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Molecular Identification and Detection of Virulence Genes among *Pseudomonas aeruginosa* Isolated from Burns Infections

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Abstract

Objective: Virulence factors are substances produced by pathogenic *Pseudomonas aeruginosa* that contribute significantly to the etiology of disease. Virulence genes encode these virulence factors on the *Pseudomonas aeruginosa* chromosome.

Methods: Between July 2021 and June 2022, at the Burn and Plastic Surgery Hospital in Duhok city, Iraq, seventy-one isolates of *Pseudomonas aeruginosa* were isolated from infected burns. The *lasB* and *toxA* genes were identified using Polymerase Chain Reaction (PCR).

Results: *P. aeruginosa* was isolated from 64.55% (71/110) of the specimens, with non-significantly higher rate from females than males (38.18% vs 26.36%), but the differences between various ages were significant ($P < 0.04$). About 38.18% of burns were due to flame and the highest rate (45.4%) of infected burns were second-degree burns. Furthermore, 76.06% (54/71) of the isolates were multidrug resistant. They demonstrated greater resistance to Piperacillin; the resistance was 98.59%. Of the isolates examined, 35 (64.81%) were positive for *toxA* and 27 (50%) were positive for *lasB* genes.

Conclusion: Due to the limited number of effective medications against this bacterium that are currently available, testing for antimicrobial susceptibility must be performed on all isolates. By doing this, you can help manage the treatment plan and stop the emergence of resistance in burn units.

Keywords: *Pseudomonas aeruginosa*, *toxA*, *lasB*, burn wound infection

Introduction

Pseudomonas aeruginosa is a ubiquitous, rod-shaped aerobic, non-fermentative, Gram-negative bacterium that inhabits soil, water, plants, and humans.[1] It is a resistant microbe that can survive at temperatures ranging from 4 to 42°C and grow in nutrient-poor environments. *P. aeruginosa* persistent adaptation and survival enable it to live up to 6 months on dry, abiotic surfaces in hospitals.[2] In the community and hospitals, it is one of the most pervasive opportunistic bacteria associated with nosocomial infections, otitis media, burns, and respiratory tract infections.[3] Multidrug-resistant (MDR) isolates of *P. aeruginosa* are becoming more prevalent in hospitalized patients, and *P. aeruginosa* infections are becoming more and more common.[4,5] High rates of morbidity and mortality are produced by this bacterium's capacity to infect virtually all tissues.[6] The ideal habitat for opportunistic microbes, both exogenous and endogenous, is provided by infected burn wounds. Burn victims may become infected due to a number of causes, such as exposed body surfaces, immunocompromised conditions, invasive hospital treatments, and protracted hospitalization.[7]

Several virulence factors are present in *Pseudomonas aeruginosa*, including flagella, pili, and LPS, which help the bacteria adhere to and colonize the host, proteases and toxins that destroy tissue, secretion systems that transport effectors and poisons into the host, and quorum-sensing and biofilm, which help the bacteria communicate and resist medication.[8] Exotoxin A inhibits protein synthesis by preventing the eukaryotic elongation factor 2 from being ADP-ribosylated.[9] Connective tissue constituents like elastin, collagen, fibronectin, and laminin are among the substrates for the zinc metalloprotease known as *las B.* Lung tissue is elastolytically affected by *las B.*[10]

The purpose of this work was to identify *toxA* and *lasB* virulence genes in *Pseudomonas aeruginosa* isolates.

Materials and Methods

Bacterial Isolates

During the period of July 2021 to June 2022, one hundred and ten clinical samples were obtained from individuals who attended the Burn and Plastic Surgery Hospital in Duhok City, Iraq. These samples were obtained from all genders and ages of hospitalized patients. And then transferred to the laboratory. As part of the sampling technique, swabs were taken from clinically deep burn wound sites that showed clinical symptoms of wound infection after changing the wound dressing. By employing cultures (preliminary isolation on MacConkey agar and Blood agar) followed by subculture on Cetrimide agar the isolates were determined to be *P. aeruginosa* according to a study by Leboffe et al.,[11] these pure colonies were identified based on their physical and biochemical traits. In addition, the species-specific gene confirmed the genotype of each identified *P. aeruginosa* strain (16S rDNA).[12]

