 μg), Ciprofloxacin (5 μg), Erythromycin (15 μg), Clindamycin (2 μg), linzolid(10μg), azithromycin(5 μg). The tests were performed according to the guidelines of clinical and Laboratory Standards Institute (CLSI). It also detected the meca gene of MSRA strains. 470 Staphylococcus aureus samples from patients hospitalized in different departments of Ahvaz Hospitals included 283 blood culture samples, 75 wound samples, 72 body fluid samples and 40 catheter samples, and 321 (68.3%) MRSA isolates were reported. All these 321 MRSA isolates were tested with ampicillin, ciprofloxacin, clindamycin, linezolid, gentamicin, erythromycin, and azithromycin antibiotics. Also, the results of molecular identification of the mec A gene in 321 strains of S. aureus showed that 312 strains carry the mec A gene. The high prevalence of S. aureus samples can be caused by long-term hospitalization of patients in the ward and excessive use of antibiotics to treat the infection and increased resistance in isolates. As a result, more monitoring of the hospital’s infection control department, as well as the expansion of the correct use of antibiotics, seems necessary and important.

Keywords: Antibiotic Resistance Pattern, Gram-Positive Cocci, Linezolid Antibiotic, meca Gene, Staphylococcus aureus

1. Introduction

Staphylococcus aureus (S. aureus), gram-positive cocci, facultative anaerobes and opportunistic bacteria, is one of the most common hospital and acquired infections, due to its ability to adapt to different environmental conditions and increasing resistance to antimicrobial drugs, it is considered a pathogen with high concern. Its clinical manifestations are very variable and include bacteremia, pneumonia, osteomyelitis, skin scaling syndrome, toxic shock syndrome, endocarditis, etc [1, 2].

Before the discovery of penicillin, 80% of S. aureus infections led to death. During the 1940s, the death rate caused by this pathogen decreased significantly with the use of penicillin. But the incorrect and excessive use of antibiotics in 1942 and only two years after

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the use of penicillin, the first resistant strain appeared first in the hospital and then in the community, so that currently more than 90% of *S. aureus* strains are resistant to penicillin. This resistance is due to the acquisition of a plasmid that encodes the penicillin hydrolyzing enzyme. Therefore, to deal with this resistance, researchers introduced methicillin, which is a semi-synthetic antibiotic-resistant to this enzyme, for clinical use. In 1961, shortly after the use of methicillin in England, methicillin resistance was also created in methicillin-resistant *S. aureus* (MRSA) strains due to the acquisition of the mecA gene. In this way, MRSA strains spread rapidly in different parts of the world and became one of the most important hospital pathogens [3].

The mecA gene is located on a common chromosome between the genes coding for protein A (spa) and the protein that biosynthesizes purines, and it encodes a 78 KDa protein called (PBP2) [4], which has affinity for binding to methicillin is less than other proteins that bind to penicillin in the bacterial wall. PBP proteins play a role in the construction of the bacterial cell wall. Therefore, the presence of such a new protein will not be affected by the antibiotic and the bacterium can easily continue its life. These resistant isolates can cause various clinical situations varying between superficial infections and serious life-threatening infections. Therefore, *S. aureus* should be frequently isolated from the community and hospital-acquired infections [5].

Hospital-acquired methicillin-resistant *S. aureus* (MRSA) infections are the factors held most responsible for mortality and morbidity in Iran and the world. MRSA can easily be spread from patient to patient through the hands of the staff and can lead to frequent epidemics [6, 7]. MRSA colonization is common in hospitals [8].

Hand hygiene and isolation measures can be preventive for MRSA. These measures have been shown to reduce hospital-acquired MRSA infections. However, effort has been expended for a long time toward preventing hospital-acquired MRSA infections, and effective infection control measures have been put into practice [9]. In sensitive bacteria (lacking the mec-A gene), methicillin binds with greater affinity to the PBP protein in the cell wall, which causes the lysis of the bacterial cell wall, and finally its death.

