



DNA Analysis of *qacE* Gene in *Pseudomonas Aeruginosa* Isolated from Iraqi Patient

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Article information

Article history:

Received: August, 19, 2021

Accepted: February, 16, 2022

Available online: June, 10, 2022

Keywords:

Pseudomonas aeruginosa,

QacE,

Phylogenetic tree

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Abstract

Pseudomonas aeruginosa is an opportunistic pathogen that can cause different infections such as nosocomial bacterial infection, Urinary tract infection, Meningitis, Eye infection, Otitis Media, Respiratory infections, and Pneumonia, especially in people with Cystic fibrosis, Bone and joints infection, gastrointestinal infection, Skin infection and soft tissue inflammation. Resistance of *P. aeruginosa* to antibiotics and disinfectants is a major problem all over the world due to the random and unrestricted use of antibiotics. This resistance is also likely to occur due to several reasons, including the production of beta-lactamase enzymes and the lack of permeability of their cell wall, possibility of it containing resistance genes and the occurrence of genetic mutations leading to the development of resistance to antiseptics and causing failure in treating infections that caused by *P. aeruginosa*. This study aims to detect and sequence analysis of *qacE* gene in *P. aeruginosa* that responds to the resistance of Quaternary ammonium compounds and detect the mutations in this sequence and detection protein synthesis in this gene. A hundred swabs were collected from patients with burns and wounds infection from many Hospital in Baghdad including Al- Kindi Teaching Hospital, Al-Yarmouk Teaching Hospital, Baghdad Teaching Hospital, National Centre for Educational Laboratories, Al-Shahid Ghazi Hariri Specialist Specialized Burns Centre (Medical City), and Al-Zaafarana General Hospital during the period October 2017 for the end of December 2017. The current study detected 69 isolates of *P. aeruginosa* from wound 68.6% (24/35) and burn samples 69.2% (45/65). The study was investigated *QacE* gene in 97.1% that responses of *P. aeruginosa* resistance to Quaternary ammonium compounds. The sequencing analysis for *QacE* genes were determined and the results appeared multiple mutations including Missense mutations, insertion mutations and several silent mutations, some mutations effected in the translation of protein while others not affected. This study also included the analysis of phylogenetic tree of *QacE* gene and the results showed that the tree consisted of only one species which is *P. aeruginosa*, and the isolates in this study did not deviate of this bacteria even if their mutations were more than 22.

P. aeruginosa is one of the important bacterial. It is found in water, soil, plants, animals, hospitals and on the skin of natural persons. The skin layer provides natural protection for body tissues, so when any burn or wound leads to damage to this tissue and thus provides an environment for the growth and multiplication of microorganisms, including *P. aeruginosa*, which represents a threat to many patients, including patients suffering from burn and wound inflammation Leukemia. Bacteraemia Septicaemia Immunodeficiency [1, 2, 3] *P. aeruginosa* is an opportunistic pathogen that can cause various infections such as nosocomial bacterial infection, Urinary tract infection, Meningitis, Eye infection, Middle ear infections, Otitis Media, Respiratory infections, and Pneumonia, especially in people with Cystic fibrosis [4], Bone and joints infection, gastrointestinal infection, Skin infection and soft tissue inflammation [5, 6, 7].

P. aeruginosa is a disease-causing harmful bacterium because it has many virulence factors that impact its pathogenicity, including flagellum, biofilm, pili IV, the enzyme Exoenzyme S that helps it adhere the in tissue [8, 9], and the enzyme proteolytic enzyme and protein. In addition, Hemolysine, phospholipase, Elastase [10], and an alginate-like exopolysaccharide responsible for the mucoid phenotype, pilus, non-pilus adhesins, flagellum [11] toxins represented by Exotoxin A and Endotoxin, which is responsible for fever and shock associated with Sepsis. Furthermore, *P. aeruginosa* can secrete many pigments, including green-blue pyocyanin, and the culture medium of the bacteria acquires the color of the pyocyanin like Muller Hinton agar and nutrient agar. Bacteria also have a greenish-yellow pyoverdine pigment, and these pigments are dissolved in water [12, 13] in addition to having a layer of alginate and a biofilm, which increases its resistance to antibiotics [14]

