Current Research in Microbiology and Biotechnology
Vol. 5, No. 3 (2017): 1062-1070
Research Article
Open Access

ISSN: 2320-2246

Nucleotide sequences of the *Pseudomonas aeruginosa* algD gene isolated from Iraqi patients with otitis media

Rana M. Abdullah Al-Shwaikh[1], Arwa M. Abdullah Al-Shuwaikh[2,*] and Abbas Falih Al-arnawtee[1]

[1] *Department of Biology, College of Education for pure science Ibn-Al Haitham, University of Baghdad, Baghdad.*
[2] *Department of Microbiology, College of Medicine, Al-Nahrain University, Baghdad, Iraq.*

* Corresponding author: Arwa M. Abdullah Al-Shuwaikh; e-mail: arwa_alshwaikh_2004@yahoo.com

Received: 25 March 2017 Accepted: 24 April 2017 Online: 04 May 2017

ABSTRACT

Out of 28 isolates *of Pseudomonas aeruginosa* from otitis media. The result revealed that the *algD* virulent gene was present in 21 isolates with 75 percentage. The gel electrophoresis showed that the molecular weight of *algD* gene was 1310 bp. DNA sequences of *algD* gene was done, and the results showed presence of some gene mutations like substitution, addition and deletion with 97% identity with the Refseq gene. From the other side, the results of identities of translated nucleotide sequence with the original sequence of amino acids revealed that there were effects of gene mutations on translation of the product protein.

Keywords: *Pseudomonas aeruginosa*, *algD* gene, Sequencing.

1. INTRODUCTION

Pseudomonas aeruginosa (*P. aeruginosa*) is a non-lactose fermentative, aerobic, motile, gram-negative, rod-shaped bacterium [1]. Its > 6 Mbp genome contains at least 5500 open reading frames (ORFs), encoding hundreds of virulence determinants [*2*]. It can cause acute and chronic infections in human. It typically infects the pulmonary tract, urinary tract, burns, wounds, ear and also cause other blood infections [*3*]. *P. aeruginosa* able to grow and survive in almost any environment, lives primarily in water, soil, and vegetation. However, despite abundant opportunities for spread, it rarely causes community-acquired infections in immunocompetent patients. As a result, the pathogen is viewed as opportunistic [*4*]. *P. aeruginosa* produces a number of virulence factors, which, after colonization, can cause extensive tissue damage, bloodstream invasion, and dissemination. Pathogenesis is based on multiple virulence factors: endotoxin, exotoxins, enzymes and an alginate-like exopolysaccharide that is responsible for the mucoid phenotype as well as pilus and non-pilus adhesins and flagellum. The importance of these putative virulence factors depends upon the site and nature of infection [1,5,6].

P. aeruginosa is able to form multiple types of biofilms, which allow them to persistently colonize a variety of surfaces, thereby making their eradication extremely challenging [*2*]. The alginate is a slimy exopolysaccharide layer surrounding *P. aeruginosa* which believed to facilitate bacterial colonization and play an important role in protection of infecting cells from the host immune defenses and antibiotic therapy [7,8,9,10]. The *P. aeruginosa algD* gene encodes GDPmannose dehydrogenase, an enzyme which converts GDPmannose to GDP-mannuronic acid, a precursor of the exopolysaccharide alginate [11]. Most of the genes involved in alginate biosynthesis are clustered at 34 min on the *P. aeruginosa* chromosome and probably form a single operon [12].
Alginate overproduction is regulated at two general levels: at the post-translational level as a response to

various environmental conditions such as high osmolarity, nitrogen, iron, phosphate or carbon limitation, and ethanol-induced dehydration [12,2], and at the genetic level from mutations in the chromosome [2]. Conversion to mucoid in laboratory and cystic fibrosis strains of *P. aeruginosa* can occur via frameshift or nonsense mutations in the second gene of the algU mucA mucB cluster [13].

