TRACKING BLA_{SPM} AND BLA_{NDM} GENES IN CLINICAL ISOLATES OF MBL-PRODUCING PSEUDOMONAS AERUGINOSA AND DETERMINATION OF ANTIBIOTIC RESISTANCE PATTERN IN BURN SAMPLES IN KERMAN (2018)

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ABSTRACT

Class B beta-lactamases, like NDM and SPM, are among the most important factors in the development of resistance to gram-negative bacteria, including Pseudomonas aeruginosa. Due to the resistance of this group of beta-lactamases to beta-lactam inhibitors. They are clinically significant in this bacterium. Therefore, in this research, we investigated the prevalence of NDM and SPM beta-lactamase genes in Pseudomonas aeruginosa isolates of burn wounds. In this descriptive cross-sectional study, we collected and cultured 125 burn wound samples from patients admitted to educational hospitals. We identified the colonies suspected of Pseudomonas aeruginosa by routine biochemical methods. We used Kirby Bauer method to measure antibiotic susceptibility; we evaluated the MIC of these strains against ceftazidime by E.test method. Then, The above enzymes using a hybrid disk. Genotypically, we analyzed NDM and SPM beta-lactamase genes in the above strains by PCR. In this study, out of 125 Pseudomonas aeruginosa isolates, 32 (26%) were extended spectrum beta-lactamase-producing isolates. Totally, the Pseudomonas aeruginosa resistance to the antibiotics imipenem, piperacillin, ceftazidime, cefotaxime, ceftriaxone, levofloxacin, ciprofloxacin, gentamicin, amikacin, streptomycin, tetracycline and Carbenicillin was respectively 88%, 93%, 89%, 98%, 97%, 89%, 84%, 82%, 97%, 91%, 90% and 95%. According to PCR test, among 32 isolates with extended spectrum beta-lactamase enzyme, 28.12% had SPM and 43.75% had NDM beta-lactamase genes. Given the prevalence of antibiotic resistance by mechanism. Diverse in high teaching hospitals need actions such as asmeasuring antibiotic susceptibility, rational prescribing of antibiotics, and controlling contributing factors. The results show that most of the samples are drug resistant and among the MBL producing strains, the frequency of SPM gene was higher than NDM.

Keywords: MBL, Pseudomonas aeruginosa, Antibiotic resistance, NDM, SPM

I. INTRODUCTION

Pseudomonas aeruginosa is one of the most important organisms that causes disease in humans and animals (Estahbanatani et al., 2002). It is a non-fermentative gram-negative bacterium and plays a major role in causing opportunistic infections and nosocomial infections. It is a cause of 10 to 15% of nosocomial infections worldwide. In people with burns, they are susceptible to this bacterium due to the loss of the skin's defense level and a severe reduction in the strength of the body's defense system (Hsueh et al., 1998). The high resistance of this bacterium to antimicrobial substances such as antibiotics has made the treatment of infections caused by this bacterium more complicated and has turned it into one of the major medical problems. The mechanism of resistance can be intrinsic or acquired (Pedersen et al., 1990).

Due to its resistance to many drugs and disinfectants, this bacterium has grown in some consumedsststances (alcohol, dilute betadine, etc.) used to disinfect patients; using them is the main sources of contamination and
infections by this bacterium. Swimming pools are also the most important source for the growth of *Pseudomonas aeruginosa* due to the favorable conditions for the growth of this bacterium, including water, heat, aeration and human pollution. More than 7% of healthy humans carry the bacterium in their nasal, skin, or throat mucosa and 24% have it in their stool samples (Driscoll et al., 2007; Haidari & Akya, 2015).

*Pseudomonas aeruginosa* has numerous factors that lead to increased adhesion to host cells, damage to host tissues, inflammation, and impaired defense mechanisms. Problems of high prevalence, severity of infection and resistance of *Pseudomonas aeruginosa* to antimicrobial therapy are important. Although the bacterium is widely known, the mortality rate for people with the bacterium is about 50%. The incubation period of the disease is usually between 24 and 72 hours (Lyczak et al., 2000; Rumbaugh et al., 2000).