The mecA gene is located on a mobile genomic island called staphylococcal chromosomal cassette (SCCmec). SCCmec elements are one of the unique genomic islands that contain the mec gene complex [10].

*S. aureus* strains, having this gene, show resistance too many other antibiotics (multidrug resistance), (Cfr). Linezolid antibiotic resistance gene is one of these genes, which was first identified in 2000, Staphylococcus species isolated from animals were reported. The gene of this type of resistance is transmitted through a plasmid. The site of the antibiotic effect of linezolid is on the large ribosomal subunit, and by acting on this part, it prevents bacterial protein synthesis, for the treatment of serious and dangerous infections such as those caused by methicillin-resistant *S. aureus*[11].

The first clinical isolate of methicillin-resistant *S. aureus* that showed resistance to linezolid was introduced in 2007. MRSA isolates are resistant to linezolid, in addition to the Cfr gene, also receive the erm gene from the plasmid, which induces resistance to macrolides, lincosamide, and streptogramin B by rRNA methylase produced by this gene. Therefore, MRSA has become a type of staphylococcus that shows resistance to all antibiotics effective on the large ribosomal subunit and will cause many difficulties and problems in health and treatment [11], such as the CM-05 type that appeared in the year 2007 was reported in Colombia [12].

Therefore, this important fact should be kept in mind that the emergence of linezolid antibiotic resistance creates resistance to all antibiotics effective against *S. aureus* [13]. When multi-drug resistance phenotypes of MRSA strains and intrinsic resistance to beta-lactam antibiotics appear [14]. It causes colonization and spread in the hospital environment, increasing the duration of hospitalization of patients and as a result, increasing treatment costs [15].
The spread of MRSA strains in different types of hospital infections is one of the most important clinical problems, as well as in patients with immune system defects, especially patients with various malignancies, who form a very large group of hospitalized patients for a long time. Infection with bacteremia and as a result death caused by this opportunistic bacterium are due to the reduction of white blood cells and gamma globulins [16].

The prevalence of MRSA strains among hospital isolates of S. aureus has been increasing rapidly in the community and hospitals in recent years. Since the antimicrobial resistance of S. aureus varies from one hospital to another, Therefore, controlling and preventing the spread of this pathogen through the screening of patients and the hospital environment is a major priority in the infection control programs of medical centers, and genotyping of strains can be used as an important tool to identify the genetic characteristics of isolates. They are a very important help for epidemiological investigations in order to trace the source and find the ways of MRSA transmission from the hospital and the community [17].

One of the basic ways to determine the prevalence of MRSA strains is to investigate the spread of specific clones of this bacterium. Therefore, genetic investigation and evolutionary paths of clones in different regions are very important and valuable [17].

In Iran, due to the lack of a monitoring and control system for the spread of drug resistance, it is necessary and important to carry out research to identify the important local patterns of drug resistance and to determine the In the present study, the drug resistance of MRSA strains isolated from different hospital samples to the antibiotic linezolid in Ahvaz city was investigated, and the resistance pattern of this antimicrobial in patients with malignancy and other hospitalized patients was also compared. It will be, the great interest in hospitalized patients due to drug interactions and necessary precautions in the use of this expensive antibiotic.

One of the reasons for the importance of this research is that in southwest Iran, especially Ahvaz metropolis, there has been no research on MRSA resistance to the antibiotic linezolid. This study aims to retrospectively determine the resistance rates of S. aureus strains, isolated from clinical samples in hospitals, against methicillin and other antibiotics and to set out the changes in detail.