Antimicrobial Susceptibility Test

The sensitivity of the purified isolates to various antimicrobial drugs was assessed using the Kirby-Bauer disk diffusion method, according to Hudzicki.[13] There were ten antibiotics utilized, provided by (Bioanalyses, Turkey). Antimicrobials that be used for testing included: Colistin (CL; 10 mg), Ciprofloxacin (CIP; 5 mg), Levofloxacin (LEV; 5 mg), Amikacin (AK; 30 mg), Gentamicin (CN; 10 mg), Imipenem (IPM; 10 mg), Meropenem (MEM; 10 mg), Ceftazidime (CAZ; 30 mg), Cefepime (CPM; 30 mg), and Piperacillin (PI; 100 mg). The

Clinical and Laboratory Standards Institute[14] estimated the diameter of the zone of inhibition surrounding antibiotic disks.

Bacterial DNA Extraction from P. aeruginosa

A high-yield DNA Purification Kit was used to extract genomic DNA from bacterial isolates according to the manufacturer's instructions (Genomic DNA mini kit, Favorgen, Taiwan). The purity of the bacterial DNA was assessed using a (NanoDropTM One UV-Vis Spectrophotometer, Thermo Fisher Scientific, Waltham, MA, USA) and then stored in a freezer at –20°C, in preparation for PCR amplification. Table 1 displays the primer sequences and amplification band sizes for 16SrDNA, *las*B, and *tox*A.

The PCR mixtures (20 µl) contained 3 µl of DNA, 10 µl of PCR master mix (ADDBIO.INC, South Korea), 5 µL of distilled deionized water, and 1 µl of each primer. Reaction conditions for all the primers were described in Table 2. Amplicons were made visible using electrophoresis on 1.5% agarose gel and subsequent UV light (Cleaver Scientific Ltd., Rugby, UK). Additionally, the amplicon's size was measured against a 1500–100 bp DNA ladder (Guangzhou Dongsheng Biotech Co., Ltd., Guangzhou, China).[15]

Ethics Declarations

The Duhok Directorate General of Health, Directorate of Planning, Scientific Research Division, and Institutional Ethics Committee received and accepted the study protocol (approval No. 13072021-7-10).

Statistical Analysis

GraphPad Prism version 9.3.1 (471) was used for statistical analysis. Statistical significance was set at $P < 0.05$.

Results

During the study period of July 2021–June 2022, a total of 110 samples from burn patients of both genders and varying ages were obtained. *P. aeruginosa* was the most common pathogenic bacteria, it was isolated from 64.55% (71/110) of the specimens. The isolated *P. aeruginosa* was confirmed using biochemical and phenotypic assays including staining, MacConkey, Blood, and Cetrimide agars (Oxidase, Citrate, TSI). Furthermore, the species-specific gene was used to confirm the genotype of all *P. aeruginosa* isolates (16S rDNA).

A higher rate of this bacterium was isolated from females accounting for 38.18 (42/71) vs 26.36% (29/71) with a statistically non-significant ($P > 0.05$) difference between both genders. As regards age, the differences between various ages were statistically significant ($P < 0.04$) as shown in Table 3. Concerning the causes of the burns, 38.18% of the patients had flame burns, whereas 2.73% of them had electrical burns, making flame burns the most prevalent form of the burn wound, statistically the differences between burn causes were highly significant ($P < 0.01$). The second-degree burn showed the highest rate of infection which was 45.4% as compared with third- and mixed-degree burns (6.36% and 12.73% respectively). Differences in burn severity were found to be statistically highly significant ($P < 0.01$). Rates of infection were greater in patients with TBSA burns between 20% and 40% (48.18%) compared to those with burns of less than 20% (7.27%) and more than 40% (9.09%), although the difference was statistically non-significant ($P > 0.05$) (Table 4).

The majority of *P. aeruginosa* isolates, 76.06% (54/71), were determined to be multidrug-resistant (MDR) based on the results of antimicrobial susceptibility testing, displaying resistance against aminoglycosides, β-lactams, and/or fluoroquinolones (at least three classes of antimicrobial medications).

