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DNA Sequences of *qacEΔ1* Gene in *Pseudomonas Aeruginosa* Isolated from Wounds and Burns Infections.

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Abstract

A total of 69 isolates of *Pseudomonas aeruginosa* were obtained from different clinical samples including wounds 24 (35%) and burns 45 (65%)، the genes that responsible for *Pseudomonas aeruginosa* resistance isolates was detected, the results showed that quaternary ammonium compound delta 1 *(qacE*Δ1) gene was 97.1% in *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 *qacE*Δ1 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 *qacEΔ1* gene with their relatives from the nucleotide sequences as containing variations occurring at one distance and equally in *Pseudomonas aeruginosa* and other bacterial species.

Keywords: *Pseudomonas aeruginosa*, *qacEΔ1*, Sequencing analysis.

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التحليل التتابعي لجين *qacEΔ1* لبكتريا *Pseudomonas aeruginosa* المعزولة من التهابات الجروح والحروق

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الملخص

تم عزل 69 عزلة من بكتريا *Pseudomonas aeruginosa* المعزولة من حالات سريرية تضمنت 24 عزلة من الجروح (35%) و 45 عزلة من الحروق (65%). وتم عزل الجين المسؤول عن مقاومة بكتريا *Pseudomonas aeruginosa* وبينت النتائج ان 97.1% من بكتريا *Pseudomonas aeruginosa* تمتلك الجين *qacEΔ1* وكانت 24 (35%) من الجروح و 43 (62.31%) من الحروق وكان حجم الجين 285 زوج قاعده واظهرت نتائج التحليل التتابعي لجين *qacEΔ1* حصول طفره مفرده .

واظهرت نتائج تحليل الشجره التطورية ان هنالك تنوع وراثي للجين *qacEΔ1* في تسلسا النكيوتيدات وكانت تمتلك نفس البعد بين الانواع الاخرى من عزلات بكتريا *Pseudomonas aeruginosa* والانواع الاخرى من البكتريا.

الكلمات الدالة: *Pseudomonas aeruginosa*، *qacEΔ1*، التسلسل التتابعي.

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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 *qacEΔ1*, 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 *qacEΔ1* gene which is responsible for the resistance of these bacteria to disinfectants. DNA sequencing analysis of *qacEΔ1*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.

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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^{TM}Minig 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* Δ*1* 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.

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2.8. DNA Sequencing analysis :

After initial amplification of *P. aeruginosa qacE*Δ1 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. aeurginosa* 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 *qacE*Δ1 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 *qacE*Δ1 gene in *P. aeruginosa* is due to the number of samples and the concentration of the disinfectant [13,14]. Fig 1 and 2.

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The sequencing analysis of the *qacE*Δ1 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].

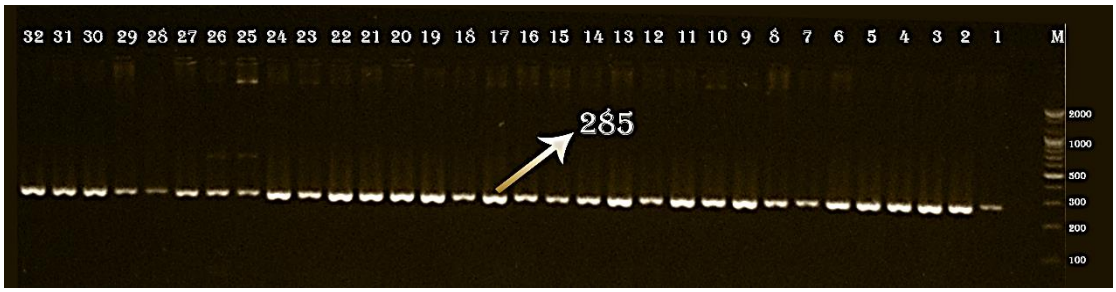

Fig. 1: The electrophoresis of *qacE*Δ1 gene (285bp) produced by PCR technique of *P.aeruginosa* on agarose 2% volt 100 for 60 min. M: ladder (100-2000bp) line (1, 2,3 ,4, 5,6 ,7,8 ,9,10 ,11, 12, 13, 14,15,16 ,17 ,18,19,20,21,22, 23,24,25,26,27,28,29, 30,31,32) (positive isolate).