2. Materials and Method

2.1. Isolation of S. aureus

In this cross-sectional-analytical method, samples of blood cultures, fluids and wounds of patients were cultured on blood agar and EMB upon arrival to the microbiology laboratory and incubated at 37 degrees for 24 hours and then read if Positive culture, grayish-white colonies were removed from the culture medium for Gram staining, phenotypic tests, catalase, mannitol salt agar, Dnase and tubular coagulase tests. After determining the identity of S. aureus, the samples were placed in TSB medium with glycerol and -70 degrees Celsius, 470 samples of S. aureus were isolated and stocked for 6 months. This research is on S. aureus isolated from patients hospitalized in different departments of hospitals (Imam, Golestan, Razi Abuzar and Baqei) in Ahvaz.

The duration of the research was from the beginning of April to the end of September 2023, from the samples sent and prescribed by the doctor. It was done by an expert.

2.2. Isolation of S. aureus resistant to methicillin

After collecting the required sample size by disc diffusion agar method and based on CLSI for cefoxetine antibiotics (30 micrograms), to isolate the methicillin-resistant S. aureus strain, the antibiogram method was performed on Hilton Muller Agar medium with 470 samples.

A sterile swab or loop was removed from a turbidity equivalent to the McFarland standard turbidity (1.5 x 108 bacterial cells per ml), and cultured, and using sterile tweezers, antibiogram disks were placed on the surface of the environment. And for 24
hours at 37°C. Incubation and halo formation and according to Kirby Baer, it was reported as sensitive, semi-sensitive and resistant.

2.3. Methicillin-resistant S. aureus antibiotic sensitivity and resistance test

After obtaining 76 MRSA isolates, all MRSA isolates were cultured on Muller’s medium to check the resistance to antibiotics ciprofloxacin (5 micrograms), tetracycline (30 micrograms), gentamicin (10 micrograms), linezolid (30 micrograms), clindamycin (2 micrograms), erythromycin (15 micrograms), and ampicillin (5 micrograms) from Patan Teb company. This method is the same as the above method for these different standard antibiograms, half of McFarland was removed, and cultured, and the antibiograms were placed on the surface of the medium, incubated for 24 hours at 37°C and halo formation and according to Kirby-Bauer as sensitive and semi-sensitive. It was reported as resistant. Antimicrobial susceptibility testing was performed by the Kirby-Bauer disk diffusion method and according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) in 2023.

2.4. Detection of mecA by PCR technique

2.4.1. DNA extraction

Parstous kit was used to extract the DNA of methicillin-resistant S. aureus bacteria. The volume of the PCR reaction was considered to be 20 microliters. To perform the PCR reaction, the above compounds were added to a 2 ml microtube. This reaction was carried out in a thermocycler, the number of PCR reaction cycles for the amplification of genes. The materials and compounds used for the PCR reaction were as follows: chemical mixture: Master mix PCR2x (amplicon) 10 µmol, forward primer 0.5 microliter, reverse primer 0.5 microliter, DNA (100 nanograms) 3 microliter, Distilled water 6 microliter. TM for this reaction was 53.5°C.

The number of PCR reaction cycles for gene amplification was 35 cycles, and the reaction steps were considered in the following order: Initial denaturation: temperature of 35°C for 5 minutes, Denaturation: temperature of 94°C for 45 seconds, Connection of primers: temperature of 58°C for 45 seconds, and Final elongation: 72°C for 5 minutes.

Along with the studied samples, a negative control without cDNA was used in each series of PCR reactions. The Pairs of specific primers used in this study were as follows: Primer name: Mec, Sequence: F-GTAGAAATGACTGAACTCGGATAA, R-CAAATTCCACATTGTTGCTCAA, Length 310 (pb) [18].