Resistance of *P. aeruginosa* to antibiotics and disinfectants is a major problem all over the world due to the random and unrestricted use of antibiotics [15]. This resistance is also likely to occur due to several reasons, including the production of beta-lactamase enzymes and the lack of permeability of their cell wall, as well as the possibility of it containing and acquiring resistance genes from another bacterial genus by plasmids and transposons via bacterial conjugation and the occurrence of genetic mutations in the genes leading to the development of resistance to these antiseptics and causing failure in treating infections caused by *P.aeruginosa* bacteria [16,17].

This study aims to detect and sequence analysis of *qacE* gene in *P. aeruginosa* that responds to the resistance of Quaternary ammonium compounds and detect the mutations in this sequence and detection protein synthesis in this gene.

2. Materials and Methods

2.1. Specimen Collection

A hundred swabs were collected from patients with burns and wounds infection from Al- Kindi Teaching Hospital, Al-Yarmouk Teaching Hospital, Baghdad Teaching Hospital, National Centre for Educational Laboratories, Al-Shahid Ghazi Hariri Specialist Specialized Burns Centre (Medical City), and Al-Zaafarana 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 (France) [18].

2.3. Genomic DNA Extraction

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

2.4. DNA Purity

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

2.5. QacE Gene Primer Design

Primers were designed using the Primer 3 plus program from the NCBI website primer sequence F: (ATGACCAACTATCTCTACCT) R: (AACAACTGGATCAC CAGCA) size 311 bp.

2.6. Prepare PCR Mixture

The reaction mixture consisted (GO Taq Green Master Mix Bioneer (Korea) 5µl, F-Primer 2 µl, R-Primer 2 µl, DNA template 5 µl, Deionized Sterile Distilled Water 11 µl. 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

DNA product 5 µl was transferred to the gel electrophoresis system using agarose gel 2% with 100 volts for 60 minutes, then imaging using ultraviolet radiation at a wavelength of 260 nanometres and photographed with a high-resolution camera.

2.8. DNA Sequencing Analysis

After initial amplification of *P. aeruginosa qacE* gene, the PCR product of (20µl) DNA of each F primer and R primer was sent to US NICEM Company for sequencing by a Genetic analyzer. DNA sequence data were analysed using NCBI (National Centre for Biotechnology Information) database and using BioEdit program (V.7.2.5) [19]

Analysis Phylogenetic tree by using the NCBI-BLASTn software then the results were combined with each other and the merging sequences were viewed by tree Fig. [20, 21].

3. Result and Discussion

The current study detected sixty-nine of *Pseudomonas aeruginosa isolate* from a wound and burn samples. The rate isolates of *Pseudomonas aeruginosa* from wound and burn samples was 68.6% (24/35) and 69.2% (45/65), respectively. *P. aeruginosa* is one of the most important species of the genus *Pseudomonas* due to its widespread and severe pathogens. In addition, they are opportunistic pathogens and the main cause of infection in Intensive Care Units (ICUs), especially in patients admitted to burn centers. Studies have shown that 75% of deaths are related to Sepsis-related burn injuries, especially in developing countries [7, 22, 23].

The current study was agreement with many studies, these study showed that *P. aeruginosa* was most common bacteria isolated from burn and wounds in Baghdad hospitals [7, 24] where the 76% isolation, as well as that results by Pruitt *et al.* (2001) [25] in another study in the United States a rate of 51.2% of *P. aeruginosa* isolate, the reasons of the difference in rates is due to the difference in the source of the sample, number of samples, geographical location, method of sterilizing wounds or burns, sterilization times, as well as the common and indiscriminate use of antibiotics that had a major role in the emergence of resistance.

Detection antiseptic resistance gene (*QacE gene*) by using a PCR polymerase chain reaction in all *P. aeruginosa* isolates using Specialized (*QacE*) gene, as the results showed that 67 isolates (97.1%) to *P. aeruginosa* possessing the *QacE* gene, while that only isolates no.50 and isolate no.54 do not contain this gene. Furthermore, when comparing the replicated with Ladder, it was found that the resulting band has a molecular weight (311 bp) and as shown in Figure (1).

