Silver resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolated from burn patients

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**Abstract**

**Introduction:** Burn infections are a serious obstacle to the patient’s recovery. Infection is estimated to account for 75% of burn patient mortality. Widespread and often indiscriminate administration of antibiotics and lack of basic infection control methods are major factors in the emergence of drug-resistant bacteria. Due to the growing and serious threat of antibiotic resistance, interest in silver compounds has grown in modern medicine.

**Objectives:** The aim of this descriptive study was to evaluate the frequency of *sil* genes and its phenotypic expression in *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates isolated from the burn ward of Imam Khomeini hospital in Urmia.

**Methods:** Patients and Methods: *P. aeruginosa* (n=16) and *A. baumannii* (n=32) isolates were collected from burn wound samples in an 8-month period from August to March 2017 from the burn ward of the hospital. The minimum inhibitory concentration of silver nitrate on the clinical isolates was determined using microdilution method. The presence of *silP*, *silE* and *silS* silver resistance genes was investigated by polymerase chain reaction (PCR).

**Results:** The results of minimum inhibitory concentration (MIC) test showed that 62.5% (n = 10) of *P. aeruginosa* isolates and 56.25% (n = 18) of *A. baumannii* isolates showed MIC above 512 mg/ml. Polymerase chain reaction results revealed that only one *P. aeruginosa* isolate had *silE* gene and among *A. baumannii* isolates, 20 isolates had *silE* gene and six isolates had *silS* gene. None of the isolates showed positive results for the *silP* gene.

**Conclusion:** Based on the results, *A. baumannii* was the most common microorganism of burn wounds in the burn ward of our hospital in Urmia. This study showed a high degree of phenotypic resistance to silver in *A. baumannii* and *P. aeruginosa* isolates which *silE* and *silS* genes were also observed in some isolates.

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**Key point**

This study showed a high degree of phenotypic resistance to silver in *Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolates, which *silE* and *silS* genes were also observed in some isolates.
antimicrobial effects, a large number of medical products containing silver such as catheters, bandages, shunts and surgical devices have been prepared (8). Bacterial resistance to silver may develop with its increasing use for medical and non-medical applications in the same way that it has developed for antibiotics (9). Previously, Gupta et al identified the sil operon, which is the genetic basis of silver resistance, in Salmonella plasmid PMG101 (10). PMG101 is a 180 kb plasmid that causes resistance to several antibiotics and heavy metals, including silver. The silver resistance gene cluster includes nine genes, silR, silA, silB, silC, silR, silE, silS, ORF105, and silABC (ORF96) (11). Clinically, sil genes have been identified in Salmonella, E. coli, Pseudomonas and methicillin-resistant staphylococci (8).

Objectives
Concerns on the excessive use of silver and the possible emergence of the bacterial resistance to silver have increased, especially in the clinical setting. Although this is a global concern, no study related to silver resistance has been yet initiated in Urmia. This study aimed to investigate phenotypic and genotypic evidence of the silver resistance in some medically important bacteria isolated from burn wounds. This is the first study in Urmia to show phenotypic and genotypic evidence of bacterial resistance to silver in burn wound swab samples.

Patients and Methods
The method of collecting samples
In this descriptive study, the samples were taken from 125 burn patients in the burn department of Imam Khomeini general medical center in Urmia during the 8-month period from August to March 2018.

Sampling of the wounds of hospitalized patients was conducted with sterile swabs while the dressing change team opened them and the surface of the burn wounds were completely clean and also free of disinfectants and surgical devices have been prepared (8). Bacterial resistance to silver may develop with its increasing use for medical and non-medical applications in the same way that it has developed for antibiotics (9).

The genomic DNA of 48 isolates of P. aeruginosa and A. baumannii were extracted by the phenol-chloroform method. The amplicons were profiled into PCR with primers from Bioneer Company in South Korea (Table 1) to check the presence of silver resistance genes including silE, silP and silS based on previous studies was conducted (12). Accordingly, the minimum inhibitory concentration (MIC) test by broth micro-dilution method. Susceptibility to silver nitrate in the clinical isolates was investigated by broth micro-dilution method based on previous studies (13).

Results
Frequency of the number of samples collected
About 110 bacteria isolated from 120 patients were confirmed through different laboratory tests and the test results were observed and recorded (Table 2).