Fast recognition of isolates is essential for subsequent treatment choice for patients. Polymerase Chain Reaction (PCR) was essential for recognizing etiological genus quickly by magnification of the unique series of nucleotides (sequence) to a specific being [6]. In this study, we analyze the *algD* gene coding for GDP mannose dehydrogenase which control the mechanisms leading to the emergence of mucoid *P. aeruginosa* in otitis media infection.

2. MATERIALS AND METHODS

2.1 Specimen collection

A total of 28 isolates *of P. aeruginosa* from otitis media were obtained from different hospitals in Baghdad, Iraq. This study was done in accordance with the ethical guidelines of the institution. Written informed consent was not required since all data were collected as part of routine diagnosis and treatment, and were retrospectively analyzed.

2.2 Identification of bacteria

The bacteria were cultivated on a selective medium: MacConkey agar, Cetrimide agar, Pseudomonas agar and CHROMagar Orientation then identified by performing biochemical tests including oxidase and catalase test and further identification by using an API20E system [14].

2.3 DNA extraction: Bacteria were collected directly from the culture plate and DNA extracted using DNA extraction kit (Geneaid Biotech kit system, UK) according to the manufacturer's instructions. The DNA concentration was then assessed at 260 nm in a Nano drop spectrophotometer (Biogroup, UK).

2.4 Detection of *alg D* gene

To identify *algD* gene of *P. aeruginosa*, we use PCR primer pair: F (5′–ATGCGAATCAGCATCTTTGGT–3′) and R (5′– CTACCAGCAGATGCCCTCGGC–3′) which produces a specific 1310-bp PCR product. DNA amplification reactions were performed with a 25µl reaction mixture that consisted of 5µl GO Taq Green Master Mix (Bioneer, Korea), 5µl template DNA, 2µl F-Primer, 2µl R-Primer and 11µl deionized sterile D.W. (Bioneer, Korea). The polymerization reaction of *algD* gene was carried out according to [15] in three steps: first initial denaturation at 94ºC for 5 min (1 cycle) and then the DNA amplification by sequentially heated for denaturation of DNA template at 94°C for 30 sec., annealing at 60 ºC for 45 sec. and extension at 72 ºC for 45sec. (30 cycles) and then for addition final extension at 72 ºC for 7 min (1 cycle).

2.5 Separation of DNA bands

PCR products were electrophoresed on a 2% agarose gel with 5µl Ethidium bromide in TBE buffer for 2 hours at 50 vol. Then the DNA bands were visualized and photographed under UV light [16].

2.6 DNA sequence determination and analysis

After initial amplification of *P. aeruginosa algD* gene by conventional polymerase chain reaction, the PCR product of DNA was sent with (20µl) of each F primer and R primer to US NICEM company for sequencing by Genetic analyzer. DNA sequence data were handled and analyzed using NCBI (National Center for Biotechnology Information) database and BioEdit program (V.7.2.5) [17].

3. RESULTS AND DISCUSSION

Out of 28 isolates *of P. aeruginosa* from otitis media, the result revealed that the *algD* virulent gene was present in 21 (75%) isolates. The results are in disagreement with [3] who showed that *algD* and *lasB* genes were widely disseminated (100%) in all studied isolates with no difference according to site of infection. Bulgarian investigations revealed that the prevalence of *algD* gene was 91.1% [15]. Other study found that the prevalence of *P. aeruginosa algD* was (62.5%) in strains isolated from urine samples of Iranian patients who suffered from UTIs, which was very similar to our results [1].

The specificity of PCR with the primer pair was tested when a specific 1310-bp fragment produced, which is the expected result of amplification with the *algD* gene of *P. aeruginosa* [15] Fig (1). The *P. aeruginosa algD* gene was chosen because of its importance to the *P aeruginosa*. It codes for GDP mannose dehydrogenase, a pivotal enzyme of the alginate biosynthetic pathway and this polymer represents an important protective mechanism for the bacteria [18].