All isolates of this study were completely sensitive to Colistin. In contrast, they showed significant resistance to Piperacillin, Cefepime, Ceftazidime, and Meropenem at rates of 98.59%, 85.92%, 84.50%, and 74.65%, respectively (Table 5).

Using PCR amplification, the prevalence of the virulence genes *toxA,* and *lasB* in the *P. aeruginosa* isolates were examined and confirmed by agarose gels based on the results

Table 1. **Sequences of the primer used and the molecular weights of the genes**

Primer name	Primer sequence (5′–3′)	Detected gene	Molecular weight	References
16S rDNA	F: GGGGGATCTTCGGACCTCA R:TCCTTAGAGTGCCCACCCG	16S rDNA	956	12
toxA	F GACAACGCCCTCAGCATCACCAGC R CGCTGGCCCATTCGCTCCAGCGCT	*toxA*	396	42
lasB	F GGAATGAACGAAGCGTTCTC R GGTCCAGTAGTAGCGGTTGG	*lasB*	300	43

Table 2. **Reaction conditions for PCR amplification of *Pseudomonas* sp. *toxA* and *lasB* genes**

Gene name	Temperature (°C) /Time				
	Initial denaturation	Cycling condition			Final extension
		Denaturation	Annealing	Extension	
16S rDNA	95°C; 2 min; 1 cycle	94°C; 20 sec	54°C; 20 sec 25 cycle	72°C; 40 sec	72°C; 5 min 1 cycle
toxA	94°C; 2 min 1 cycle	94°C; 2 min	68°C; 1 min 30 cycle	72°C; 1 min	72°C; 7 min 1 cycle
lasB	94°C; 3 min 1 cycle	94°C; 30 sec	60°C; 1 min 30 cycle	72°C; 90 sec	72°C; 5 min 1 cycle

Table 3. **The correlations between the causes of burns in both genders and various age groups**

Factors		No. of specimens	No. of positive isolates with (%)	Odd (ratio 95% CI)	*P* value
Gender	Females	62	42 (38.18%)	1.376 (0.6099 to 3.114)	0.5469
	Males	48	29 (26.36%)		
	Total	110	71 (64.55%)		
Age group	1 month-10 Y	29	13 (11.82%)	Reference group	–
	11–20	22	12 (10.90%)	0.6771 (0.2077 to 2.076)	0.5771
	21–30	23	15 (13.64%)	0.4333 (0.1510 to 1.314)	0.1707
	31–40	18	14 (12.73%)	0.2321 (0.07210 to 0.9406)	0.0359
	41–50	10	9 (8.18%)	0.09028 (0.007819 to 0.6224)	0.0240
	51–60	4	4 (3.64%)	0.000 (0.000 to 1.069)	0.1026
	61–70	3	3 (2.73%)	0.000 (0.000 to 1.083)	0.2258
	71–80	0	0	–	–
	81–90	1	1 (0.91%)	0.000 (0.000 to 7.875)	0.4667
	Total	110	71 (64.55%)	–	–
Cause of burn*	Flame	76	42 (38.18%)	Rreference group	–
	Scald (hot water)	22	17 (15.45%)	0.3633 (0.1370 to 1.058)	0.0841
	Hot liquids	9	9 (8.18%)	0.000 (0.000 to 0.5348)	0.0098
	Electrical	3	3 (2.73%)	0.000 (0.000 to 1.501)	0.2551
	Total	110	71 (64.55%)	–	–

*Cause of burn: Chi-square = 11.01, 3 and **P* value 0.0117; *Age group: Chi-square = 14.50, 7 and **P* value 0.0430.

Table 4. **The relationships between burn infection, the distribution of burned TBSA, and depth (degree)**

Variables and their distributional patterns		Total no.	Infected No. and (%)	Chi-square	*P* value
TBSA group	<20	17	8 (7.27%)	3.410	0.1818
	20–40	76	53 (48.18%)		
	>40	17	10 (9.09%)		
	Total	110	71 (90.91%)		
Degree of burn	Second degree	65	50 (45.45%)	17.73	0.0001
	Third degree	8	7 (6.36%)		
	Mixed	37	14 (12.73%)		
	Total	110	71 (90.91%)		

indicated that 35 (64.81%), and 27 (50%) of the isolates comprised *toxA*, and *lasB* related genes, respectively and 24 of the *P. aeruginosa* isolates possessed both *toxA* and *lasB* genes.