*Pseudomonas aeruginosa* is inherently resistant to many antibiotics, which is why it is a resistant bacterium and is a very dangerous and terrifying pathogen. When the natural flora is destroyed or the body's immune system becomes inadequate or dysfunctional, it becomes more important and causes a local or general infection. However, the following therapies can be usable (Levin et al., 1999):

1. Mixture of gentamicin and carbolicillin, piperacillin or ticaricillin
2. Mixture of tobramycin and carbolicillin, piperacillin or ticaricillin
3. Third and fourth generation cephalosporins or new quinolones

Gentamicin and carbolicillin are mostly used to treat severe infections. The best antibiotic that we can introduce according to bacteriological studies to treat *Pseudomonas* infections, especially critical ones, is imipenem. Daily inhalation of 300 mg tobramycin (in the absence of drug resistance) is recommendable for further treatment. A small number of antibiotics are effective against *Pseudomonas*, including fluoroquinolones, gentamicins, imipenem, and even not all of these antibiotics are of effect on all methods. Polymyxin E and carbapenems are the most effective antibiotics against resistant multidrug isolates (Rossolini & Mantengoli, 2005).

Treating patients and choosing the right antibiotic is difficult for them because this bacterium is resistant to different types of antibiotics by different mechanisms. In addition, it is inherently resistant to many antibiotics, so in patients it can lead to death. The highest acquired resistance in *Pseudomonas aeruginosa* concerns beta-lactams, carbapenems and fluoroquinolones. In patients admitted to the ICU and burn hospital, *Pseudomonas aeruginosa* is more resistant to common antibiotics (Lee et al., 2005). Since antibiotic resistance genes can be transmitted between bacteria through motile genetic elements such as plasmids, integrons and transposons, the occurrence of drug resistance is of particular importance. It is important to be aware of the pattern of antibiotic resistance among pathogens. These strains transmit resistance factors to other strains and even susceptible species of bacteria and develop an exponential population of resistant bacteria. Due to the importance of beta-lactamase-producing strains in hospitals, rapid identification and tracking of these strains can be an important and fundamental step in treating and controlling the infection caused by these strains.

One thing that makes the *Pseudomonas aeruginosa* resistant to beta-lactams is to produce the enzyme beta-lactamase, which has caused many problems in the treatment of infections caused by this bacterium. Among these beta-lactamases are the beta-lactamases derived from the blaNDM and blaSPM genes. Determining the frequency of these genes in the population can be very effective in controlling the pattern of resistance (Shahcheraghi et al., 2009).

Fatemeh Akhavan Tafti et al. (2012) conducted a study at the Burn Injury Hospital in Yazd under the title *a phenotypic study of Pseudomonas aeruginosa beta-lactamase and metallo-β-lactamase enzymes isolated from burn wounds*. According to the results of 180 cultured samples, 54 isolates were identified as *Pseudomonas aeruginosa*, of which 22% (12 samples) had ESBL and 29.5% (16 samples) had MBL. 42 (79%), 40 (74%), 38 (70%), 35 (66%) and 34 isolates (62%) were resistant to ceftizoxime, imipenem, gentamicin, piperacillin, cefpirome, meropenem and Ertapenem, respectively (Tafti et al., 2014).

Neha et al. (2014) in India conducted a study entitled *the presence of ESBL-producing shv, tem, ctx-m genes in isolated strains* at Mangolar Hospital. 75 samples of Enterobacteriaceae family that were isolated after determination of *Pseudomonas* and Acinetobacter phenotype and after determination of specific primer by PCR showed that genes encoding beta-lactamase and metallo-β-lactamase are transferable in hospitals (Neha & Shenoy, 2014).
Dr. Bekaian et al. (2013) conducted a study at Imam Ali (AS) Educational Hospital in Zahedan under the title of examining the frequency of per, veb, shv, tem, ctx-m genes in resistance to Pseudomonas aeruginosa producing extended spectrum beta-lactamase (ESBL). In the results, ciprofloxacin and piperacillin had the greatest effect against Pseudomonas aeruginosa. The results show that 19 isolates (16.37%) are multidrug resistant (MDR) and 8 isolates (6.89%) are ESBL positive. Out of 116 isolates, 30 isolates (25.86%) had the lowest resistance to the antibiotics ceftazidime, ceftriaxone, cefotaxime and aztronom. Among them 30 (100%), 4 (3.13), 2 (6.6), 2 (6.6) are the boosters of beta-lactamases tem, veb, per, shv, respectively. Out of 30 isolated samples, 22 ESBL isolates are negative. According to the results obtained from 116 samples of Pseudomonas aeruginosa, the highest amount of ESBL isolates was about beta-lactamase tem gene (Bokaeian et al., 2015).