The result found the differed with [26] who determined the prevalence of the *QacE* gene in 42.3% of *P. aeruginosa* for disinfectants, including quaternary ammonium compounds, which include Cetramide, the researcher Kucken *et al.* [27] determined the prevalence of the *QacE* gene at 32.8%. On the other hand, Mahzounieh *et al.* [28] found that the prevalence of the *QacE* gene is 50% in *P. aeruginosa* isolated from burn hospitals in Tehran and Isfahan. The difference in *QacE* gene concentrations in *P. aeruginosa* isolates is due to the number of samples taken, the source of the isolate, the type, and the concentration of the disinfectant [29, 30]. As for inside Iraq, there are no studies similar to this current study.

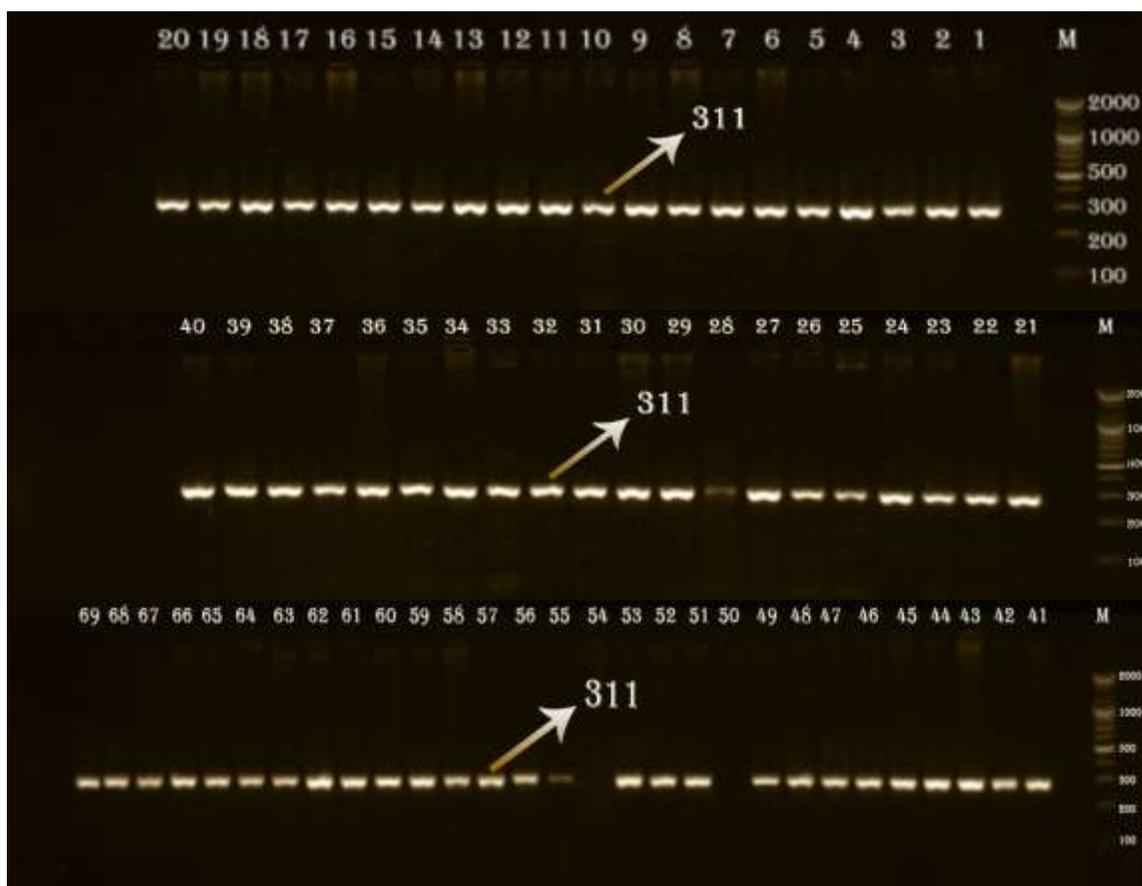


Figure (1): Electrophoresis of PCR Product of *QacE* gene (311 b p) in *P. aeruginosa* at 2% Agarose 100 volt at 60 mint Line M: DNA ladder (100 - 2000 bp), Line 1,2, 3, 4 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21,22,23,24,25,26,27,28,29,30,31,32 ,33,34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 51, 52, 53, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69 (*P. aeruginosa* isolates possessing the *QacE* gene).

