DNA Sequences of \textit{qacE}Δ\textit{1} Gene in \textit{Pseudomonas Aeruginosa} Isolated from Wounds and Burns Infections.

Shahla Najim Abed Al-Azzawi\textsuperscript{1}, Rana Mujahid Abdullah\textsuperscript{2}

\textsuperscript{1,2} Department of Biology, College of Education for Pure Science (Ibn Al-Haitham), University of Baghdad, Baghdad, Iraq.

\textsuperscript{1}shahlanajim33@gmail.com, \textsuperscript{2}dr.rana_alshwaikh@yahoo.com

**Abstract**

A total of 69 isolates of \textit{Pseudomonas aeruginosa} were obtained from different clinical samples including wounds 24 (35\%) and burns 45 (65\%), the genes that responsible for \textit{Pseudomonas aeruginosa} resistance isolates was detected, the results showed that quaternary ammonium compound delta 1 (\textit{qacE}Δ\textit{1}) gene was 97.1\% in \textit{Pseudomonas aeruginosa} isolation. 24 (35\%) in the wounds and 43 (62.31\%) in burns, the size of the gene showed 285 bp, the results of the \textit{qacE}Δ\textit{1} analysis showed a single mutation of the mutation type.

The results of genetic analysis tree were showed after comparing presence of genetic variations of the \textit{qacE}Δ\textit{1} gene with their relatives from the nucleotide sequences as containing variations occurring at one distance and equally in \textit{Pseudomonas aeruginosa} and other bacterial species.

**Keywords:** \textit{Pseudomonas aeruginosa}, \textit{qacE}Δ\textit{1}, Sequencing analysis.

**DOI:** http://doi.org/10.32894/kujss.2019.14.3.2
Pseudomonas aeruginosa لبكتريا qacEΔ1

التحليل التتابعي لجين

المعزولة من التهابات الجروح والحروق

شله نجم عبد العزاوي1، رنا ميجاد عبدالله2

قسم علوم الحياة، كلية التربية للعلوم الصرفة/إبن الييثم، جامعة بغداد، بغداد، العراق.

1shahlanajim33@gmail.com، 2dr.rana_alshwaikh@yahoo.com

المتخص

تم عزل 69 عزلة من بكتريا Pseudomonas aeruginosa المعزولة من حالات سريرية تضمنت 24 عزلة من Pseudomonas في الجروح (35%) و 45 عزلة من الحروق (65%). وتم عزل الجين المسؤول عن مقاومة بكتريا Pseudomonas aeruginosa qacEΔ1، ويمكن أن يشكل الجين Pseudomonas aeruginosa 97.1% من بكتريا aeruginosa في الدراسة. وبينت النتائج أن 75% من الحروق و 35% من الجروح كانت حجم الجين qacEΔ1 زوج قاعد وزوج قاعد وزوج قاعد وزوج قاعد وزوج قاعد وزوج قاعد وزوج قاعد وزوج قاعد وزوج قاعد وزوج قاعد وزوج قاعد وزوج قاعد وزوج قاعد وزوج قاعد وزوج قاعد وزوج قاعد وزوج قاعد وزوج قاعد وزوج قاعد وزوج قاعد وزوج قاعد وزوج قاعد وزوج قاعد وزوج قاعد وزوج قاعد وزوج قاعد وزوج قاعد وزوج قاعد وزوج قاعد وزوج قاعد وزوج قاعد وزوج قاعد وزوج C. وظهرت النتائج تحليل الشجرة التطورية أن هناك تنوع وراثي للجين qacEΔ1 في تسلسل التكبيترات وكانت تمتلك Pseudomonas aeruginosa نفس النوع بين الأنواع الأخرى من عزلات بكتريا Pseudomonas aeruginosa والأنواع الأخرى من البكتريا.

الكلمات الدالة: Pseudomonas aeruginosa، qacEΔ1، التحليل التتابعي

DOI: http://doi.org/10.32894/kujss.2019.14.3.2
1. Introduction:

*Pseudomonas aeruginosa* is a major hospital–acquired pathogen and is an opportunistic pathogen. Its morbidity increased for people having cancer, immunodeficiency diseases and people with Acquired Immune Deficiency Syndrome AIDS [1]. As *Pseudomonas aeruginosa* is considered the first pathogen which causes many inflammation of the burns, wounds, bacteremia in serious injuries and urinary tract infection, Otitis media, eye infection, inflammation of the brain membranes (Meningitis), endocarditic, respiratory treat infection such as Pneumonia especially in people with cystic fibrosis patients and joints infection, gastrointestinal infection and skin and soft tissue infection [2, 3].