Due to the many health problems and the vast economic losses that this bacterium causes in terms of Manpower disability in the country, it seems that any study and research in this regard can be useful for controlling and preventing the disease. Antibiotic resistances occur during treatment. They are not isolated by conventional methods. They show a high prevalence of resistance-generating genes and relatively high phenotypic expression of gene-producing genes. They need to be identified by specific methods to control disease and infection. Molecular epidemiological analysis of drug-resistant strains is very important. Rapid detection of isolates with antibiotic resistance is necessary to make therapeutic and managerial decisions. Since no research existed in Kerman on the frequency of blaNDM and blaSPM genes in Pseudomonas aeruginosa isolated from burn wounds, so in this research, we studied the frequency of these genes for the first time in Kerman.

II. MATERIALS AND METHODS

This is a descriptive cross-sectional study. Sampling is at convenience. The population under study was Pseudomonas aeruginosa bacteria isolated from burn wound samples of patients admitted to the burn ward of Kerman educational hospitals. We collected Pseudomonas aeruginosa isolates from burn wound samples of patients admitted to the burn ward; they were transferred to the microbiological laboratory for further investigation to determine the genus and species. We estimated the sample size according to different studies and based on the percentage of frequency of reported genes and 95% confidence interval of 125 Pseudomonas aeruginosa isolates.

The antibiotic discs and E-test strips used to determine the pattern of antimicrobial susceptibility and the minimum inhibitory concentration of the isolates under study are as follows:

**Antibiotic disc**

- Imipenem (10μg)
- Meropenem (10μg)
- Ceftazidime (30μg)
- Cefepime (30μg)
- Cefotaxime (30 μg)
- Ceftriaxone (30μg)
- Ciprofloxacin (5 μg)
- Gentamicin (10μg)
- Ticarcillin / clavulanic acid (10.75 μg)
- Ceftazidime / clavulanic acid (75/30 μg)
- Cefotaxime / clavulanic acid (75/30 μg)

**E-test Seftazidim**

We received the primers used by the ordering company as lyophilized. For preparation at a final concentration of 100 pmol/μl, we added sterile injected distilled water to each vial according to the manufacturer's instructions.
To prepare a working primer, we mixed 4 μl of each Forward and Reverse primer with 92 μl of injected distilled water to bring the final concentration to 8 pmol/μl. We then stored these primers in a -20 °C freezer.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence 3'-5'</th>
<th>Gene</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPM-F</td>
<td>TCAGCGAAAAACACCTTG</td>
<td>472bp</td>
<td>Liu et al., 2015(</td>
</tr>
<tr>
<td>SPM-R</td>
<td>TCCCGCAGATAAATCACC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NDM-F</td>
<td>CTTCTCTTTTTGCTCACC</td>
<td>636bp</td>
<td>Neyestanaki et al., 2014(</td>
</tr>
<tr>
<td>NDM-R</td>
<td>AGCAATAAACCAGCCAGC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Isolation and identification of the genus *Pseudomonas aeruginosa*

During a period of 6 months, from June 2018 to the end of December 2018, referring to the educational hospitals of Kerman, we collected the isolates suspected of *Pseudomonas aeruginosa* burn wound samples. After completing the questionnaire form, we transferred the isolates to Kerman Microbiological Laboratory; after reculture on McConkey agar medium and incubation at 35 °C for 24 hours, we identified the suspicious isolates using standard biochemical tests such as oxidase, culture on TSI medium, Simon citrate, Motion test in SIM medium and OF medium. *Pseudomonas aeruginosa* is a gram-negative, non-fermenting lactose, positive and mobile oxidase. We performed the following steps to identify the phenotype of the isolates.

**OXIDASE TEST**

Using a wooden handle, we take a sterile cotton swab from a single-grown colony on McConkey agar medium and transfer it to wet filter paper to the oxidase reagent. If we observe a color change to dark purple up to 10 seconds later, we consider the test result positive and include it in the study for further tests. We used *Pseudomonas aeruginosa* ATCC 27853 as positive control and *Acinetobacter baumannii* ATCC 19606 as negative control.