In order to study molecular details of *P. aeruginosa algD* gene isolated from otitis media, we first determined the complete nucleotide sequence for the corresponding DNA region by sending the *algD* gene DNA PCR product samples with the Primer F and Primer R to the US NICEM company. Then we analyzed the *algD* gene DNA sequence results by BLAST program (BLAST Basic Local Alignment Search Tool) which is available on the website NCBI to determine the number of nucleotides and to find out the number and type of mutations and then compared with the isolation of *Pseudomonas aeruginosa* strain VA-134, complete genome, sequence ID: gb|CP013245.1| and *Pseudomonas aeruginosa* strain 12-4-4(59), complete genome, sequence ID: gb|CP013696.1| (Schematically represented in Fig. 2 and 3, respectively).

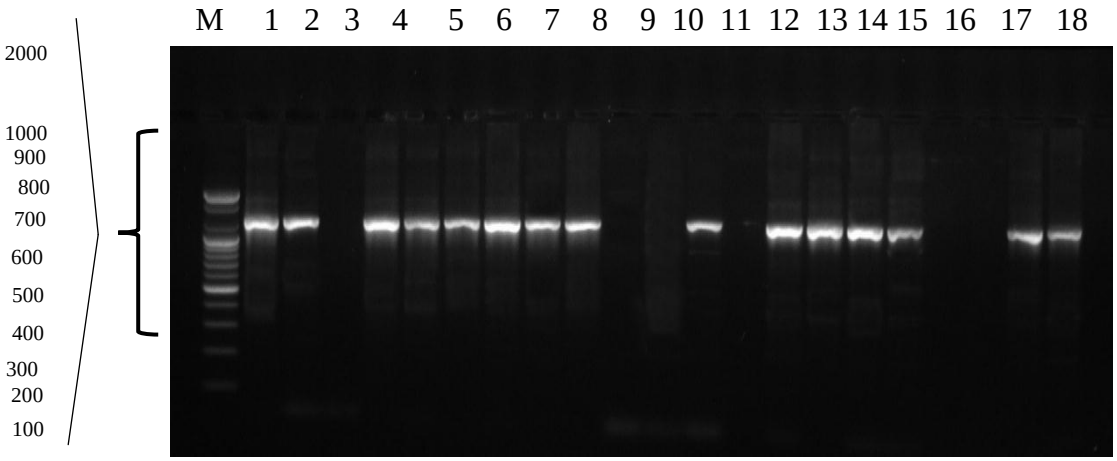

Figure 1: Agarose gel electrophoresis of PCR products. **Line M:** DNA marker (100bp -2000 bp ladder, Promega, USA); **Lanes 1,2, 4, 5, 6, 7, 8, 9,12, 14, 15, 16, 17, 20, 21, 22** *Pseudomonas aeruginosa* PCR-positive isolates; **Lanes 3, 10, 11, 13, 18, 19** *Pseudomonas aeruginosa* PCR- negative isolates.

The results showed that there are mutations in the DNA of the gene *algD*, these mutations were mostly point mutation of substitution and insertion (Table 1 and 2). For the D1-DF isolate, the sequence analysis showed that DNA substitution mutations were found to be transition and transversion. Transition replacement of purine base with another purine (G>A) at the positions 674183, 674192, 674207 and 674218. Also there were transversion replacement of a purine with a pyrimidine (G>C) at the positions 674162,674196 and 674216. In addition the results showed that insertion of pyrimidine base thymine at the positions 647064, 647163 and 674211 for subject and insertion of cytosine at the positions 674153, 674198 and 674229 for subject. Also insertion of purine base guanine at the positions 674103, 674135, 674202 and 674239 for subject and insertion of Adenine at the positions 674180, 674208, 674220 and 674236 for subject. The similarity between our sample and original gene in the NCBI database was 98%, as shown in Figure (2).

The results of sequence analysis for isolation D2-DF showed transition replacement of purine base with another purine (G>A) at the positions 3075101, 3075128, 3075031 and 3079987. In addition the results showed transition replacement of pyrimidine base with another pyrimidine (C>T) and (T>C) at the positions 3075005 and 3075006, respectively. Also there were transversion replacement of a purine with a pyrimidine (G>T) at the position 3075088, (G>C) at the positions 3075099 and 3074977, (A>C) at the positions 3075119, 3075975 and 3074978 for subject. While, transversion replacement of a pyrimidine with a purine (T>A) and (C>G) were found at the positions 3075046 and 3075013, respectively. In addition to deletion mutation at the positions 3076068 and 3075052 and many insertion of nitrogenous-bases at different sites. The percentage of similarity with the original gene in the NCBI database was 96%, as shown in Figure (3).

