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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.
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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Introduction

As one of the largest organs of the human body, the skin is vital for maintaining body temperature and protecting the host against infection. Burn damage creates a gap in the surface of the skin and destroys vital actions that are necessary to maintain life and also as a complex injury, it requires multidisciplinary and continuous treatment (1,2). Despite all the advances, infection remains the leading cause of death among burn patients. Loss of skin barrier and immunodeficiency associated with large burns make these patients particularly susceptible to sepsis. In recent years, multidrug-resistant bacterial and fungal strains have caused an unexpected increase in burn wound infections, sepsis and death worldwide(3). Although the most important infectious bacteria in burn wounds is *Staphylococcus aureus*, a study conducted in Texas detected that the main cause of death due to infection is now multiple resistant organisms, including *Pseudomonas* and *Acinetobacter* (4).

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.

Microorganisms resistant to several antibiotics can cause special problems in the wounds of patients hospitalized in the burn department (5, 6). Repurposing metal-based antimicrobials to combat the current crisis is a promising alternative strategy (6). Silver and its compounds have been used as antimicrobial agents for a longtime. The most important silver compound currently used, is silver sulfadiazine. In recent years, silver compounds were utilized to prevent burn infection and some eye infections and also to remove warts (7). In addition, due to their

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, *silP, 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 topical antibiotics.

These samples are employed to identify isolates of *P. aeruginosa* and *A. baumannii* using standard microbiological methods such as gram staining, colony morphology examination, movement examination, growth on MacConkey agar medium and differential culture mediums and also biochemical tests such as oxidase test to determine, they were identified. Then the samples were stored in a freezer at -20°C for the next steps.

DNA extraction and polymerase chain reaction (PCR)

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

Primer name	Primer sequence	Product length (bp)	Reference
silE (F)	GTACTC CCCCGGACATCACTAATT	400	12
silE (R)	GGCCAGACTGACCGTTATT	400	12
silS (F)	GGAGATCCCGGATGCATAGCAA	1500	12
silS (R)	GTTTGCTGCATGACAGGCTAA AGACATC	1500	12
silP (F)	CATGACATATCCTGAAGA CAGAAAATGC	2500	12
silP (R)	CGGGCAGACCAGCAATAACAGATA	2500	12

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

Bacteria	No. of isolates	Percent
Acinetobacter baumannii	32	29.1
Acinetobacter lwoffii	9	8.18
Pseudomonas aeruginosa	16	14.54
Escherichia coli	3	2.72
Klebsiella pneumoniae	5	4.54
Enterobacter species	5	4.54
Staphylococcus aureus	15	13.63
Coagulase negative staphylococci	14	12.72
Corynebacterium species	11	10
Total	110	100

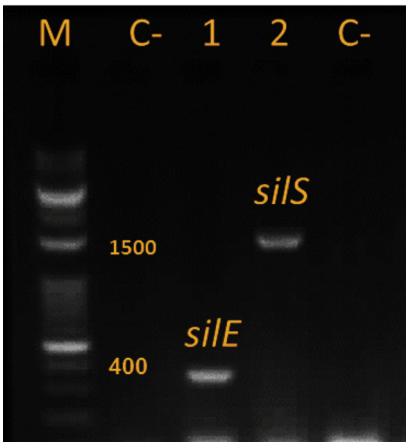

Figure 1. Agarose gel electrophoresis picture of amplified *sil* gene bands. Lane M: Size marker 10 k, Lane 1: *silE* gene, Lane 2: *silS* gene.

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 comparing 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 high 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

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 two out of eleven 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, silP, 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.

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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.UMSU.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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References