3. Results

With the arrival of different samples to the microbiology laboratory of each hospital, in the first stages of diagnosis, all samples are cultured on blood agar and EMB media. After identifying the S. aureus isolates, samples were collected from different hospitals. Specifically, from Imam Hospital, there were 25 wound isolates, 59 blood cultures, 27 body fluids, and 9 isolates from catheter swap samples. From Golestan Hospital, there were 24 wound isolates, 70 blood cultures, 16 body fluids, and 9 isolates from catheter samples. Razi Hospital had 26 wound isolates, 41 blood cultures, 19 body fluids, and 11 catheter isolates. Abuzar Hospital had 78 blood culture isolates, 8 body fluids, and 7 catheter swap isolates. Baqai Hospital had 35 blood culture isolates, 2 body fluids, and 4 catheter isolates. Among these samples, the highest number of isolates were found in blood cultures, totaling 283 isolates. Out of the patients, 210 samples were from females and 260 samples were from hospitalized males. The youngest patient was a 3-year-old child with acute lymphoid leukemia, whose catheter sample was confirmed. The oldest patient was 89 years old and was hospitalized due to old age with a positive pleural fluid sample.

Out of 470 isolates of S. aureus, 25.3% isolates were sensitive to methicillin and 68.3% showed resistance to methicillin (Table 1).
Among the 7 investigated antibiotics, the highest resistance to ampicillin was found in Baqai Hospital with 100%, followed by Abuzar Hospital with 96.6%, and the lowest resistance to Linezolid antibiotic was reported in Abuzar and Baqai Hospitals, although no resistance was reported (Table 2).

Out of 321 isolates of *S. aureus* resistant to methicillin in polymerase chain reaction technique and separation of products in agarose gel electrophoresis, 312 (97.2%) isolates had the mec gene and 9 (2.8%) isolates did not have the gene. Statistical analysis by Chi-square test revealed the absence of significance between individual mec type (*p* value<0.0018)(Figures 1 and 2).

Out of 321 isolates resistant to methicillin, 195 (60.74%) isolates showed resistance to more than 3 antibiotics (Table 3).

**Table 1.** The pattern of resistance and sensitivity of *S. aureus* to the antibiotic cefoxitin

<table>
<thead>
<tr>
<th>Hospital</th>
<th>Antibiotic</th>
<th>Cefoxitin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitive</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Imam</td>
<td>35(29.2%)</td>
<td>9(7.5%)</td>
</tr>
<tr>
<td>Golestan</td>
<td>29(24.4%)</td>
<td>10(8.4%)</td>
</tr>
<tr>
<td>Razi</td>
<td>17(17.5%)</td>
<td>7(7.2%)</td>
</tr>
<tr>
<td>Abodhar</td>
<td>32(34%)</td>
<td>2(2%)</td>
</tr>
<tr>
<td>Bagai</td>
<td>6(14.6%)</td>
<td>2(4.9%)</td>
</tr>
<tr>
<td>Total</td>
<td>119(25.3%)</td>
<td>30(6.3%)</td>
</tr>
</tbody>
</table>

**Table 2.** MRSA resistance pattern to different antibiotics by hospital

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Erythromycin</th>
<th>Ciprofloxacin</th>
<th>Clindamycin</th>
<th>Linezolid</th>
<th>Gentamycin</th>
<th>Azithromycin</th>
<th>Ampicillin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imam</td>
<td>61(80.2%)</td>
<td>34(44.7%)</td>
<td>41(53.9%)</td>
<td>3(3.9%)</td>
<td>43(56.6%)</td>
<td>17(22.4%)</td>
<td>59(77.6%)</td>
</tr>
<tr>
<td>Golestan</td>
<td>54(67.5%)</td>
<td>40(50%)</td>
<td>43(53.75%)</td>
<td>4(5%)</td>
<td>40(50%)</td>
<td>21 (26.2%)</td>
<td>62 (77.5%)</td>
</tr>
<tr>
<td>Razi</td>
<td>48(65.7%)</td>
<td>40(54.8%)</td>
<td>31(42.6%)</td>
<td>3(4.1%)</td>
<td>29(39.7%)</td>
<td>13(17.8%)</td>
<td>56(76.7%)</td>
</tr>
<tr>
<td>Abodhar</td>
<td>43(72.9%)</td>
<td>27(45.9%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4(6.8%)</td>
<td>57(96.6%)</td>
</tr>
<tr>
<td>Bagai</td>
<td>27(81.8%)</td>
<td>13(39.4%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>15(45.5%)</td>
<td>33(100%)</td>
</tr>
</tbody>
</table>