The result of the minimum inhibitory concentration of silver nitrate MIC
Out of 32 isolates of A. baumannii, 16 isolates had MIC of 1024 mg/mL, two isolates had MIC of 512 µg/mL, 10 isolates had MIC of 256 µg/mL and four isolates had MIC of 128 µg/mL. Out of 16 isolates of P. aeruginosa, four isolates showed MIC 1024, six isolates MIC 512, three isolates MIC 256 and three isolates MIC 128 µg/mL.

The results of investigating the presence of silver resistance genes
For examining the presence of silver resistance genes (silP, silS, silE) in A. baumannii and P. aeruginosa isolates, the following results were obtained.

Of 16 isolates of P. aeruginosa tested, with the studied primers, the silE gene was detected in only one (6.2%) strain, and out of 32 isolates of A. baumannii, 6 (18.75%) isolates contained the silS gene and 20 (62.5%) isolates contained silE gene. None of the isolates was positive for silP gene (Figure 1).

Table 1. The primers used in the PCR test

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Primer sequence</th>
<th>Product length (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>silE (F)</td>
<td>GTACTC...</td>
<td>400</td>
<td>12</td>
</tr>
<tr>
<td>silE (R)</td>
<td>GCCAGACTGACGTTT</td>
<td>400</td>
<td>12</td>
</tr>
<tr>
<td>silS (F)</td>
<td>GGAGATCCGGATCAGCAA</td>
<td>1500</td>
<td>12</td>
</tr>
<tr>
<td>silS (R)</td>
<td>GTTGGTCATGAGCTTGAAGAAGATC</td>
<td>1500</td>
<td>12</td>
</tr>
<tr>
<td>silP (F)</td>
<td>CATGACATCTGCTGAGAAGATC</td>
<td>2500</td>
<td>12</td>
</tr>
<tr>
<td>silP (R)</td>
<td>CGGGCACGACGAGAAATGC</td>
<td>2500</td>
<td>12</td>
</tr>
</tbody>
</table>
Discussion

Adequate adherence to infection control measures and preparation of an antibiotic stewardship program will help reduce the number of infections and the emergence of resistant strains in patients with burn wounds. The emergence of resistant strains in patients with burn wounds, and identifying their antimicrobial susceptibility patterns will help guide the antibiotic policy needed to minimize acquired infections be designed among these vulnerable patients. In our study, A. baumannii was the most common pathogen isolated from burn injuries, while these results were similar to the studies conducted by Bayram et al (14), ALfadli et al (15), Hegde et al (16), and Chim et al (17). The presence of Acinetobacter species as a normal flora of the skin, the ability to easily transfer and the ability to persist in the hospital environment due to its multi-drug resistance and several other factors are involved in increasing the incidence of hospital infections caused by this organism. Comparing of the findings of the present study to the similar studies indicates an increasing trend in the frequency of Acinetobacter in burn wounds, which has become one of the important causes of hospital infections.

In our study, P. aeruginosa was the second most common gram-negative isolate, with a frequency of 14.5%, which is not a considerable rate for this bacteria compared to this kind of studies (18,19). A study conducted in a Burn Care Unit in India showed that P. aeruginosa was the most common type of gram-negative bacteria isolated from burn patients. K. pneumonia and A. baumannii were the second and third most common species (20). While in the study conducted by Li et al, S. aureus and P. aeruginosa were reported as the first and third organisms (21). Possible explanations for differences in microbiological profiles may be due to differences in geographic location or patient age (children versus adults), differences in hygiene practices applied in health care facilities and cross-infection related to the hands of health care personnel.

Identifying the microbiological profile in burn wounds is of great importance epidemiologically and in terms of clinical outcome improvement, as these data may guide effective empiric antibiotic therapy.

In our study, the most common microorganism identified was A. baumannii. In the present study, the MIC of silver nitrate was determined by broth micro dilution method for 32 clinical isolates of A. baumannii and 16 isolates of P. aeruginosa. The results showed that the minimum inhibitory concentration is relatively higher compared to silver nitrate, therefore 62.5% of P. aeruginosa isolates and 56.25% of A. baumannii isolates demonstrated a minimum inhibitory concentration above 512 µg/mL.

The minimum inhibitory concentration in our study is higher compared to the study by Hosny et al in Egypt in 2019, in which the study of 150 clinical isolates obtained from burns and wounds displayed 19 bacterial isolates with an MIC above 512 µg/mL to silver nitrate(22).

Investigating the prevalence of silver resistance is important because of the possibility of plasmid transfer and, as a result, cross-resistance in other bacteria. Therefore, the wound provides an ideal environment for the transfer of plasmids, which may contain silver resistance genes between strains (23).