Discussion

Pseudomonas aeruginosa is one of the most common causes of hospital-acquired infections. It frequently causes serious and potentially fatal infections that are difficult to cure since this organism develops inherited multidrug resistance (MDR) and is capable of developing resistance to the majority of effective antimicrobial medications. In addition, burn patients are more susceptible to infection than other patients because of their compromised skin barrier and depressed immune systems, as well as their longer hospital stays and more intrusive therapeutic and diagnostic procedures.[16]

We found that 71 out of 110 (64.55%) burn patients treated at Duhok Burn Hospital had *P. aeruginosa* isolates, consistent results with the present study were also found in a study in Algerian burn patients, where the recorded rate was 62%.[17] Furthermore, another study carried out in Iraq revealed a high incidence of *P. aeruginosa* isolates at 97.6%.[18] While, studies from Morocco[19] and Egypt[20] demonstrated lower incidence rates of 15.1% and 19.8%, respectively. This variation may be explained by antibiotic misuse, various infection control methods used by hospitals, hygienic conditions, and regional climate.

Table 5. **Antimicrobial agent susceptibility patterns**

Antimicrobial agents	S (%)	I (%)	R (%)
CL	71 (100)	0	0
IMP	27 (38.03)	1 (1.41)	43 (60.56)
CIP	22 (30.99)	3 (4.23)	46 (64.79)
LEV	22 (30.99)	1 (1.41)	48 (67.61)
CN	20 (28.17)	2 (2.82)	49 (69.01)
AK	18 (25.35)	8 (11.27)	45 (63.38)
MEM	15 (21.13)	3 (4.23)	53 (74.65)
CAZ	10 (14.08)	1 (1.41)	60 (84.50)
FEP	8 (11.27)	2 (2.82)	61 (85.92)
PI	1 (1.41)	0	70 (98.59)

S, Sensitive; I, Intermediate; R, Resistance; CL, Colistin; IMP, Imipenem; CIP, Ciprofloxacin; LEV, Levofloxacin; CN, Gentamicin; AK, Amikacin; MEM, Meropenem; CAZ, Ceftazidime; FEP, Cefepime; PI, Piperacillin.

Burns from open flames constitute the highest rate (38.18%) among other injuries, with scald (hot water) coming in second (15.45%). When comparing gender and age, females showed a higher incidence of burn injuries (38.18%), with the highest percentage (13.64%) among age of 21–30 years. These results are consistent to some extent with those of research conducted in Basra/Iraq,[21] which found that women experienced a higher incidence of burns than men (57.5% vs. 19.16%). Burns from flames accounted for 76.6% of all burns, followed by burns from hot water at 19.1%. Similarly, studies from Iraq and Iran showed higher rates in females but at lower rates than the current study, which were: 57% in the city of Suleimani, Iraq,[22] 54.84%, and 56% in Iran[23,24] respectively. The vast majority of fire-related injuries happen in the home, and the vast majority of women in our culture do routine household duties like heating and cooking in high-risk sections of the kitchen. This considerably increases their risk for contracting burn injuries.

While a study carried out in Suleimani, Iraq, revealed that 56.2% of males had the greatest infection rates, with scald (hot water) being the most common cause (72.5%), followed by flame (22.8%).[25] The results of this study showed that the rate of second-degree burns was highest at 45.45%. Similar findings were conducted in Saudi Arabia,[26] which found that 72.1% of burn patients had second-degree burns. Patients with burns of 20–40% of total body surface area (TBSA) had a greater infection risk at 48.18%, as seen in the present study. This result agrees with the findings conducted in Suleimani/Iraq.[25] The greater the total body surface area, the greater the chance that bacteria may colonize and multiply, leading to a deeper, thicker wound and eventually letting the infection spread throughout the body via circulation.[27]

The prevalence of *P. aeruginosa* infection was more frequently related to female patients between the ages of 21 and 30 years, regardless of the cause of the burns. In our study, MDR *P. aeruginosa* infection was found in 76.06% of patients, which is higher than the percentages found in previous research in Iran (16.5–41%), Iraq (12.4%),[28-30] Brazil (71.4%)[31] and Egypt (70%).[32] However, a second Iranian study found a substantially higher proportion (89.24%) of people having MDR *P. aeruginosa* infection.[24]