Table 1: Genetic mutations in the nitrogenous bases of *P. aeruginosa algD gene* (D1-DF) strain.

	Type of Mutation	Nucleotide Base*	Nucleotide Position**
1	Insertion	▼ T	674064
2	Insertion	▼ G	674103
3	Insertion	▼G	674135
4	Insertion	▼C	674153
5	Substitution "Transversions"	G > C	674162
6	Insertion	▼ T	647163
7	Insertion	▼ A	674180
8	Substitution "Transitions"	G > A	674183
9	Substitution "Transitions"	G > A	674192
10	Substitution "Transversions"	G > C	674196
11	Insertion	▼ C	674198
12	Insertion	▼ G	674202
13	Substitution "Transitions""	G > A	674207
14	Insertion	▼ A	674208
15	Insertion	▼ T	674211
16	Substitution "Transversions"	G > C	674216
17	Substitution "Transitions"	G > A	674218

18	Insertion	▼ A	674220
19	Insertion	▼ C	674229
20	Insertion	▼ A	674236
21	Insertion	▼ G	674239

*▼= Insertion, ▲= deletion, Subject > Query = Institution, **Size of gene= 1030pb.

Table 2: Genetic mutations in the nitrogenous bases of *P. aeruginosa algD gene* (D2-DF) strain.

	Type of Mutation	Nucleotide Base*	Nucleotide Position**
1	Deletion	▲ A	3076068
2	Insertion	▼ C	3075155
3	Insertion	▼G	3075166
4	Insertion	▼A	3075182
5	Insertion	▼C	3075081
6	Substitution "Transversions"	G > T	3075088
7	Insertion	▼A	3075091
8	Substitution "Transversions"	G > C	3075099
9	Substitution "Transitions"	G > A	3075101
10	Insertion	▼ C	3075112
11	Substitution "Transversions"	A > C	3075119
12	Insertion	▼G	3075121
13	Insertion	▼A	3075103
14	Substitution "Transitions"	G > A	3075128
15	Insertion	▼T	3075131
16	Insertion	▼T	3075027
17	Substitution "Transitions"	G > A	3075031
18	Insertion	▼ G	3075034
19	Insertion	▼ A	3075038
20	Substitution "Transversions"	T > A	3075046
21	Insertion	▼ A	3075048
22	Deletion	▼G	3075052
23	Insertion	▼C	3075955
24	Insertion	▼G	3075958
25	Insertion	▼C	3075964
26	Insertion	▼C	3075971
27	Substitution "Transversions"	A>C	3075975
28	Insertion	▼A	3075983
29	Substitution "Transversions"	G > C	3074977
30	Substitution " Transversions"	A > C	3074978
31	Insertion	▼G	3074980
32	Insertion	▼T	3074982
33	Substitution "Transitions"	G > A	3079987
34	Insertion	▼A	3074989
35	Insertion	▼T	3074991
36	Insertion	▼T	3075001
37	Substitution "Transitions"	C > T	3075005
38	Substitution "Transitions"	T > C	3075006
39	Insertion	▼T	3075011
40	Substitution "Transversions"	C > G	3075013

*▼= Insertion, ▲= deletion, Subject > Query = Institution, **Size of gene = 1139pb.