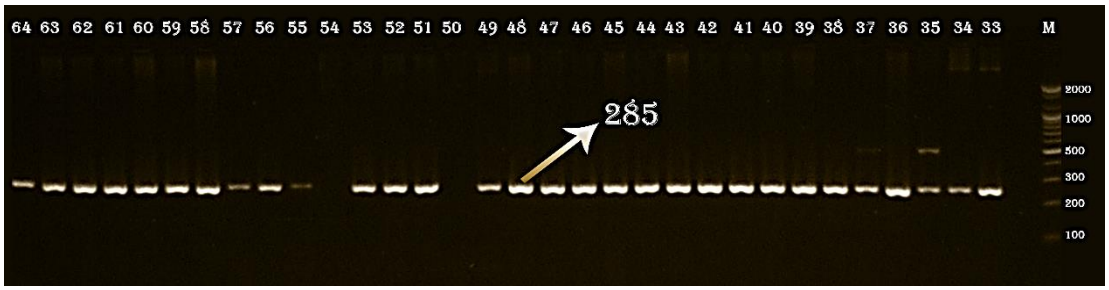

Fig. 2: Tlectrophoresis of *qacE*Δ1 gene(285bp) produced by PCR technique of *P.aeruginosa* on agarose 2% volt 100 for 60 min. M: ladder (100-2000bp) line (34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,51,52,53,55,56,57,58,59,60,61,62 ,63,64,) (positive isolate).

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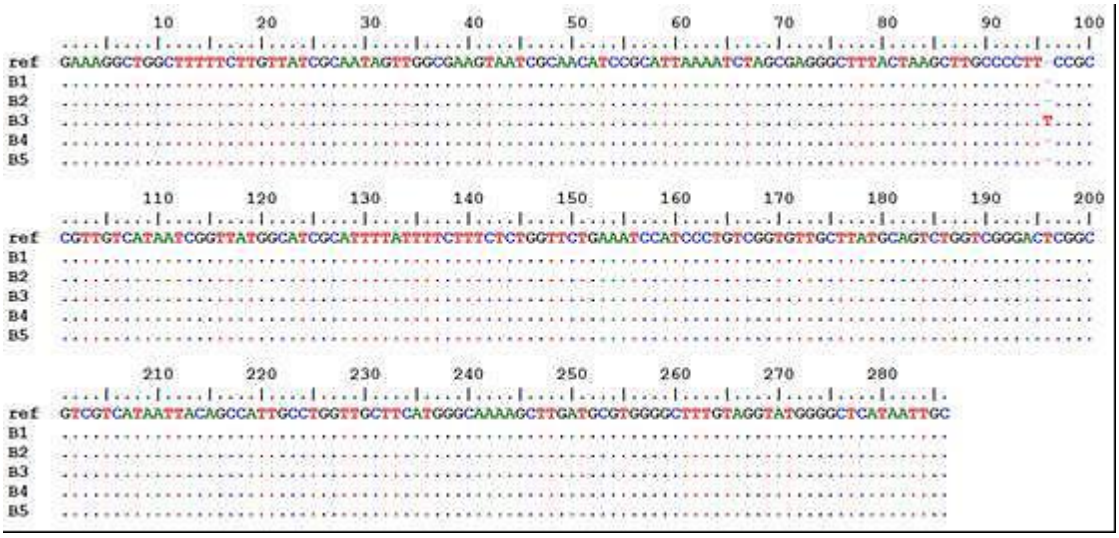
Table 2: Change in nitrogen bases and their effect on the translation of the amino acid of *qacEΔ1* gene in isolate Ps-42 of *P. aeruginosa.*