The global spread of multidrug-resistant *Pseudomonas aeruginosa* may be attributable to the misuse of antibiotics in healthcare institutions and communities, as well as the evolution of several resistance mechanisms.[18]

Colistin sensitivity was demonstrated by 100% of the isolates in the current study. In contrast, Jalil *et al.*[21] observed a significantly lower percentage of Colistin-sensitive patients in Iraq (53.1%), another study in Iraq found 7.4% colistin resistance.[18] However, Colistin susceptibility against *P. aeruginosa* remains exceptionally high, accounting to 100% in the majority of Middle Eastern and North African countries.[33]

Imipenem resistance was present in 60.56% of the *P. aeruginosa* isolates in this investigation, which is greater than the rates reported in Iran (41.3%)[34] and Iraq (47%).[35] On the other hand, lower rates have been reported in Iran (83.90%)[24] and Iraq (68.40%).[18] Carbapenem resistance in *P. aeruginosa* can arise from a number of factors, including an increase in carbapenemase production, a mutation in the *operD* gene, the overexpression of the efflux pump AmpC, and a change in the distribution of drug targets.[36]

Piperacillin resistance was shown to be the highest among *P. aeruginosa* strains in this investigation (98.59%). In contrast in earlier research carried out in Iran, a lower rate of 74.8% was reported.[37] While in South Africa, a slightly lower rate (94%) than the present study was reported.[38] These results highlight serious concerns that necessitate Health Authorities working quickly to develop rapid and precise diagnostic processes, by limiting the distribution of antibiotics, and strengthening hospital microbiological control systems.

According to PCR results, the present study findings revealed that the *toxA* gene was found in 35 (64.81%) of the *P. aeruginosa* isolates examined. These results were slightly lower than those obtained by Qader *et al.*[35] in Iraq, who used identical primers for the *toxA* gene and produced a band with a similar molecular weight of 86%. Whereas another study carried out in Iraq by Aljebory[16] revealed that 100% possessed this gene.

The results of this investigation also showed that the *lasB* gene, which has an amplified size of 300 bp, was detected in 50% of the 54 *P. aeruginosa* isolates. This result is comparable to the study of Qader *et al.*[35] in Iraq that yielded a band with an identical molecular weight of 82% using the same primers for the *lasB* gene. While other studies in Iraq by AL-Shamaa *et al.*[39] and Al-Dahmoshi *et al.*[40] found that 87% and 69.23% of the isolates from burns, respectively carried this gene, they used different primer sequence for the *lasB* gene and produced a band with a different molecular weight. The frequency of *Pseudomonas aeruginosa* and the proportion of virulence factors genes are influenced by a variety of factors, such as environmental factors, patient immunological health, levels of contamination, strain type and virulence.[41]

Conclusion

Evidence from this study suggests that the presence of multivirulence factors may be responsible for the delay in healing and the severity degree associated with infections caused by *P. aeruginosa*. This bacterium exhibits a variety of virulence traits that allow it to adapt to different conditions and cause a wide range of infections that are difficult to cure. The findings of this investigation also demonstrated the presence of a significant rate of MDR *P. aeruginosa*, that probably brought

on by the misuse and overuse of antibiotics. Therefore, the present results might offer advice on how to prescribe the proper antibiotics to the patient. The simultaneous use of specific primers for the *P. aeruginosa* genes *lasB* and *toxA* seems to result in a more precise identification of it by PCR.

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Conflicts of Interest

The authors have no conflicts of interest to declare for this study.