3.1. DNA Sequencing

The results of the *QacE* gene's DNA sequence analysis showed that the Ps-1 isolate did not have any genetic mutation. While genetic mutations occurred in the DNA of the *QacE* gene for the isolates PS-2, Ps-3, Ps-4, Ps- 5 isolated from burns of *P. aeruginosa*. As (4) genetic mutations occurred in the isolate Ps-2 of *P. aeruginosa* isolated from burns, the first was a substitution of the nitrogenous base Adenine with Guanine at the site 195 subjects 56117843, and also the nitrogen base of Cytosine was replaced by the guanine at position 5611 in subject 19846 The substitution of the Cytosine with guanine was obtained at the position 258 in subject 56117906. The Cytosine was replaced by Thymine at position 270 at the subject o56117918. That all mutations were of the type of silent mutation, which produced the same amino acid and did not affect the protein translation as in Table (1), Figure (2), and Figure (3).

Table (1): Changes in Nitrogenous Bases and Their Effect on Translating Amino Acid of the *QacE* gene for Ps-2 Isolate of *P. aeruginosa*.

Nitrogen base	Change in nitrogen base	Position	Subject	Amino acid	Change in amino acid	Type of mutation
Adenine	Guanine	195	56117843	-	-	Silent
Cytosine	Guanine	198	65117846	-	-	Silent
Cytosine	Guanine	258	6117906	-	-	Silent
Cytosine	Thymine	270	56117918	-	-	Silent

As for the isolation of Ps-3, there was a (19) mutation of *P. aeruginosa*. The nitrogen base Cytosine replaced the nitrogen base of Adenine at position 78 subjects, 56117726. The nitrogen base of adenine was replaced by guanine at position 90 for subject 56117738, and the nitrogen base was replaced by nitrogenous base Cytosine at the guanine at position 141 for subject 56117789. The nitrogenous base replaced the nitrogenous base Adenine with nitrogenous base Adenine at position 144, nitrogenous base Adenine at position 144, and nitrogenous base Adenine at the position of the residue 56117804. The nitrogen base was replaced by the nitrogenous base of guanine at position 168 56117816. The nitrogen base replaced the nitrogenous base of guanine at position 168 56117819, and the nitrogen base replaced the nitrogenous base cytosine with the nitrogen base of guanine at position 192 of the liquor 56117840. The genetics of adenine with the nitrogen base of guanine at the nitrogen base 195 for alcohol 56117843, the nitrogen base replaced cytosine with the nitrogen base of guanine at the position 198 at the residue 56117846, the nitrogen base replaced the nitrogenous base Cytosine with the nitrogenous base Thymine substituted the nitrogenous base Thymine at position 20755 at the site of Thymine 5611, the nitrogen base of Thymine was at the site 222 at the residue 56117870, the nitrogenous base replaced the nitrogenous base Cytosine with the nitrogenous base of guanine at position 255 at the residue 56117903, the nitrogenous base replaced the cytosine with the nitrogenous base of the guanine at the position 26794.

Mutation did not affect the translation of the protein, and the nitrogenous base of guanine was replaced by the nitrogenous base of thymine at position 73 at the inundation of 56117721, and the mutation was of the type of missense mutation as the amino acid was converted to the amino acid serine. The substitution of the thymine base with the nitrogen base cytosine arrived at the residue 79 sites 56117727, and the mutation was of the Missense type. The amino acid was converted from Phenylalanine to the amino acid Lucien. Missense, i.e., the amino acid was replaced by Leucine to the amino acid Valine, and the nitrogenous base Cytosine replaced the nitrogen base with the nitrogen base Adenine at the residue 196 sites 56117844, which led to the replacement of the amino acid Leucine with the amino acid Methionine. This mutation is of the Missense type, as shown in Table (2), Figures (2 & 3).