Score	Expect	Identities	Gaps	Strand
1748 bits(946)	0.0	995/1016(98%)	14/1016(1%)	Plus/Plus

Features:
GDP-mannose dehydrogenase

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Query  15      GTATGTGCTGGCTGCCTGTCGGCACGCGGTCATGAAGTCATTGGTGTGGATGTCTCCAGC  74
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  673234  GTATGTGCTGGCTGCCTGTCGGCACGCGGTCATGAAGTCATTGGTGTGGATGTCTCCAGC  673293

Query  75      ACCAAGATCGACCTGATCAACCAGGGCAAGTCGCCCATCGTCGAACCGGGCCTGGAAGCG  134
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  673294  ACCAAGATCGACCTGATCAACCAGGGCAAGTCGCCCATCGTCGAACCGGGCCTGGAAGCG  673353
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Query  135     TTGTTGCAGCAAGGCCGGCAGACCGGACGGCTGTCGGGCACCACCGACTTCAAGAAGGCC  194
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  673354  TTGTTGCAGCAAGGCCGGCAGACCGGACGGCTGTCGGGCACCACCGACTTCAAGAAGGCC  673413

Query  195     GTGCTGGACTCCGACGTATCGTTCATCTGCGTCGGCACGCCGAGCAAGAAGAACGGCGAC  254
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  673414  GTGCTGGACTCCGACGTATCGTTCATCTGCGTCGGCACGCCGAGCAAGAAGAACGGCGAC  673473

Query  255     CTGGACCTGGGCTACATCGAGACCGTCTGCCGCGAGATCGGCTTCGCCATCCGCGAGAAG  314
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  673474  CTGGACCTGGGCTACATCGAGACCGTCTGCCGCGAGATCGGCTTCGCCATCCGCGAGAAG  673533

Query  315     TCCGAACGCCACACCGTGGTGGTGCGCAGCACCGTACTGCCGGGCACCGTCAACAACGTG  374
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  673534  TCCGAACGCCACACCGTGGTGGTGCGCAGCACCGTACTGCCGGGCACCGTCAACAACGTG  673593

Query  375     GTGATCCCGCTGATCGAGGACTGCTCGGGCAAGAAGGCCGGGGTCGACTTCGGCGTCGGC  434
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  673594  GTGATCCCGCTGATCGAGGACTGCTCGGGCAAGAAGGCCGGGGTCGACTTCGGCGTCGGC  673653

Query  435     ACCAACCCCGAATTCCTCCGCGAGAGCACCGCGATCAAGGACTACGACTTCCCGCCGATG  494
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  673654  ACCAACCCCGAATTCCTCCGCGAGAGCACCGCGATCAAGGACTACGACTTCCCGCCGATG  673713

Query  495     ACCGTGATCGGCGAACTGGACAAGCAGACCGGCGACCTTCTCGAGGAAATCTACCGCGAG  554
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  673714  ACCGTGATCGGCGAACTGGACAAGCAGACCGGCGACCTTCTCGAGGAAATCTACCGCGAG  673773

Query  555     CTGGACGCGCCGATCATCCGCAAGACCGTCGAGGTCGCCGAGATGATCAAGTACACCTGC  614
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  673774  CTGGACGCGCCGATCATCCGCAAGACCGTCGAGGTCGCCGAGATGATCAAGTACACCTGC  673833

Query  615     AACGTCTGGCACGCCGCCAAGGTCACCTTCGCCAACGAGATCGGCAACATCGCCAAGGCG  674
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  673834  AACGTCTGGCACGCCGCCAAGGTCACCTTCGCCAACGAGATCGGCAACATCGCCAAGGCG  673893

Query  675     GTCGGCGTCGACGGCCGCGAGGTGATGGACGTGATCTGCCAGGACCACAAGCTCAACCTG  734
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  673894  GTCGGCGTCGACGGCCGCGAGGTGATGGACGTGATCTGCCAGGACCACAAGCTCAACCTG  673953

Query  735     TCGCGCTACTACATGCGTCCCGGCTTCGCCTTCGGCGGCTCCTGCCTGCCCAAGGATGTA  794
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  673954  TCGCGCTACTACATGCGTCCCGGCTTCGCCTTCGGCGGCTCCTGCCTGCCCAAGGATGTA  674013

Query  795     CGCGCCCTCACCTATCGCGCCAGCCAGCTGGACGTCGAGCACCCGATGCTTCGGTTCGTT  854
               |||||||||||||||||||||||||||||||||||||||||||||| ||||||||||
Sbjct  674014  CGCGCCCTCACCTATCGCGCCAGCCAGCTGGACGTCGAGCACCCGATGC-TCGGTTCGTT  674072