References

1. Wu M, Li X. *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. In Molecular medical microbiology. 2015; 3:1547–1564. Academic Press. https://doi.org/10.1016/B978-0-12-397169-2.00087-1
2. Diggle SP, Whiteley M. Microbe Profile: *Pseudomonas aeruginosa*: opportunistic pathogen and lab rat. Microbiology. 2020;166(1):30. doi: 10.1099/mic.0.000860
3. Pachori P, Gothalwal R, Gandhi P. Emergence of antibiotic resistance *Pseudomonas aeruginosa* in intensive care unit; a critical review. Genes & Diseases. 2019;6(2):109–119. https://doi.org/10.1016/j.gendis.2019.04.001
4. Pang Z, Raudonis R, Glick BR, Lin TJ, Cheng Z. Antibiotic resistance in *Pseudomonas aeruginosa*: mechanisms and alternative therapeutic strategies. Biotechnology Advances. 2019;37(1):177–192. https://doi.org/10.1016/j.biotechadv.2018.11.013
5. Abdelrahman DN, Taha AA, Dafaallah MM, Mohammed AA, El Hussein AR, Hashim AI, Hamedelnil YF, Altayb HN. β-lactamases (bla TEM, bla SHV, bla CTXM-1, bla VEB, bla OXA-1) and class C β-lactamases gene frequency in *Pseudomonas aeruginosa* isolated from various clinical specimens in Khartoum State, Sudan: a cross sectional study. F1000Research. 2020;9:774. doi: 10.12688/f1000research.24818.3
6. Najem SA. (2022). Bacteriological Study on Multidrug Resistance Genes in *Pseudomonas aeruginosa* Isolated from Different Clinical Samples in Al-Najaf Province. MS.C. Thesis, Faculty of Science, University of Kufa.
7. Chaudhary NA, Munawar MD, Khan MT, Rehan K, Sadiq A, Bhatti HW, Rizvi ZA. Epidemiology, bacteriological profile, and antibiotic sensitivity pattern of burn wounds in the burn unit of a tertiary care hospital. Cureus. 2019;11(6):e4794. DOI: 10.7759/cureus.4794
8. Newman JW, Floyd RV, Fothergill JL. The contribution of *Pseudomonas aeruginosa* virulence factors and host factors in the establishment of urinary tract infections. FEMS Microbiology Letters. 2017;364(15):fnx124. https://doi.org/10.1093/femsle/fnx124
9. Hossein HM, Mehdi RM, Masoumeh A, Gholamreza A, Masoud DM. Molecular evaluation of *Pseudomonas aeruginosa* isolated from patients in burn ward. ICU and ITU in a number of hospital in Kerman province. 2015;5(S2):1428–1431.
10. Nikbin VS, Aslani MM, Sharafi Z, Hashemipour M, Shahcheraghi F, Ebrahimipour GH. Molecular identification and detection of virulence genes among *Pseudomonas aeruginosa* isolated from different infectious origins. Iran J Microbiol. 2012;4(3):118–123.
11. Leboffe MJ, Pierce BE. Photographic Atlas for the microbiology laboratory. 4th editio. USA: Douglas N. Morton. 2011.
12. Spilker T, Coenye T, Vandamme P, LiPuma JJ. PCR-based assay for differentiation of *Pseudomonas aeruginosa* from other *Pseudomonas* species recovered from cystic fibrosis patients. Journal of Clinical Microbiology. 2004; 42(5):2074–2079. DOI: https://doi.org/10.1128/jcm.42.5.2074-2079.2004
13. Hudzicki J. Kirby-Bauer disk diffusion susceptibility test protocol. American Society for Microbiology. 2009; 15:55–63.
14. CLSI (Clinical and Laboratory Standards Institute). Performance Standards for Antimicrobial Susceptibility Testing, Twentieth Informational Supplement, CLSI Document M100- Ed32 February 2022 Replaces M100-Ed31.
15. Maniatis T, Fritsch EF, Sambrook J. Molecular cloning a Laboratory Manual Gold Spring Harber Laboratory. New York, Biotechnology. 1982; 5(6):257–261.
16. Aljebory IS. PCR detection of some virulence genes of *Pseudomonas aeruginosa* in Kirkuk city, Iraq. Journal of Pharmaceutical Sciences and Research. 2018;10(5):1068–1071.
17. Meradji S, Barguigua A, cherif Bentakouk M, Nayme K, Zerouali K, Mazouz D, Chettibi H, Timinouni M. Epidemiology and virulence of VIM-4 metallo-beta-lactamase-producing *Pseudomonas aeruginosa* isolated from burn patients in eastern Algeria. Burns. 2016; 42(4):906–918. https://doi.org/10.1016/j.burns.2016.02.023