Tube biochemical tests of TSI, OF, SIM and citrate

From pure oxidase-positive colonies, we take some bacteria and inoculate it into tubular media, and after incubation at 35 °C for 24 hours, we analyzed the results.

**Measurement of sensitivity to antibiotics**

In this study, we determined antibiotic susceptibility to 13 antibiotics from different groups by disk diffusion (Kirby-Bauer) method. In this method, we used CLSI criteria (Dudley et al., 2013).

We determined the pattern of antibiotic susceptibility of *Pseudomonas aeruginosa* isolates by disk diffusion method. We measured the results in terms of diameter of the growth inhibition zone by a ruler in millimeters and reported as sensitive, semi-sensitive and resistant according to CLSI standard criteria (Dudley et al., 2013). We used the standard strain of *Pseudomonas aeruginosa* ATCC 27853 for quality control.

**Phenotypic confirmation test for the determination of extended spectrum beta-lactamases by the combined disk method:** To confirm the production of beta-lactamase by the Combination Disk method, we used cefotaxime (30μg), cefotaxime-clavulanic acid (10.30 μg), ceftazidime (30μg) and Ceftazidime-clavulanic acid (10.30 μg) (MAST, UK). For control, we used standard strain of Escherichia coli ATCC 35218.

We did the lowest inhibitory concentration (MIC) of ceftazidime by E.test method.

The method used to extract genomic DNA in this study was the salting out method.

Quantitative and qualitative analysis of the extracted DNA: We used respectively spectrophotometry and agarose gel electrophoresis to evaluate the concentration, purity and quality of the extracted DNA.

Amplification of blaNDM and blaSPM genes was performed by PCR and simple polymerase chain reaction method.
III. RESULTS

In this study, we isolated 125 isolates of *Pseudomonas aeruginosa* from burn wound samples. Out of 125 samples under study, 73 samples (58%) were about men and (42%) 52 samples were about women.

Antimicrobial susceptibility test results

We performed the antibiotic susceptibility measurement by disk diffusion method on 125 isolates of *Pseudomonas aeruginosa*. In total, the resistance of *Pseudomonas aeruginosa* isolates to antibiotics of imipenem, piperacillin, ceftazidime, cefotaxime, ceftriaxone, levofloxacin, Ciprofloxacin, gentamicin, amikacin, streptomycin, tetracycline and carbonicillin were 88%, 93%, 89%, 98%, 97%, 89%, 84%, 82%, 97%, 91%, 90% and 95%, respectively (Table 2).

![Figure 1: Antimicrobial resistance pattern of *Pseudomonas aeruginosa* in one of the isolates under study](image)

**Table 2: Sensitivity of *Pseudomonas aeruginosa* isolates to antibiotics examined by disk diffusion method**

<table>
<thead>
<tr>
<th>Antibiotic (µg)</th>
<th>Sensitive Number (%)</th>
<th>Mediating limit Number (%)</th>
<th>Resistant Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imipenem (10)</td>
<td>9(7%)</td>
<td>3(3%)</td>
<td>113(88%)</td>
</tr>
<tr>
<td>Piperacillin (100)</td>
<td>3(3%)</td>
<td>4(4%)</td>
<td>118(93%)</td>
</tr>
<tr>
<td>Ceftazidime (30)</td>
<td>9(7%)</td>
<td>2(2%)</td>
<td>114(89%)</td>
</tr>
<tr>
<td>Cefotaxime (30)</td>
<td>1(1%)</td>
<td>1(1%)</td>
<td>123(98%)</td>
</tr>
<tr>
<td>Ceftriaxone (30)</td>
<td>-</td>
<td>3(3%)</td>
<td>122(97%)</td>
</tr>
<tr>
<td>Levofloxacin (5)</td>
<td>6(5%)</td>
<td>5(4%)</td>
<td>114(89%)</td>
</tr>
<tr>
<td>Ciprofloxacin (5)</td>
<td>17(14%)</td>
<td>2(2%)</td>
<td>107(84%)</td>
</tr>
<tr>
<td>Gentamicin (10)</td>
<td>19(15%)</td>
<td>3(3%)</td>
<td>103(82%)</td>
</tr>
<tr>
<td>Amikacin (30)</td>
<td>-</td>
<td>4(3%)</td>
<td>121(97%)</td>
</tr>
<tr>
<td>Streptomycin (10)</td>
<td>8(6%)</td>
<td>4(3%)</td>
<td>113(91%)</td>
</tr>
<tr>
<td>Tetracycline (30)</td>
<td>8(6%)</td>
<td>5(4%)</td>
<td>112(90%)</td>
</tr>
<tr>
<td>Carbonicillin (100)</td>
<td>2(2%)</td>
<td>4(3%)</td>
<td>119(95%)</td>
</tr>
</tbody>
</table>

According to the 2015 CLSI protocol, if MIC ≤8 mg/ml, we consider it as ceftazidime sensitive and if MIC ≥32 mg / ml we consider it as ceftazidime resistant.