*Pseudomonas aeruginosa* has many mechanisms for resistance to both antibiotic and antiseptics, making them widely spread and difficult to treat it owns the structural changes in the membrane a of bacteria that causes absence of outside membrane porins, as well as the presence of ESBLs β-lactamase, efflux system pumps and plasmid resistance, it also owns *Pseudomonas aeruginosa* the genes of resistance to disinfectants, including the \(qacE\Delta1\), represent a mutation of the \(qac\) gene, which acts as a multidrug transfer gene. This gene is located in the integron class 1 which allows it to transport between the plasmid and the chromosome and make of *Pseudomonas aeruginosa* resistance to disinfectants that used in hospitals [4].

The aim of this study was isolate and diagnose *Pseudomonas aeruginosa* from wounds and burns infection with detection of \(qacE\Delta1\) gene which is responsible for the resistance of these bacteria to disinfectants. DNA sequencing analysis of \(qacE\Delta1\)gene and detection the mutation and protein translate.

2. Materials and methods:

2.1. Specimen collection:

One hundred swab was collected from patients with burns and wounds infection from Al-Kindi Teaching Hospital, Al-Yarmouk Teaching Hospital, Baghdad Teaching Hospital, National Center for Educational Laboratories, Al-Shahid Ghazi Hariri Specialist Hospital, Specialized Burns Center (Medical City) and Al-Zaafarania General Hospital.
2.2. Isolation and diagnosis:

The samples were cultured on the Cetrimide agar medium, MacConkey agar, and blood agar. Biochemical tests (oxidase and catalase) were performed for the final detection of isolates using API20E system according to the instruction by BioMerieux Company [5].

2.3. Genomic DNA Extraction:

A Bioneer extraction kit was used for DNA Bacteria Kit extraction according to manufacture instruction Presto™Mini the supplier of the company (Bioneer, Korea) to extract DNA from the isolates P. aeruginosa according to the instructions of the company.

2.4. DNA purity:

The purity of DNA output in the Nano-drop device was measured the purity of obtained was between (1.7-2).

2.5. qacE Δ1 gene primer design:

Primers were designed using the Primer 3 plus program from the NCBI website primer sequence (5-3)

F: (GAAAGGCTGGCTTTTTCTTG),
R: (GCAATTATGAGCCCATACC) which size 285 bp.

2.6. Prepare PCR mixture:

The reaction mixture consisted (5μl GO Taq Green Master Mix Bioneer (Korea), 2 μl of F-Primer, 2 μl of R-Primer, 5 μl of DNA template, 11 μl of Deionized Sterile Distilled Water. The optimum conditions for the detection of this gene were one cycle for 5 minutes at 95° C for initiation 30 cycles for 30 seconds at 95 ° C for DNA denaturation, 30 seconds at 55.5° C for annealing to DNA, 45 seconds at 72° C to elongate and then only one cycle for 5 minutes and at 72° C for final elongation.

2.7. Electrophoresis:

5μl of DNA product was transferred to the gel electrophoresis system using 2% of agaros gel strength 12 cm with 100 volts for 60minutes. Then imaging by using ultraviolet radiation at a wavelength of 260 nanometers and then photographed with a high-resolution camera.
2.8. DNA Sequencing analysis:

After initial amplification of *P. aeruginosa* qacEΔ1 gene, the PCR product of (20μl) DNA of each F primer and R primer was sent to US NICEM Company for sequencing by Genetic analyzer. DNA sequence data were analyzed using NCBI (National Center for Biotechnology Information) database and using BioEdit program (V.7.2.5) [6]

3. Results and discussion:

After the laboratory diagnosis, 69 isolates of *P. aeruginosa* including 24 wounds isolates (35%) and 45 burns isolates (65%). *P. aeruginosa* is most bacteria causes burn infections because it lives in the humid environment and hospitals and is a major cause of both burns and wound infections [7], the present study is similar to that of the researchers [8,9]. Table 1

Table 1: The number and percentage of *P. aeruginosa* isolated from wound and burns infection.

| Sours of isolates | No. of *P. aeruginosa* | Total of samples | (%) |
|-------------------|------------------------|------------------|-----|
| Wounds            | 24                     | 35               | 35  |
| Burns             | 45                     | 65               | 65  |
| Total             | 69                     | 100              | 10  |