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



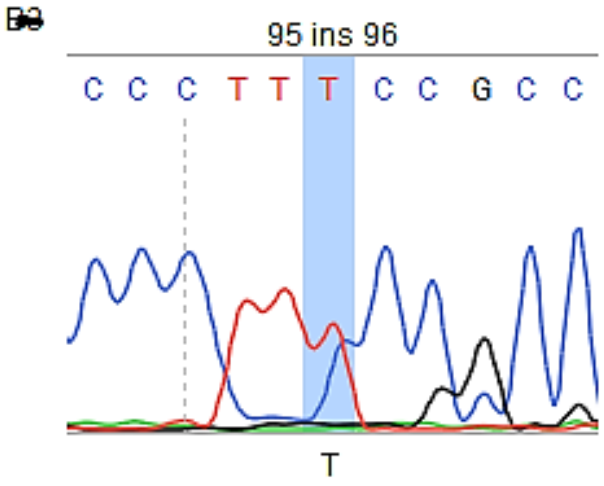


Fig. 3: Sequence analysis of the *qacE*Δ1 reference gene in Ps-42 isolate of *P. aeruginosa* dependent DNASTAR laser gene Edit Seq. program.

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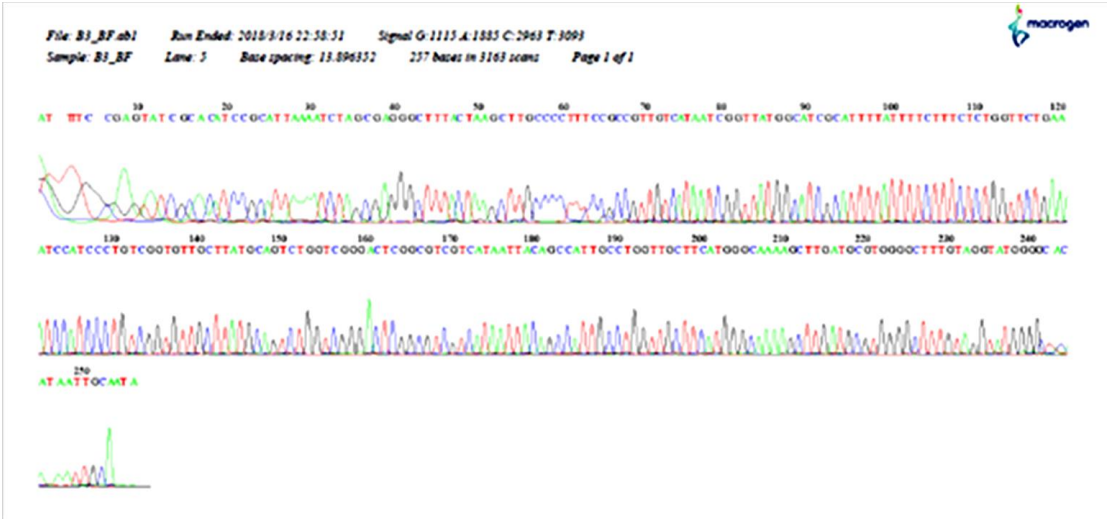

Fig. 4: The number and location of genetic mutations of the *qacE*Δ1 gene (285 bp) in Ps-42 isolate of *P. aeruginosa.*

The *qacE*Δ1 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 . The genetic tree analysis of the *qacE*Δ1 gene (285 bp) was analyzed using a program in NCBI – BLASTn. (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch) [18]. the products were than combined with each other and sequences were seen combined with a program a Fig tree tool. (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*Δ1gene is responsible for resistance *P. aeruginosa isolated* from burns and wounds for disinfectant.