![Figure 1](image)

**Determination of the Minimum inhibitory concentration (MIC) of ceftazidime**

We determined the Minimum inhibitory concentration (MIC) of ceftazidime antibiotic for 125 *Pseudomonas aeruginosa* isolates by E-test and presented the results in Table 3.

**Table 3: Determination of MIC of ceftazidime antibiotic in 125 isolates of *Pseudomonas aeruginosa***

<table>
<thead>
<tr>
<th>Minimum inhibitory concentration (µg/ml)</th>
<th>Sensitive</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-8</td>
<td>&gt;8-&lt;32</td>
<td>≥32</td>
</tr>
<tr>
<td>Number</td>
<td>14</td>
<td>4</td>
</tr>
<tr>
<td>Percentage</td>
<td>14%</td>
<td>4%</td>
</tr>
</tbody>
</table>

According to the 2015 CLSI protocol, if MIC ≤8 mg/ml, we consider it as ceftazidime sensitive and if MIC ≥32 mg / ml we consider it as ceftazidime resistant.
Results of phenotypic study of the presence of extended spectrum beta-lactamases

Considering that 98% of the samples were resistant to at least one of the third generation cephalosporins, we selected all samples for the combined disk confirmation test. Of the 125 isolates under study, 32 isolates (26%) produced beta-lactamase enzymes (Table 4).

Table 4: Determination of the frequency of *Pseudomonas aeruginosa* isolates producing MBL

<table>
<thead>
<tr>
<th></th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (%)</td>
<td>32 (26%)</td>
<td>93 (74%)</td>
<td>125 (100%)</td>
</tr>
</tbody>
</table>

Quantitative and qualitative examination results of extracted DNA

The quality of DNA extracted by 0.8% agarose gel method was confirmed (Figure 4). The quantity of extracted DNA was evaluated by spectrophotometry. The OD ratio was 260 to 280 for the samples (1.6±0.2) and the DNA concentration was estimated to be 50±5 on average.
Results of frequency distribution of bla_{NDM} and bla_{SPM} genotypes producing beta-lactamases in *Pseudomonas aeruginosa* isolates

As shown in Table 5, the frequency distributions of bla_{SPM} and bla_{NDM} genes in *Pseudomonas aeruginosa* isolates are 28.12% and 43.75%, respectively.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Positive Number (%)</th>
<th>Negative Number (%)</th>
<th>Total Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>bla_{SPM}</td>
<td>9(28/12%)</td>
<td>23(71/87%)</td>
<td>32(100%)</td>
</tr>
<tr>
<td>bla_{NDM}</td>
<td>14(43/75%)</td>
<td>18(56/25%)</td>
<td>32(100%)</td>
</tr>
</tbody>
</table>

Results of regional amplification of target genes using specific primers

We have given the results of regional amplification of the genes under study using the polymerase chain reaction technique in Figure 4-5. To control and compare the specificity of the obtained bands along with each of the genes, we used a standard sample with the specified sequence.

IV. DISCUSSION

Burn infections as a nosocomial infection are an important factor in patient mortality and post-burn disability. *Pseudomonasaeruginosa* is a non-fermenting gram-negative bacterium that plays a major role in causing
opportunistic infections and severe infections in burn patients and is a cause of 10 to 15% of nosocomial infections worldwide. According to studies, it is clear that the prevalence of different patterns of drug resistance in Pseudomonas aeruginosa strains, from one country to another, from one geographical area to another, and even among different hospitals, can be different. So due to the clinical importance of strains produced with multidrug resistance in different hospitals, it is necessary to identify these strains in order to treat them and control their spread in hospitals as much as possible. Due to its genetic nature, Pseudomonas aeruginosa has a wide range of antibiotic resistance, accepting a variety of genes (such as plasmids and transposons) and a variety of mutations, and can therefore become rapidly resistant to a variety of antibiotics. Improper use of antibiotics, especially fluoroquinolones and carbapenems, is a risk factor for the bacterium to become resistant to these drugs (Rossolini & Mantengoli, 2005). Therefore, due to the high ability of this organism to acquire resistance to various antibiotics, continuous monitoring of susceptibility changes in this bacterium is essential (Church et al., 2006). In this study, we investigated the frequency of blaNDM and blaSPM genes in Beta-lactamase producing Pseudomonas aeruginosa isolates and the determination of antibiotic resistance pattern in burn wound samples.