In the present study, 16 isolates of P. aeruginosa and 32 isolates of A. baumannii were tested for the presence of silver resistance genes including silS, silE and silP using PCR technique. Out of 16 isolates of P. aeruginosa, only one isolate had silE gene, and out of 32 isolates of A. baumannii, 20 isolates had silE gene and six isolates had silS gene. None of the strains showed positive results for the silP gene. These results were consistent with the results of Sütterlin et al, which conducted in Sweden in 2012. silS and silE genes were reported in two strains of Enterobacter cloacae and P. aeruginosa in their study (13). Another study conducted by Sütterlin et al in Sweden in which out of 836 investigated isolates, 176 isolates (21%) had at least one sil gene. sil genes showed the highest frequency in Enterobacter and Klebsiella. At the species level, the

Table 2. Frequency of the bacterial pathogens isolated from the study cases

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>No. of isolates</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter baumannii</td>
<td>16</td>
<td>29.1</td>
</tr>
<tr>
<td>Acinetobacter lwoffii</td>
<td>12</td>
<td>22.0</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>10</td>
<td>26.3</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>3</td>
<td>12.7</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>8</td>
<td>21.5</td>
</tr>
<tr>
<td>Enterobacter species</td>
<td>5</td>
<td>14.3</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>4</td>
<td>10.6</td>
</tr>
<tr>
<td>Coagulase negative staphylococci</td>
<td>3</td>
<td>2.7</td>
</tr>
<tr>
<td>Corynebacterium species</td>
<td>10</td>
<td>25.0</td>
</tr>
<tr>
<td>Total</td>
<td>110</td>
<td>100</td>
</tr>
</tbody>
</table>
highest abundance was reported in *Enterobacter cloacae*, *K. pneumoniae*, and *K. oxytoca* (24).

A study was conducted in Bangladesh in 2017 by Safain et al on 11 isolates of *K. pneumoniae*. In the phenotypic test, it was found that out of two to fourteen isolates were resistant to silver nitrate. The result of polymerase chain reaction and sequencing for silver resistance genes confirmed the presence of silE gene in an isolate of *K. pneumoniae* (11). Another study conducted by Hosny et al in 2019 in Egypt in which 150 bacterial isolates from burn wounds were tested for the presence of silP, silF, silE, silA, silB, silIP, silCBA and silRS genes.

Silver sensitive isolates were negative in phenotypic test for all sil genes tested. Nevertheless, all isolates resistant to silver showed positive results for at least three tested sil genes. All tested sil genes were detectable in 6 of 19 silver-resistant isolates. Other isolates were positive for only some of the sil genes tested (22). Moreover, in a study conducted by Percival et al in Sweden, 112 clinical isolates obtained from patients with diabetic foot ulcers were screened for the presence of silS silE and silP genes. 1.8% of the isolates contained silver resistance genes (12). This is the first study carried out in Iran that reports the ability to detect resistance to silver and sil genes in clinical isolates of *P. aeruginosa* and *A. baumannii* isolated from the burn section. This study is alarming considering the increase of MIC and spread of resistance to phenotypic silver and sil genes, especially in *A. baumannii* species.

**Conclusion**

According to the obtained results, *A. baumannii* was the most common microorganism in burn wounds in the burn department of our hospital in Urmia. This study showed a high level of phenotypic resistance to silver in *A. baumannii* and *P. aeruginosa* isolates and silver resistance genes were found in some collections.

**Limitations of the study**

No limitations were observed in this study.

**Acknowledgements**

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**Authors’ contribution**

Conceptualization: FF, KD, JK and SAY.
Methodology: FF, KD, JK and SAY.
Validation: FF, KD, JK and SAY.
Investigation: FF, KD, JK and SAY.
Resources: FF.
Data curation: SAY.
Writing—original draft: FF and SAY.
Writing—review and editing: JK and SAY.
Supervision: SAY.
Project administration: SAY.

**Conflicts of interest**

The authors declare that they have no competing interests.

**Ethical issues**

The research followed the tenets of the Declaration of Helsinki. The Ethics Committee of Urmia University of Medical Sciences approved this study (IR.UUMSU.REC.1398.373). Accordingly, written informed consent was taken from all participants before any intervention. This study is extracted from the MSc of microbiology thesis of Fatemeh Farajzadeh (thesis #9847). Moreover, Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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