Query  855     GATGCGCAGCAACTCCAACCAGGTGCAGAAGGGCCTTCGATCTCATCACCAGCCACGACA  914
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                ||||||||||||||||||||||||||||||| ||||||||||||||||||||||||||||||
Sbjct  674073  GATGCGCAGCAACTCCAACCAGGTGCAGAA-GGCCTTCGATCTCATCACCAGCCACGACA  674131

Query  915     CCCGGCAAGGTCGGCCTGCTCCGGCCTGTCCTTTCAAGGCCGGCACCGAACAATTTGCGC  974
                ||| |||||||||||||||| ||||||||  ||||||||||||||| || ||||||||
Sbjct  674132  CCC-GCAAGGTCGGCCTGCTC-GGCCTGTCG-TTCAAGGCCGGCACCG-ACGATTTGCGC  674187

Query  975     GAAAACCCCCCTGGGTGGAAACTTGGCCCAAAATGCTCATCCGGCAAGAGGGCTAC  1030
                |||| ||| | ||| ||||  || |||| | | |||||||| |||||| || ||||
Sbjct  674188  GAAAGCCCGC-TGG-TGGAG-CT-GGCCGAGA-TGCTCATC-GGCAAG-GG-CTAC  674235
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Figure 2: DNA sequences of *P. aeruginosa algD gene* (D1-DF) strain comparing with VA-134 strain (complete genome, sequence ID: gb|CP013245.1|)

Score	Expect	Identities	Gaps	Strand
1829 bits(990)	0.0	1083/1123(96%)	26/1123(2%)	Plus/Minus

Features:
type II secretion system protein GspIGDP-mannose dehydrogenase

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Query  19       GTGCTGGCTGCCTGTCGGCACGCGGTCATGA-GTCATTGGTGTGGATGTCTCCAGCACCA  77
                ||||||||||||||||||||||||||||||| ||||||||||||||||||||||||||||
Sbjct  3076037  GTGCTGGCTGCCTGTCGGCACGCGGTCATGAAGTCATTGGTGTGGATGTCTCCAGCACCA  3075978

Query  78       AGATCGACCTGATCAACCAGGGCAAGTCGCCCATCGTCGAACCGGGCCTGGAAGCGTTGT  137
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3075977  AGATCGACCTGATCAACCAGGGCAAGTCGCCCATCGTCGAACCGGGCCTGGAAGCGTTGT  3075918

Query  138      TGCAGCAAGGCCGGCAGACCGGACGGCTGTCGGGCACCACCGACTTCAAGAAGGCCGTGC  197
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3075917  TGCAGCAAGGCCGGCAGACCGGACGGCTGTCGGGCACCACCGACTTCAAGAAGGCCGTGC  3075858

Query  198      TGGACTCCGACGTATCGTTCATCTGCGTCGGCACGCCGAGCAAGAAGAACGGCGACCTGG  257
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3075857  TGGACTCCGACGTATCGTTCATCTGCGTCGGCACGCCGAGCAAGAAGAACGGCGACCTGG  3075798

Query  258      ACCTGGGCTACATCGAGACCGTCTGCCGCGAGATCGGCTTCGCCATCCGCGAGAAGTCCG  317
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3075797  ACCTGGGCTACATCGAGACCGTCTGCCGCGAGATCGGCTTCGCCATCCGCGAGAAGTCCG  3075738

Query  318      AACGCCACACCGTGGTGGTGCGCAGCACTGTACTGCCGGGCACCGTCAACAACGTGGTGA  377
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3075737  AACGCCACACCGTGGTGGTGCGCAGCACTGTACTGCCGGGCACCGTCAACAACGTGGTGA  3075678

Query  378      TCCCGCTGATCGAGGACTGCTCGGGCAAGAAGGCCGGGGTCGACTTCGGCGTCGGCACCA  437
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3075677  TCCCGCTGATCGAGGACTGCTCGGGCAAGAAGGCCGGGGTCGACTTCGGCGTCGGCACCA  3075618