In this study, out of 125 isolates of Pseudomonas aeruginosa isolated from burn wounds, the resistance of antibiotics imipenem, piperacillin, ceftazidime, cefotaxime, ceftriaxone, levofloxacin, ciprofloxacin, gentamicin, amikacin, streptomycin, respectively was 88%, 93%, 89%, 98%, 97%, 89%, 84%, 82%, 97%, 91%, 90%, 95%.

Mirsalehian et al. (2011) conducted a study on 170 isolates of Pseudomonas aeruginosa isolated from burn wound. The highest resistance to the antibiotics was for aminoglycoside class (amikacin 81%, gentamicin 88%, tobramycin was 84%). 52.9% of the isolates were resistant to imipenem; while in the present study, 88% of the isolates were resistant to imipenem and 93% to meropenem, which could indicate an increase in carbapenem resistance.

Fatemeh Akhavan Tafti et al. (2014) performed a study in the burn hospital in Yazd on 54 Pseudomonas aeruginosa isolates of burn wounds. According to the reports, the resistance to ceftizoxime, imipenem, gentamicin, piperacillin, cefpime, meropenem, and ertapenem were 79%, 74%, 74%, 70%, 66%, and 62%, respectively.

In a study conducted by Masoumeh Kiani et al. (2015) in Yazd, from among 90 isolates of Pseudomonas aeruginosa isolated from different clinical samples, 56, 50.6, 44.4, 48.9, 52 52, 50.7% of isolates were resistant to the antibiotics ceftazidime, ceftrixon, ciprofloxacin, imipenem, meropenem, ertapenem and gentamicin, respectively. In this study, 66.6% of isolates were resistant to several drugs. The highest number of samples in this study was from the burn section (40% of isolates) and it seems that the results are closer to those of present study.

Maryam Adabi et al. (2015) performed a study in Motahhari Hospital in Tehran on 94 isolates of Pseudomonas aeruginosa isolated from burn wounds. According to the reports, the resistance to imipenem, cefpime, ticarcillin, aztronam, tobrumycin, gentamicin, cleistine, ciprofloxacin, amikacin and piperacillin tazobactam was respectively 76%, 90%, 87%, 77%, 88%, 86%, 0%, 87%, 85% and 78%. In this study, the highest percentage of resistance is for cefpime (90%) and the lowest for cleistine (0%).

Ranjbar et al. (2011) conducted a study in Baqiyatallah Hospital of Tehran on Pseudomonas aeruginosa isolated from burn wound. Resistance to ceftazidime, amikacin, ciprofloxacin, gentamicin and imipenem was 57.5%, 90%, 65%, 67.5% and 97.5%, respectively.

The results of the resistance pattern of the isolates obtained in this study show the high resistance of the strains against the antibiotics under consideration. A brief look at previous studies suggests that resistance to different antibiotics for Pseudomonas aeruginosa isolates is relatively high; its results are different depending on the time and place of strain isolation. On the other hand, these patterns of resistance are constantly changing that we must take into account.

In this study, out of 125 Pseudomonas aeruginosa isolates of burn wounds, 26% of the isolates produced MBL.

In the study of Shojapour et al. (2011) in Shahrekord, conducted on Pseudomonas aeruginosa samples isolated from burn wound patients, 37% of the samples were ESBL-producing.

In the study of Tafti et al. (2014) in Yazd, conducted on Pseudomonas aeruginosa isolates of burn wounds, 22% of isolates were ESBL-producing.
Mana Shojapour et al. (2011) conducted a study in Arak on Pseudomonas aeruginosa isolated from burn wound patients; 37.7% of them had ESBL.