1. Church D, Elsayed S, Reid O, Winston B, Lindsay R. Burn wound infections. Clin Microbiol Rev. 2006;19:403-34. doi: 10.1128/cmr.19.2.403-434.2006.
2. Saxena N, Dadhich D, Maheshwari D. Aerobic bacterial isolates from burn wound infection patients and their antimicrobial susceptibility pattern in Kota, Rajasthan. J Evol Med Dent Sci. 2013;2:4156-61.
3. Branski LK, Al-Mousawi A, Rivero H, Jeschke MG, Sanford AP, Herndon DN. Emerging infections in burns. Surg Infect (Larchmt). 2009;10:389-97. doi: 10.1089/sur.2009.024.
4. Williams FN, Herndon DN, Hawkins HK, Lee JO, Cox RA, Kulp GA, et al. The leading causes of death after burn injury in a single pediatric burn center. Crit Care. 2009;13:R183. doi: 10.1186/cc8170.
5. van Langeveld I, Gagnon RC, Conrad PF, Gamelli RL, Martin B, Choudhry MA, et al. Multiple-drug resistance in burn patients: a retrospective study on the impact of antibiotic resistance on survival and length of stay. J Burn Care Res. 2017;38):99-105. doi: 10.1097/bcr.0000000000000479.
6. Wong TH, Tan BH, Ling ML, Song C. Multi-resistant *Acinetobacter baumannii* on a burns unit--clinical risk factors and prognosis. Burns. 2002;28:349-57. doi: 10.1016/s0305-4179(02)00012-8.
7. McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev. 1999;12:147-79. doi: 10.1128/cmr.12.1.147.
8. Finley PJ, Norton R, Austin C, Mitchell A, Zank S, Durham P. Unprecedented silver resistance in clinically isolated *Enterobacteriaceae*: major implications for burn and wound management. Antimicrob Agents Chemother. 2015;59:4734-41. doi: 10.1128/aac.00026-15.
9. Randall CP, Gupta A, Jackson N, Busse D, O'Neill AJ. Silver resistance in gram-negative bacteria: a dissection of endogenous and exogenous mechanisms. J Antimicrob Chemother. 2015;70:1037-46. doi: 10.1093/jac/dku523.
10. Sütterlin S. Aspects of Bacterial Resistance to Silver [dissertation]. Acta Universitatis Upsaliensis; 2015.
11. Safain KS. Screening of Silver Resistance Gene in Clinical Isolates and Determination of Minimum Inhibitory Concentration (MIC) for Silver Nitrate [dissertation]. BRAC University; 2017.
12. Percival SL, Woods E, Nutekpor M, Bowler P, Radford A, Cochrane C. Prevalence of silver resistance in bacteria isolated from diabetic foot ulcers and efficacy of silver-containing wound dressings. Ostomy Wound Manage. 2008;54:30-40.
13. Sütterlin S, Tano E, Bergsten A, Tallberg AB, Melhus A. Effects of silver-based wound dressings on the bacterial flora in chronic leg ulcers and its susceptibility in vitro to silver. Acta Derm Venereol. 2012;92:34-9. doi: 10.2340/00015555-1170.
14. Bayram Y, Parlak M, Aypak C, Bayram I. Three-year review of

bacteriological profile and antibiogram of burn wound isolates in Van, Turkey. Int J Med Sci. 2013;10:19-23. doi: 10.7150/ijms.4723.
15. ALfadli M, El-Sehsah EM, Ramadan MA. Risk factors and distribution of MDROs among patients with healthcare associated burn wound infection. Germs. 2018;8:199-206. doi: 10.18683/germs.2018.1147.
16. Hegde R, Bhandary S. Bacteriological profile and susceptibility pattern of burn wound isolates in a tertiary care hospital. Indian J Basic Appl Med Res. 2015;5:99-103.
17. Chim H, Tan BH, Song C. Five-year review of infections in a burn intensive care unit: high incidence of *Acinetobacter baumannii* in a tropical climate. Burns. 2007;33:1008-14. doi: 10.1016/j.burns.2007.03.003.
18. Afreen S, Tripathi S, Khan F, Khurram MF, Ahmad I. Study of bacterial isolates and their antibiotic resistance pattern in post burn patients at tertiary care centre. Int J Pharm Res. 2021;13:1586-93. doi: 10.31838/ijpr/2021.13.02.199.
19. Forson OA, Ayanka E, Olu-Taiwo M, Pappoe-Ashong PJ, Ayeh-Kumi PJ. Bacterial infections in burn wound patients at a tertiary teaching hospital in Accra, Ghana. Ann Burns Fire Disasters. 2017;30):116-20.
20. Gupta M, Naik AK, Singh SK. Bacteriological profile and antimicrobial resistance patterns of burn wound infections in a tertiary care hospital. Heliyon. 2019;5:e02956. doi: 10.1016/j.heliyon.2019.e02956.
21. Li L, Dai JX, Xu L, Chen ZH, Li XY, Liu M, et al. Antimicrobial resistance and pathogen distribution in hospitalized burn patients: a multicenter study in Southeast China. Medicine (Baltimore). 2018;97:e11977. doi: 10.1097/md.0000000000011977.
22. Hosny AE, Rasmy SA, Aboul-Magd DS, Kashef MT, El-Bazza ZE. The increasing threat of silver-resistance in clinical isolates from wounds and burns. Infect Drug Resist. 2019;12:1985-2001. doi: 10.2147/idr.s209881.
23. Davis IJ, Richards H, Mullany P. Isolation of silver- and antibiotic-resistant *Enterobacter cloacae* from teeth. Oral Microbiol Immunol. 2005;20:191-4. doi: 10.1111/j.1399-302X.2005.00218.x.
24. Sütterlin S, Dahlö M, Tellgren-Roth C, Schaal W, Melhus Å. High frequency of silver resistance genes in invasive isolates of *Enterobacter* and *Klebsiella* species. J Hosp Infect. 2017;96:256-61. doi: 10.1016/j.jhin.2017.04.017.