Query  438      ACCCCGAATTCCTCCGCGAGAGCACCGCGATCAAGGACTACGACTTCCCGCCGATGACCG  497
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                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3075617  ACCCCGAATTCCTCCGCGAGAGCACCGCGATCAAGGACTACGACTTCCCGCCGATGACCG  3075558

Query  498      TGATCGGCGAACTGGACAAGCAGACCGGCGACCTTCTCGAGGAAATCTACCGCGAGCTGG  557
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3075557  TGATCGGCGAACTGGACAAGCAGACCGGCGACCTTCTCGAGGAAATCTACCGCGAGCTGG  3075498

Query  558      ACGCGCCGATCATCCGCAAGACCGTCGAGGTCGCCGAGATGATCAAGTACACCTGCAACG  617
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3075497  ACGCGCCGATCATCCGCAAGACCGTCGAGGTCGCCGAGATGATCAAGTACACCTGCAACG  3075438

Query  618      TCTGGCACGCCGCCAAGGTCACCTTCGCCAACGAGATCGGCAACATCGCCAAGGCGGTCG  677
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3075437  TCTGGCACGCCGCCAAGGTCACCTTCGCCAACGAGATCGGCAACATCGCCAAGGCGGTCG  3075378

Query  678      GCGTCGACGGCCGCGAGGTGATGGACGTGATCTGCCAGGACCACAAGCTCAACCTGTCGC  737
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3075377  GCGTCGACGGCCGCGAGGTGATGGACGTGATCTGCCAGGACCACAAGCTCAACCTGTCGC  3075318

Query  738      GCTACTACATGCGTCCCGGCTTCGCCTTCGGCGGCTCCTGCCTGCCCAAGGATGTACGCG  797
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3075317  GCTACTACATGCGTCCCGGCTTCGCCTTCGGCGGCTCCTGCCTGCCCAAGGATGTACGCG  3075258

Query  798      CCCTCACCTATCGCGCCAGCCAGCTGGACGTCGAGCACCCGATGCTCGGTTCGTTGATGC  857
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3075257  CCCTCACCTATCGCGCCAGCCAGCTGGACGTCGAGCACCCGATGCTCGGTTCGTTGATGC  3075198

Query  858      GCAGCAACTCCAACCAGGTGCAGAAGGCCTTCGATCTCATCACCAGCCACGACACCCGCA  917
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3075197  GCAGCAACTCCAACCAGGTGCAGAAGGCCTTCGATCTCATCACCAGCCACGACACCCGCA  3075138

Query  918      AGGTCGGCCTGCTCGGCCCTGTCGTTCAAGGGCCGGCACCGACGAATTTGCGCGAAAGCC  977
                |||||||||||||||||| |||||||||| |||||||||||||||| ||||||||||||||
Sbjct  3075137  AGGTCGGCCTGCTCGGCC-TGTCGTTCAA-GGCCGGCACCGACGA-TTTGCGCGAAAGCC  3075081

Query  978      CCGCTGGTTGAAGCTGGCCCAAAATGCTCCTCCGGCAAGGGGCTACGAATTTGCGCATCT  1037
                | |||||| || ||||||| | | ||||| || |||||||| |||||| || ||||||||
Sbjct  3075080  C-GCTGGTGGA-GCTGGCCGAGA-TGCTCATC-GGCAAGGG-CTACGAGTT-GCGCATCT  3075027

Query  1038     TTCGAACGGCAAACGTCGAAAAACGC-CGCTGGTCCACCGGGGCCCAACCAGGAAATACA  1096
                | ||| || ||| ||||||| | ||| || || ||||| |||||| ||| ||||| ||||
Sbjct  3075026  T-CGACCG-CAA-CGTCGAATA-CGCGCG-TG-TCCAC-GGGGCC-AACAAGGAA-TACA  3074976