These results was agreed with Kucken *et al.* [8] who determined the prevalence of the gene 81% in *P. aeruginosa* isolated from different clinical sources which encodes quaternary ammonium compound delta qacEΔ1 which responsible for the resistance to disinfectants, that including quaternary ammonium compound and cetramide, as well as with the researcher Helal and Khan [9], who determined the prevalence of the gene 79% in *P. aeruginosa*, while the agreement with Dabiri *et al.* [10] who showed that 64.4% gene prevalence in *P. aeruginosa* isolated from different hospitals in China, while Kazama *et al.* [11] showed the prevalence of the gene was 68%, while the Mahzounieh *et al* [12] determined the prevalence of the gene 91% in *P. aeruginosa* which is isolated from burns in Tahran and Isfahan hospitals, the difference in the proportion of the qacEΔ1 gene in *P. aeruginosa* is due to the number of samples and the concentration of the disinfectant [13,14]. Fig 1 and 2.
The sequencing analysis of the qacEΔ1 gene was performed the results showed that isolation Ps-42 which is a single point mutation of type shown in Table 2 and Fig. 3, 4 the thymine amino acid was added on site 96 for reference 5368142 and this mutation did not affect the translation of protein. The mutation is known as a change in the sequence or number of nucleotides in the DNA, which leads to the formation of new sequences of nucleotides [15]. Additions or deletions occur when adding or deleting a nitrogen base and the deletion or addition area may be small since the genetic code consists of three nucleotides, adding or deleting does not affect the reading of the code RNA polymerase, thus it does not affecting the change of amino acids [16].

![Fig. 1: The electrophoresis of qacEΔ1 gene (285bp) produced by PCR technique of P.aeruginosa on agarose 2% volt 100 for 60 min. M: ladder (100-2000bp) line (1, 2,3 ,4, 5,6 ,7,8 ,9,10 ,11, 12, 13, 14,15,16 ,17 ,18,19,20,21,22, 23,24,25,26,27,28,29, 30,31,32) (positive isolate ).](image1)

![Fig. 2: Tlectrophoresis of qacEΔ1 gene(285bp) produced by PCR technique of P.aeruginosa on agarose 2% volt 100 for 60 min. M: ladder (100-2000bp) line (34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,51,52,53,55,56,57,58,59,60,61,62 ,63,64,) (positive isolate ).](image2)
Table 2: Change in nitrogen bases and their effect on the translation of the amino acid of *qacEΔ1* gene in isolate Ps-42 of *P. aeruginosa*.

| No. | Native | Allele | Strain | Position in the PCR fragment | Position in the reference genome |
|-----|--------|--------|--------|-------------------------------|----------------------------------|
| 1   | -      | Thymine| Ps-42  | 95ins96                       | 5368141ins5368142                |

Fig. 3: Sequence analysis of the *qacEΔ1* reference gene in Ps-42 isolate of *P. aeruginosa* dependent DNASTAR laser gene Edit Seq. program.

Web Site: [www.uokirkuk.edu.iq/kujss](http://www.uokirkuk.edu.iq/kujss)  E-mail: kujss@uokirkuk.edu.iq, kujss.journal@gmail.com
**Fig. 4:** The number and location of genetic mutations of the $qacE\Delta 1$ gene (285 bp) in Ps-42 isolate of *P. aeruginosa*.

The $qacE\Delta 1$ gene was registered at the American International Bank of Genes NCBI under the serial number (LC38194.1) [17]. [https://www.ncbi.nlm.nih.gov/nuccore/LC381964](https://www.ncbi.nlm.nih.gov/nuccore/LC381964). The genetic tree analysis of the $qacE\Delta 1$ gene (285 bp) was analyzed using a program in NCBI – BLASTn. ([https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch](https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch)) [18]. The products were then combined with each other and sequences were seen combined with a program a Fig tree tool. ([http://tree.bio.ed.ac.uk/software/figtree/](http://tree.bio.ed.ac.uk/software/figtree/)) [19]. A comparison of 101 sequences of NCBI was found for isolating Ps-42 isolates from *P. aeruginosa* due to the close distance between isolation, Ps-42 and the rest of other bacterial species present within this tree. This gives evidence of the identity of this local isolation studied as shown in Fig. 5.

**4. Conclusion:**

the $qacE\Delta 1$ gene is responsible for resistance *P. aeruginosa isolated* from burns and wounds for disinfectant.
Fig. 5: Genetic tree analysis of the local *P. aeruginosa* isolates. The black color refers to the isolation variations *P. aeruginosa* while the other colors indicate reference sequences for different bacteria species from NCBI.