Table (2): Changes in Nitrogenous Bases and Their Effect on Translating Amino Acid of the QacE gene for Ps-3 Isolate of *P. aeruginosa*.

Nitrogen base	Change in nitrogen base	Position	Subject	Amino acid	Change in amino acid	Type of mutation
Guanine	Thymine	73	56117721	Alanine	Serine	Missense
Adenine	Cytosine	78	56117726	-	-	Silent
Thymine	Cytosine	79	56117727	Phenyl-alanine	Leucine	Missense
Guanine	Adenine	87	56117735	-	-	Silent
Adenine	Guanine	90	56117738	-	-	Silent
Cytosine	Guanine	124	56117772	Leucine	Valine	Missense
Cytosine	Guanine	141	56117789	-	-	Silent
Guanine	Adenine	144	56117792	-	-	Silent
Cytosine	Thymine	156	56117804	-	-	Silent
Cytosine	Adenine	168	56117816	-	-	Silent
Cytosine	Guanine	171	56117819	-	-	Silent
Cytosine	Guanine	192	56117840	-	-	Silent
Adenine	Guanine	195	56117843	-	-	Silent
Cytosine	Adenine	196	56117844	Leucine	Methio-nine	Missense
Cytosine	Guanine	198	56117846	-	-	Silent
Cytosine	Thymine	207	56117855	-	-	Silent
Guanine	Thymine	222	56117870	-	-	Silent
Cytosine	Guanine	255	56117903	-	-	Silent
Cytosine	Guanine	264	56117912	-	-	Silent

These results showed many mutations, including silent mutation that did not affect any change of amino acid, and the result showed four missense mutations that change amino acid instead of the original amino acid. The change in the amino acid sequence is either acceptable when a single base change leads to the substitution of an amino acid with another similar in function to the original amino acid.

The change may be partially acceptable which results in a protein molecule with partial activity, or is unacceptable when the protein molecule is unable to function in its role and function. When a change occurs in the genetic code to one of the three genetic codes UAA, UAG, or UGA, then the nonsense mutation occurs.

When a codon nonsense appears in the mRNA molecule sequence that leads to the premature termination of the amino acids in the peptide chain, and then producing an incomplete piece of the protein molecule to be produced that cause of ineffective protein [31]. While Missense mutations are mutations lead to change in amino acid in the protein sequence. [32].

The isolation of Ps-4 replaced cytosine with thymine at position 97 at subject 56117745, and the nitrogenous base of adenine was replaced by guanine at position 195 for subject 56117843. It was found that there are two silent mutations as shown in Table (3), Figure (2), and Figure (3).

Table (3): Changes in Nitrogenous Bases and Their Effect on Translating Amino Acid of the *QacE* gene for Ps-4 Isolate of *P. aeruginosa*.

Nitrogen base	Change in nitrogen base	Position	Subject	Amino acid	Change in amino acid	Type of mutation
Cytosine	Thymine	97	56117745	-	-	Silent
Adenine	Guanine	195	56117843	-	-	Silent

Ps-5 isolation replaced Cytosine with Thymine at site 207 at subject 56117855. When analyzing the results of translating the amino acid to the *QacE* gene with the effects of the original amino acid translation, it was found that one mutation of the silent mutation as shown in Table (4), Figures (2), and Figures (3). The *QacE* gene was registered in the NCBI under serial numbers (LC381962.1 and LC381963.1).

Table (4): Changes in Nitrogenous Bases and Their Effect on Translating Amino Acid of The *QacE* gene for Ps-5 Isolate of *P. aeruginosa*.