**Fig. 1.** mec gene electrophoresis. Number 1 to 12 of *S. aureus* isolates resistant to methicillin, M; DNA Size Marker (FKN-02-420), 100 bp Ladders (100-1,500 bp)

**Fig. 2.** Abundance of mec gene typing in patients of different hospitals
Table 1. Multidrug resistance pattern of MRSA by isolate type

<table>
<thead>
<tr>
<th>Concomitant drug resistance, number of isolates</th>
<th>Blood</th>
<th>Wound</th>
<th>Fluid</th>
<th>Catheter</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 antibiotics</td>
<td>2 (0.6%)</td>
<td>1 (0.3%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6 antibiotics</td>
<td>43 (13.4%)</td>
<td>21 (6.5%)</td>
<td>11 (3.4%)</td>
<td>7 (2.2%)</td>
</tr>
<tr>
<td>5 antibiotics</td>
<td>27 (8.4%)</td>
<td>13 (4.04%)</td>
<td>7 (2.2%)</td>
<td>6 (1.9%)</td>
</tr>
<tr>
<td>4 antibiotics</td>
<td>33 (10.3%)</td>
<td>17 (5.3%)</td>
<td>8 (2.5%)</td>
<td>5 (1.5%)</td>
</tr>
<tr>
<td>4 antibiotics</td>
<td>19 (6%)</td>
<td>11 (3.4%)</td>
<td>4 (1.24%)</td>
<td>4 (1.24%)</td>
</tr>
<tr>
<td>3 antibiotics</td>
<td>39 (12.1%)</td>
<td>27 (8.4%)</td>
<td>9 (2.8%)</td>
<td>3 (1%)</td>
</tr>
<tr>
<td>3 antibiotics</td>
<td>12 (3.7%)</td>
<td>6 (1.9%)</td>
<td>5 (1.5%)</td>
<td>-</td>
</tr>
<tr>
<td>3 antibiotics</td>
<td>10 (3.1%)</td>
<td>4 (1.24%)</td>
<td>1 (0.3%)</td>
<td>3 (1%)</td>
</tr>
</tbody>
</table>

4. Discussion

After *Escherichia coli*, *S. aureus* is considered as the second cause of hospital infections, among which *S. aureus* resistant to methicillin is one of the most famous and common among them. The colonization of these bacteria in the nose and hands of nurses and hospital workers. Also, the personnel of special care units are considered the most common sources of contaminating isolates of hospitalized patients [19, 20].

In recent years, high mortality has been reported in connection with them in different geographical areas. One of the major problems in the treatment and prevention of infections caused by *S. aureus* is the resistance of this bacterium to various antibiotics such as beta-lactams, aminoglycosides, macrolides, etc., which causes the spread of infections caused by this bacteria and also the occurrence of problems such as increasing the number of injuries to hospitalized patients, increasing treatment costs due to the need for expensive antibiotics, increasing the duration of hospitalization, and most importantly, increasing the death rate, which is the problem of doctors that Has faced many limitations for the treatment of *S. aureus* infections [21, 22].

One of the important reasons for the wide development of the resistance of this bacterium against a wide range of antibiotics is related to the mobile genetic elements that transfer resistance among bacterial populations. Mainly, this transfer through four main mechanisms, namely transformation, conjugation by phage and transduction, is considered a therapeutic problem in medical centers, especially hospitals. The simultaneous presence of PBP gene mutations causing MRSA resistance phenotypic occurrence in *S. aureus* strains along with transferable gene cassettes encoding drug resistance genes to other therapeutic drugs (MDR) is another newly emerging problem in these bacteria. The recent discovery of a mechanism of linezolid resistance based on acquisition of a natural and potentially transferable resistance gene that modifies a specific rRNA nucleotide located in the site of the drug action is of particular concern and could completely change the picture of linezolid susceptibility in the future. This gene is apparently associated with mobile genetic elements which raises the possibility of its
transmission both intra-species and to other pathogenic strains [23-25].