Query  1097     TCCCGGTTCGAAAAATTCCCGCACGTTCTCTCCACTTGGTGGT  1139
                ||  | | |||| | | ||||||||| |||  |||| | ||||
Sbjct  3074975  TCGAG-T-CGAAGA-T-CCCGCACGT-CTCCTCACT-GCTGGT  3074939
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Figure 3: DNA sequences of *P. aeruginosa algD gene* (D2-DF) strain comparing with 12-4-4 (59) strain (complete genome, sequence ID: gb|CP013696.1|)

The results of protein translation in the isolation D1-DF found that missense mutations in the gene causing amino acid substitution in the protein. Arginine has been replaced by the amino acid Alanine or Proline. Serine has been replaced with one of the amino acid Phenylalanine, Glutamine, Leucine and Proline. While, the amino acids Aspargine has been replaced with the amino acid Glutamine. Aspartic acid has been replaced with Serine or Threonine. Also the replacement of Glutamin with Proline or Alanine. In addition, replacment of Histidine with Alanine, replacement of Leucine with Valine or Isoleucine, Glycine with Arginine, Methionine with Aspartic acid, Valine with Glycine, Lysine with Glutamine acid, Alanine with Glycine, Phenylanine with Proline and replacement of Isoleucine and Threonine with Serine, were shown in the table (3).

DNA sequence analysis of *algD* gene of *P. aeruginosa* isolate D2 -DF revealed several point mutations which resulted in an amino acid change from Lysine to Serine and replacement of amino acid Serine with Histidine or Leusine. Also replacement of Leucine with Tryptophan, Valine and Methionine with amino acid cysteine, Tryptophan with amino acid Glycine and replacement each of Proline and Alanine with amino acids Glutamine and Histidine, respectively. In addition, Nonsense mutation led to loss of the amino acid Proline and resulted in a truncated, incomplete, and usually nonfunctional protein product. The predicted amino acid sequence of *algD gene* was shown in the table (4).

This study showed that many mutations has been found in *P. aeruginosa algD* gene that led to replacement of some amino acids. Many Studies have shown that this gene produced by the bacteria have a role in the adhesion of bacteria and resistance to antibiotics and protect them from dehydration process and work as capsule, where warm up the bacteria in the process of phagocytosis in the tissue and reduces the effectiveness of the immune response [19,20].

Table 3: The impact of genetic mutations in *P. aeruginosa algD gene* (D1-DF) strain on the translation of protein.

Subject		Query	
Arginine	R	Glycine	G
Phenylalanine	F	Serine	S
Valine	V	Leucine	L
Aspartic acid	D	Methionine	M
Alanine	A	Arginine	R
Glutamin	Q	Serine	S
Glutamin	Q	Aspargin	N
Leucine	L	Serine	S
Glutamin	Q	Aspargin	N
Proline	P	Glutamin	Q
Glycine	G	Valine	V
Alanine	A	Glutamin	Q
Glutamine acid	E	Lysine	K
Glycine	G	Alanine	A
Proline	P	Phenylalanine	F
Serine	S	Aspartic acid	D
Isoleucine	I	Leucine	L
Serine	S	Isoleucine	I
Serine	S	Threonine	T
Proline	P	Serine	S
Alanine	A	Histidine	H
Threonine	T	Aspartic acid	D
Proline	P	Arginine	R

Table 4: The impact of genetic mutations in *P. aeruginosa algD gene* (D2-DF) strain on the translation of protein.

Subject		Query	
K	Lysine	S	Serine
S	Serine	H	Histidine
L	Leusine	W	Tryptophan
V	Valine	C	Cysteine
W	Tryptophan	G	Glycine
M	Methionine	C	Cysteine
S	Serine	L	Leusine
P	Proline	Q	Glutamin
A	Alanine	H	Histidine
P	Proline	X	Missing

Acknowledgments:
Thanks for Asifa Ali for her assistance in samples collection.

Authors' Contribution: Dr. Rana M. Abdullah and Dr. Arwa M. Abdullah were all developed the original research concept, involved in the writing and editing of this manuscript. Abbas Falih Al-arnawtee collected the samples and performed the laboratory work

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