Nitrogen base	Change in nitrogen base	Position	Subject	Amino acid	Change in amino acid	Type of mutation
Cytosine	Thymine	207	56117855	-	-	Silent

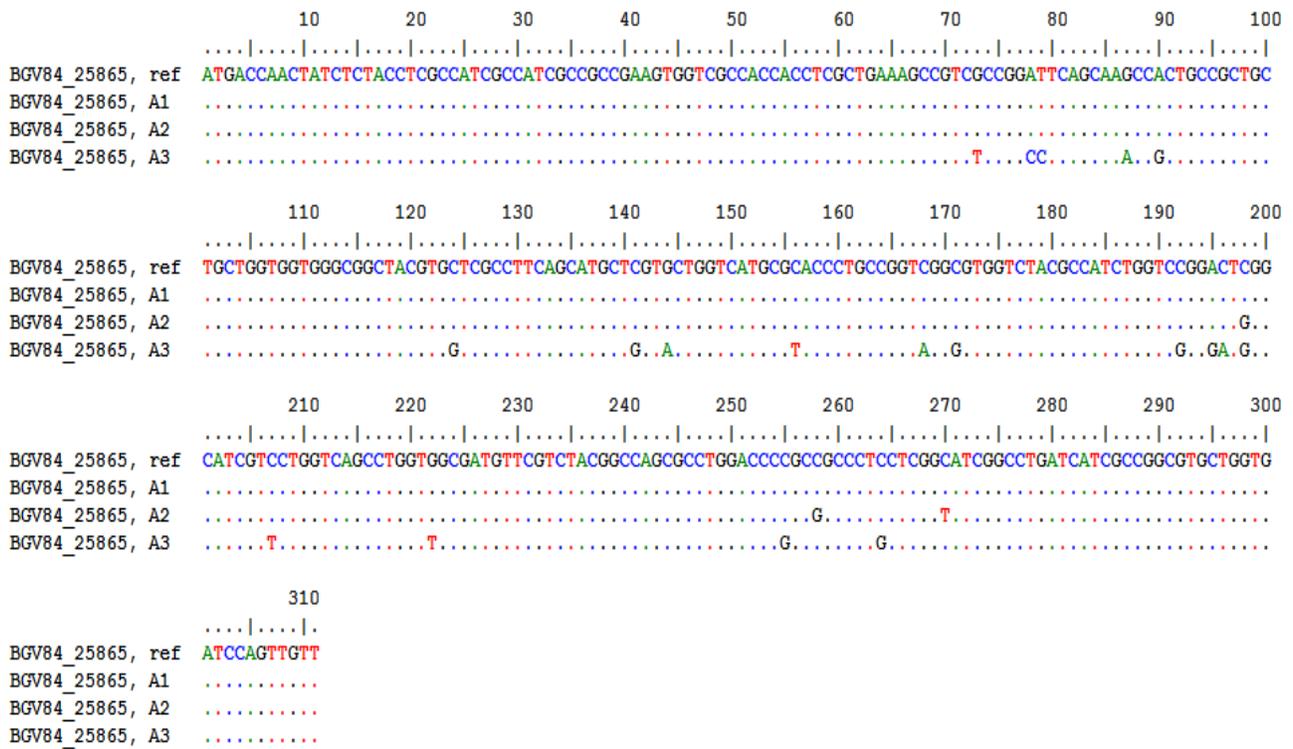


Figure (2): Sequence Analysis of The *QacE* Reference Gene with Twenty-Six Changes Occurring for *P.aeruginosa* Isolates PS-2, Ps-3, Ps-4, and Ps-5 Using The DNASTAR Lasergene Edit Seq Program.