In the study of Zahra Farshadzadeh et al. (2014) in the burn hospital in Ahvaz, conducted on Pseudomonas aeruginosa isolated from burn patients, 51.9% were ESBL-producing.

Okesola's study (Okesola & Oni, 2012) examined 90 Pseudomonas aeruginosa isolates obtained from various hospital wards in Nigeria. In this study, 22.2% of isolates were ESBL-producing. In a study conducted in India (2011), 42.3% of Pseudomonas aeruginosa isolates were ESBL-producing (Goel et al., 2013).

The results confirm the fact that in our country, due to the excessive use of beta-lactam drugs, especially cephalosporins, the frequency of ESBLs enzymes in patients admitted to burn wards is unfortunately higher than other especially developed countries. Therefore, it is necessary to take some measures to prevent the spread of ESBLs isolates in hospitals.

In the present study, the frequency of bla<sub>SPM</sub> and bla<sub>NDM</sub> genes was 28.12% and 43.75%, respectively.

In the study of Zahra Farshadzadeh et al. (2014) conducted in the burn accidents hospital in Ahvaz on samples of Pseudomonas aeruginosa isolated from burn wounds, the frequency of CTX-M gene was 1.04%.

In the study of Heidari et al. (2015) in Kermanshah, carried out on Pseudomonas aeruginosa isolated from burn wounds, the frequency of NDM gene was 21.6%.

In the study of Dr. Bokaeian et al. (2015) in Imam Ali (AS) Educational Hospital in Zahedan, conducted on Pseudomonas aeruginosa isolated from burn wound samples, the frequency of TEM and SHV genes were 100% and 6.6% respectively.

During the studies conducted in recent years in Iran, the prevalence of MBL enzymes, especially NDM and SPM, has increased. The frequency of NDM and SPM beta-lactamase enzymes in the current study was 43.75% and 28.12%, respectively. This could be a justification for the high resistance of isolates to third-generation cephalosporins.

The results of the present study and those of previous studies show that the frequency of beta-lactamase genes has increased over many years. The frequency of beta-lactamases was first abundant in the Enterobacteriaceae family and then transmitted to other bacteria, including Pseudomonas aeruginosa, through various routes.

### V. CONCLUSION

According to the results of the present study, the resistance to antibiotics used in the treatment of patients with infections caused by Pseudomonas aeruginosa, especially Cephalosporins and carbapenems, is expanding in our country. In this study, more than 80% of Pseudomonas aeruginosa isolates were resistant to ceftazidime, cefpirome, ceftaraxone, cefotaxime, ticarcillin and the prevalence of Pseudomonas aeruginosa producing MBL was 26%. This indicates an increase in the prevalence of these resistance enzymes. Therefore, in burn wards, measuring antibiotic susceptibility and examining the production of MBL-producing Pseudomonas aeruginosa strains seems necessary before drug administration. In the present study, we reported the expression of NDM and SPM genes; it showed that the SPM gene was higher than the NDM gene.

### VI. SUGGESTIONS

1. Due to the prevalence of isolates with multi-antibiotic resistance in patients with burn wounds, diagnosis and accurate determination of the pattern of antibiotic susceptibility of these strains should be done in the early times of hospitalization.

2. Since the drug resistance of bacteria, especially bacteria that cause nosocomial infections is constantly changing, it is better to conduct several studies annually on microorganisms and their drug resistance so that they are used as a guide for physicians in the treatment of patients.

3. In order to reduce the spread of microbial contamination in hospitals, we recommend careful identification and examination of the grounds and factors that spread them, such as incorrect and unprincipled methods of disinfection, the presence of peripheral reservoirs of infection, as well as the
length of hospital stay of patients. By educating staff, the officials should consider the effective methods of care and control of nosocomial infections.

4. Due to the importance of this bacterium in nosocomial infections and the high prevalence of beta-lactamase-producing strains, we recommend the use of a carbapenem with a non-beta-lactam antibiotic to treat these patients. As with piperacillin-tazobactam instead of ceftazidime, the frequency of MBL decreases.

5. Given the abundance of gram-negative bacilli producing beta-lactamases and their high resistance to most extended spectrum drugs, it seems that the identification of MBL-producing strains should be in the daily schedule of microbiology laboratories for gram-negative bacilli, including Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa.

6. By forming committees for prescribing antibiotics in hospitals, the use of extended spectrum antibiotics in hospitals should be seriously monitored.

REFERENCE