In the current study, 470 isolates of S. aureus were isolated from clinical samples of patients hospitalized in different departments of Ahvaz Hospitals. And after determining the identity with phenotypic tests, they were kept at -70°C. S. aureus isolates were taken to cefoxetine antibioticogram on Mueller Hinton agar medium and 321 isolates resistant to cefoxetine (methicillin) were reported. For all 321 MRSA isolates, 7 antibiotics were used to check multidrug resistance, and 195 isolates had resistance to more than 3 antibiotics. Out of 321 MRSA isolates in the study of mec gene, 312 had this gene and Statistical analysis by Chi-square test revealed the absence of significance between individual mec types (pvalue<0.0018). According to the comparison of antibiotic resistance patterns among MRSA isolates from teaching hospitals [26, 27].

Reports and research conducted in Iran and other parts of the world show a significant increase in the prevalence of MRSA and the development of multiple drug resistance in it. As a result, the speed of diagnosis of MRSA-related infection in clinical samples isolated from hospitals is very important in improving, and reducing the cost of treatment and the duration of hospitalization of patients, as well as the existence of resistance to the important antibiotic linezolid as a last resort treatment, is an alarm. It is considered a serious risk. The remarkable increase in the prevalence of methicillin-resistant S. aureus and the development of multidrug resistance in these strains, in Iran and the world, shows that the rate of the development of resistance is somehow dependent on the use of antibiotics [28-30].

It has been reported that out of 150 isolates of S. aureus, 55 MRSA samples were determined by disk diffusion method, and the obtained nucleotide sequence was confirmed by the PCR technique of the mecA gene in all isolates [34]. In terms of examining mec gene is consistent with the current study. It has been reported that in Nigeria isolated 81 samples of S. aureus from the samples sent to the laboratory, and in the disk diffusion agar method, using oxacillin antibiogram, it has been obtained 46.9% of methicillin-resistant S. aureus. Also, in the PCR technique, the cfr gene (resistance to linezolid) was reported by 17.4% [35]. This study is consistent in terms of technique, antibiogram method and linezolid antibiotic resistance. Mendes and
colleagues reported only 8 cases of linezolid-resistant staph aureus in the United States until 2008 [36]. In addition, in the same year, Hentschecki and his colleagues reported 2 strains resistant to linezolid in Germany [37], one case in England [38], one case in Brazil [39], comparing the studies done with the present research that linezolid antibiotic can still be used as an important antibiotic in the treatment of infections of this microorganism despite the increase in the resistance of S. aureus strains resistant to methicillin. Therefore, each hospital should not use this antibiotic without having a resistance pattern.

5. Conclusion

The prevalence of MRSA can result from long-term hospitalization of patients and overdosing on antibiotics to more effectively treat infections. As a result, monitoring and developing safe and effective infection control practices in these sectors are of great importance.

Conflict of Interests

All authors declare no conflict of interest.

Ethics approval and consent to participate

This research effectively adhered to the tenets mentioned under the Declaration of Helsinki. The authors have adhered to ethical standards, including avoiding plagiarism, data fabrication, and double publication.

Consent for publication

All authors read and approved the final manuscript for publication.

Informed Consent

The authors declare not used any patients in this research.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors’ contributions

Methodology: Elaha Tajbakhsh.

Project administration: Elaha Tajbakhsh.

Resources: All authors.

Supervision: Elaha Tajbakhsh.

Validation: Sonia Razmjou.

Visualization: Hassan Mottaz.

Writing—original draft: All authors.

Writing—reviewing & editing: All authors.

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