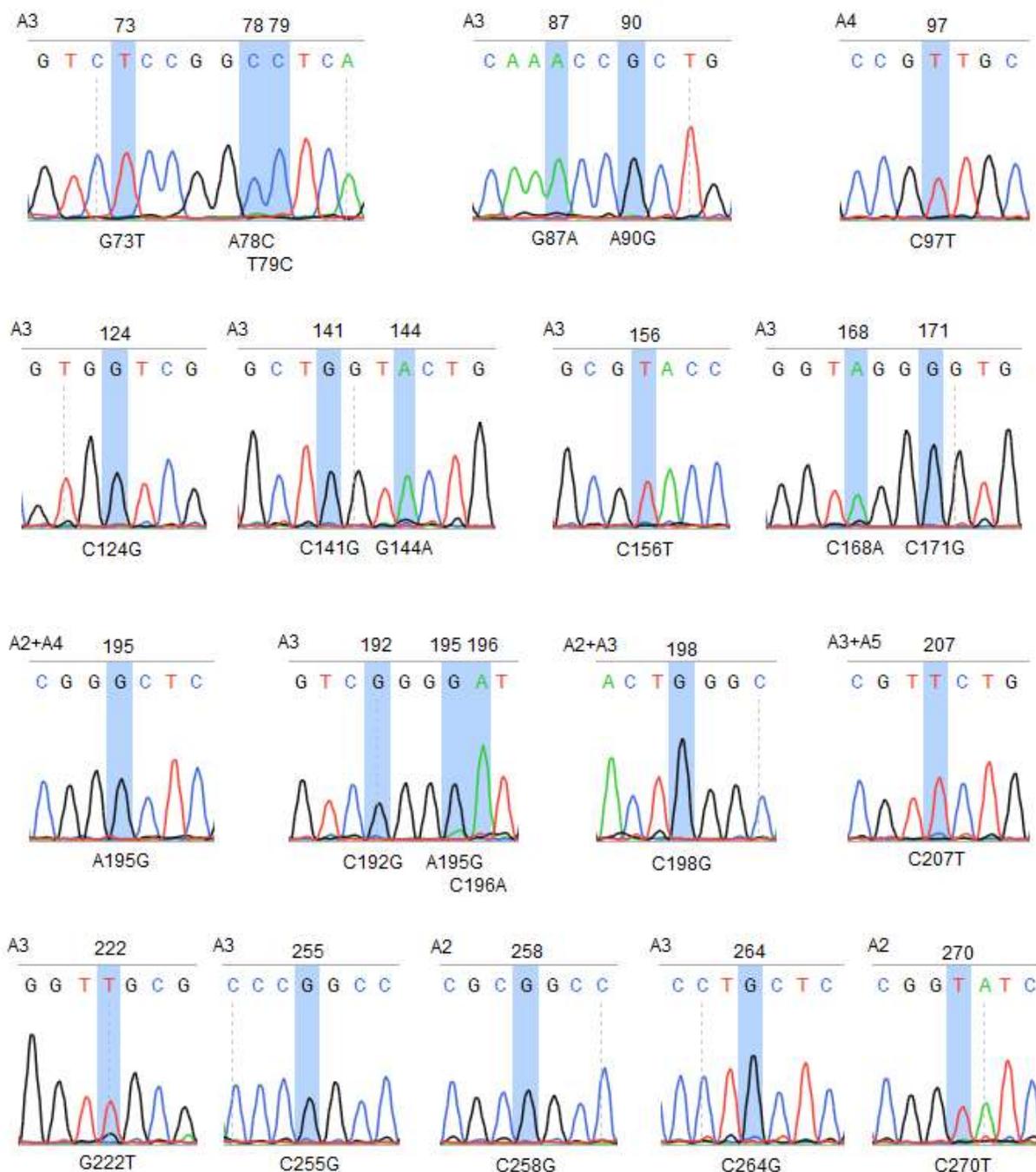


Figure (3): The Number and Location of Genetic Mutations of The *QacE* gene (311 bp) of The Isolates Ps-2, Ps-3, Ps-4, and Ps-5 within the *P.aeruginosa* Bacterial Sequences.

3.2. Analysis Phylogenetic Tree

The *QacE* gene size (311 base pairs) was analyzed using the NCBI-BLASTn software [20]. The results were then combined and the merging sequences were viewed by Fig tree and 104 sequences were used for comparison from the NCBI website of global isolates of *P. aeruginosa* and found that tree of *P. aeruginosa* local isolates have occupied several different sites within the genetic tree and species, it was found that this genetic tree consisted of only one type of *P. aeruginosa*, and it was found that all local sequences did not deviate from the species even if its mutations increased to 22 A mutation as shown in Figure (4) [21].