18. Alkhulaifi ZM, Mohammed KA. The Prevalence of Cephalosporins resistance in *Pseudomonas aeruginosa* isolated from clinical specimens in Basra, Iraq. University of Thi-Qar Journal of Science. 2023;10(1 (SI)). DOI: https://doi.org/10.32792/utq/utjsci/v10i1(SI).1010
19. Essayagh M, Essayagh T, Essayagh S, El Hamzaoui S. Epidemiology of burn wound infection in Rabat, Morocco: Three-year review. Médecine et Santé Tropicales. 2014; 24(2):157–164. DOI : 10.1684/mst.2014.0315
20. Mahmoud AB, Zahran WA, Hindawi GR, Labib AZ, Galal R. Prevalence of multidrug-resistant *Pseudomonas aeruginosa* in patients with nosocomial infections at a university hospital in Egypt, with special reference to typing methods. J Virol Microbiol. 2013; 2013:1–13. DOI: 10.5171/2013.290047
21. Jalil MB, Abdul-Hussien ZR, Al-Hmudi HA. Isolation and identification of multi drug resistant biofilm producer *Pseudomonas aeruginosa* from patients with burn wound infection in Basra province/Iraq. IJDR. 2017; 7(11): 17258–17262.
22. Othman N, Babakir-Mina M, Noori CK, Rashid PY. *Pseudomonas aeruginosa* infection in burn patients in Sulaimaniyah, Iraq: risk factors and antibiotic resistance rates. The Journal of Infection in Developing Countries. 2014; 8(11):1498–1502. doi: 10.3855/jidc.4707.
23. Khosravi AD, Taee S, Dezfuli AA, Meghdadi H, Shafie F. Investigation of the prevalence of genes conferring resistance to carbapenems in *Pseudomonas aeruginosa* isolates from burn patients. Infection and Drug Resistance. 2019; 12:1153–1159. https://doi.org/10.2147/IDR.S197752
24. Khoshnood S, Khosravi AD, Jomehzadeh N, Montazeri EA, Motahar M, Shahi F, Saki M, Seyed-Mohammadi S. Distribution of extended-spectrum β-lactamase genes in antibiotic-resistant strains of *Pseudomonas aeruginosa* obtained from burn patients in Ahvaz, Iran. Journal of Acute Disease. 2019; 8(2):53–57. DOI: 10.4103/2221-6189.254426
25. Rashid KJ, Babakir-Mina M, Abdilkarim DA. Characteristics of Burn Injury and Factors in Relation to Infection among Pediatric Patients. MOJ Gerontol Ger. 2017; 1(3): 57–66. DOI:10.15406/MOJGG.2017.01.00013
26. Al-Aali KY. Microbial profile of burn wound infections in burn patients, Taif, Saudi Arabia. Arch Clin Microbiol. 2016;7(2):1–9.
27. Sewunet T, Demissie Y, Mihret A, Abebe T. Bacterial profile and antimicrobial susceptibility pattern of isolates among burn patients at Yekatit 12 hospital burn center, Addis Ababa, Ethiopia. Ethiopian Journal of Health Sciences. 2013; 23(3):209–216. DOI:10.4314/ejhs.v23i3.3
28. Mirzaei B, Bazgir ZN, Goli HR, Iranpour F, Mohammadi F, Babaei R. Prevalence of multi-drug resistant (MDR) and extensively drug-resistant (XDR) phenotypes of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolated in clinical samples from Northeast of Iran. BMC research notes. 2020; 13:1–6.
29. Ahmadian L, Haghshenas MR, Mirzaei B, Norouzi Bazgir Z, Goli HR. Distribution and molecular characterization of resistance gene cassettes containing class 1 integrons in multi-drug resistant (MDR) clinical isolates of *Pseudomonas aeruginosa*. Infection and Drug Resistance. 2020; 13:2773–2781. https://doi.org/10.2147/IDR.S263759
30. Alkhudhairy MK, Al-Shammari MM. Prevalence of metallo-β-lactamase–producing *Pseudomonas aeruginosa* isolated from diabetic foot infections in Iraq. New microbes and new infections. 2020; 35:100661. https://doi.org/10.1016/j.nmni.2020.100661
31. de Almeida KD, Calomino MA, Deutsch G, de Castilho SR, de Paula GR, Esper LM, Teixeira LA. Molecular characterization of multidrug-resistant (MDR)