References

[1] S.L. Gellatly and R.E.W. Hancock, "*Pseudomonas aeruginosa: new insights into pathogenesis and host defenses*", Journal of Pathology Discusses, 67, 159 (2013).

[2] W. Levinson "*Medical Microbiology and Immunology*", 14th Ed., Mc Graw Hill Education, USA, 821 (2016).
[3] J. O. Falkinham, E. D. Hilborm, M. J. Arduino, A. Pruden, and M.A. Edwards, "Epidemiology and Ecology of Opportunistic Premise Plumbing Pathogens: Legionella Pneumophila, Mycobacterium avium and Pseudomonas aeruginosa", Environmental Health Perspectives, 123(8), 478 (2015).

[4] S. H. Mobaraki, M. Aghazadeh, M. H. S. Barhaghi, M.Y. Memar, H. R. Goli, P. Gholizadeh, and H. S. Kafil, "Prevalence of integrons 1, 2, 3 associated with antibiotic resistance in Pseudomonas aeruginosa isolates from Northwest of Iran", International Journal of Biomedical Science, 8(1), 12 (2018).

[5] E. J. Baron, S. M. Finegold and I. L. R. Peterson, "Bailey and Scott's Diagnostic Microbiology", 9th Ed., Mosby Company, Missouri, (2007).

[6] T. A. Hall, "Bioedit: A user friendly biological sequence alignment editor and analysis program windows", Nucleic Acids Symposium Series, 41, 95 (1999).

[7] N. A. Hassuna, A. H. Ibrahim, S. M. Abo-Eleuoon and H.A.W. Rizk, "High prevalence of Multidrug Resistant Pseudomonas aeruginosa recovered from Infected Burn Wounds in children", Archives of Clinical Microbiology, 6(4), 1 (2015).

[8] Z. H. Helal, and M. I. khan, "QacE and QacE ∆1 Genes and their correlation to Antibiotics and Biocides Resistance Pseudomonas aeruginosa", American Journal Biomedical Sciences, 7 (2), 52 (2015).

[9] J. S. Hong, E. J. Yoon, H. Lee, S. H. Jeong and K. Lee, "Clonal dissemination of Pseudomonas aeruginosa sequence type 235 isolates carrying bla IMP-6 and emergence of bla GES-24 and bla IMP-10 on novel genomic islands PAGI-15 and -16 in South Korea", Antimicrobial Agents and Chemotherapy, 60, 7216 (2016).
[10] D. Kucken, H. H. Feucht, and P. M. Kaulfers, "Association of qacE and qacEΔ1 with multiple resistance to antibiotics and antiseptics in Clinical isolates of Gram negative bacteria", FEMS Microbiology Letters, 183, 95 (2000).

[11] H. Dabiri, N. Jonaidi, and K. Dahi, "Distribution of the antiseptic –resistance gens qaEΔ1 in 331 clinical isolates of Pseudomonas aeruginosain china", Elsevier Ltd 93095, (2007).

[12] H. Kazama, H. Hamashima, M. Sasatsu, and T. Arai, "Distribution of the antiseptic-resistance gens qacE and qacEΔ1 in Gram negative bacteria", FEMS Microbiology Letters, 159(2), 173 (1998).

[13] M. Mahzounieh, S. H. Khoshnood, A. Ebrahimi, S. Habibian, .and M. Yaghoubian,"Detection of Antiseptic-Resistance Gene in Pseudomonas and Acinetobacter spp. Isolated from Bun patients", Jundishapur Journal of Natural Pharmaceutical Products, 9(2), e15402 (2014).

[14] M. Mitiku, S. Ali, and G. Kibru, "Antimicrobial Drug Resistance and Disinfectants Susceptibility of Pseudomonas aeruginosa Isolates from Clinical and Enviromental Samples in Jimma University Specialized hospital, Southwest Ethiopia", American Journal of Biomedical and Life Sciences, 2, 40 (2014).

[15] J. R Dettman, J. L. Sztepanacz, and R. Kassen, "The properties of spontanerous mutations in the opportunisitic pathogen Pseudomonas aeruginosa", BMC Genomics, 17, 27 (2016).

[16] M. Lnhof, and C. Schlotterer, "Fitness effects of Proceedings of advantageous mutation in evolving Escherichia coli pulations", Proceedings of the National Academy of Sciences of the United States of America, 98 (3), 1112 (2001).