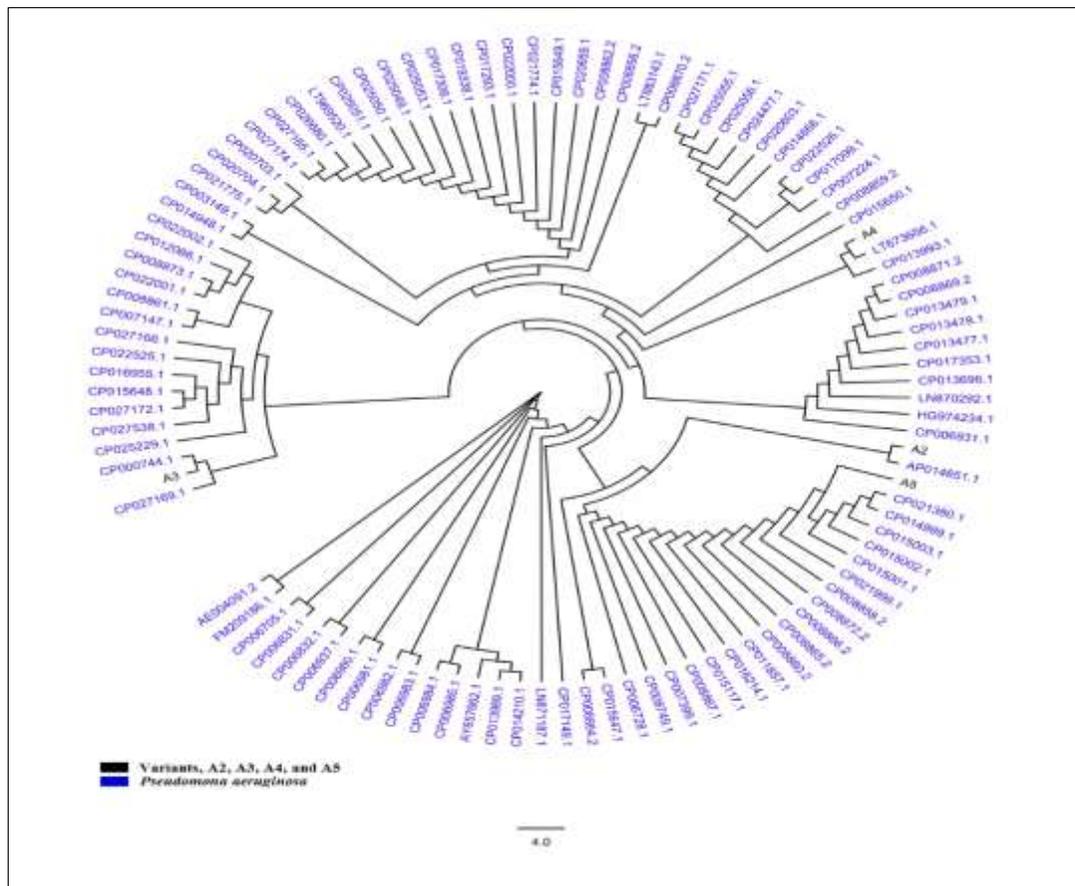


Figure (4): Analysis of Phylogenetic Tree of *QacE* gene (311 base pairs) of *Pseudomonas aeruginosa* Isolates. The Black Colour Indicates the Heterogeneities of Ps-2, Ps-3, Ps-4, and Ps-5 While the Blue Colour Indicates the Reference *Pseudomonas aeruginosa* Obtained from The NCBI Site.

4. Conclusions

The results showed many mutations in *QacE* gene, including silent mutation this mutation did not affect in translation of amino acid and result showed four missense mutations that change an amino acid instead of original amino acid.

The change in amino acid sequence is either acceptable when a single base change leads to the substitution of an amino acid with another similar in function to the original amino acid. The change may be partially acceptable which results in a protein molecule with partial or abnormal activity, or the change is unacceptable when the protein molecule is unable to function in its role and function.

Conflict of Interest

The authors declare that they have no conflict of interest.

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