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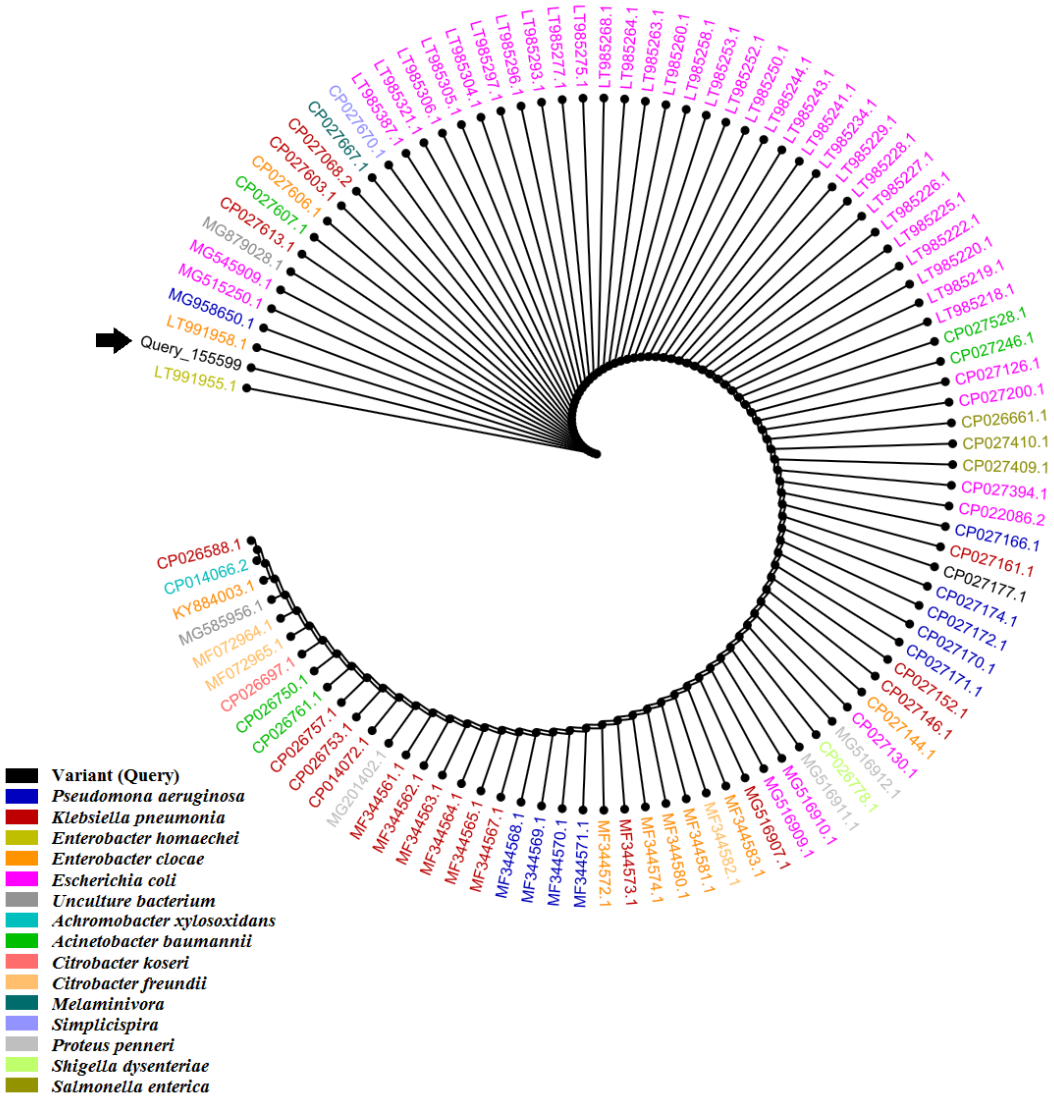

Fig. 5: Genetic tree analysis of the local *P.aeruginosa* isolates. The black color refers to the isolation variations *P. aeruginosa* while the other colors indicate reference sequences for different bacteria species from NCBI.

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