Pseudomonas aeruginosa isolated in a burn center. Burns. 2017; 43(1):137–43. https://doi.org/10.1016/j.burns.2016.07.002
32. Kishk RM, Abdalla MO, Hashish AA, Nemr NA, El Nahhas N, Alkahtani S, Abdel-Daim MM, Kishk SM. Efflux MexAB-mediated resistance in *P. aeruginosa* isolated from patients with healthcare associated infections. Pathogens. 2020; 9(6):471. https://doi.org/10.3390/pathogens9060471
33. Al-Orphaly M, Hadi HA, Eltayeb FK, Al-Hail H, Samuel BG, Sultan AA, Skariah S. Epidemiology of multidrug-resistant *Pseudomonas aeruginosa* in the Middle East and North Africa Region. Msphere. 2021; 6(3):e00202-21. DOI: https://doi.org/10.1128/msphere.00202-21
34. Peymani A, Naserpour-Farivar T, Zare E, Azarhoosh KH. Distribution of blaTEM, blaSHV, and blaCTX-M genes among ESBL-producing *P. aeruginosa* isolated from Qazvin and Tehran hospitals, Iran. J Prev Med Hyg. 2017; 58(2): E155–E160.
35. Qader MK, Solmaz H, Merza NS. Molecular Typing and Virulence Analysis of *Pseudomonas aeruginosa* Isolated From Burn Infections Recovered From Duhok and Erbil Hospitals/Iraq. UKH Journal of Science and Engineering. 2020; 4(2):1–10. DOI:10.25079/ukhjse.v4n2y2020.pp1-10
36. Doi Y. Treatment options for carbapenem-resistant gram-negative bacterial infections. Clinical Infectious Diseases. 2019; 69(Supplement_7):S565–S575. https://doi.org/10.1093/cid/ciz830
37. Haghighifar E, Dolatabadi RK, Norouzi F. Prevalence of blaVEB and blaTEM genes, antimicrobial resistance pattern and biofilm formation in clinical isolates of *Pseudomonas aeruginosa* from burn patients in Isfahan, Iran. Gene Reports. 2021; 23:101157. https://doi.org/10.1016/j.genrep.2021.101157
38. Adjei CB, Govinden U, Essack SY, Moodley K. Molecular characterisation of multidrug-resistant *Pseudomonas aeruginosa* from a private hospital in Durban, South Africa. Southern African Journal of Infectious Diseases. 2018; 33(2):38–41. https://hdl.handle.net/10520/EJC-107ba37322
39. AL-Shamaa NF, Abu-Risha RA, AL-Faham MA. Virulence genes profile of *Pseudomonas aeruginosa* local isolates from burns and wounds. Iraqi Journal of Biotechnology. 2016;15(3):31–39.
40. Al-Dahmoshi HO, Al-Khafaji NS, Jeyad AA, Shareef HK, Al-Jebori RF. Molecular detection of some virulence traits among *Pseudomonas aeruginosa* isolates, Hilla-Iraq. Biomedical and Pharmacology Journal. 2018; 11(2):835–42. DOI: https://dx.doi.org/10.13005/bpj/1439
41. Khan AA, Cerniglia CE. Detection of *Pseudomonas aeruginosa* from clinical and environmental samples by amplification of the exotoxin A gene using PCR. Applied and Environmental Microbiology. 1994; 60(10):3739–3745. DOI: https://doi.org/10.1128/aem.60.10.3739-3745.1994
42. Rawya B, Magda el N, Amr el S, Ahmed el D. *P aeruginosa* exotoxin A as a virulence factor in burn wound infections. EJMM-Egyptian Journal of Medical Microbiology. 2008; 17: 125–133.
43. Stover CK, Pham XQ, Erwin AL, Mizoguchi SD, Warrener P, Hickey MJ, Brinkman FS, Hufnagle WO, Kowalik DJ, Lagrou M, Garber RL. Complete genome sequence of *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen. Nature. 2000; 406(6799):959–64.