

DNA sequencing and phylogeny of *Pseudomonas aeruginosa* isolated from nosocomial infections' in Iraq

Abstract

PCR product of *16SrRNA* gene was used for partial sequencing, and registration in gene bank-NCBI and phylogeny. Ten accession numbers were obtained from registration of ten sequences of *16srRNA* gene bank-NCBI: The accession numbers were: KX963356.1, KX963357.1, KX963358.1, KX963359.1, KX963360.1, KX963361.1, KX963362.1, KX963363.1, KX963364.1 and KX963365.1.

The phylogenetic analysis of local isolates of *P. aeruginosa* showed a close related to NCBI-BLAST *P. aeruginosa* strain(KR81540.1) except the Iraq isolates 2 (burns source) and Iraq isolates 8 from (ear source) which showed genetically differences as unique isolates.

Based on the update local literatures, this is the first study in Iraq which employed sequencing, registration of sequences in gene bank-NCBI, and carrying out phylogeny of local, clinical isolates and world strains of *P. aeruginosa*, in addition to that, a new records of two strains of local isolates from ear (KX963362.1) and burns (KX963357.1) are established in Gene bank locus.

introduction

Pseudomonas aeruginosa is a Gram's negative opportunistic pathogen has emerged as one of the most problematic of the nosocomial pathogens; it is considered Multi-resistant infections in both communal and hospital settings. It is an opportunistic pathogen that causes infections in immunocompromised, cancer, burn, urinary

tract, surgical wound, eye, blood, ear infection, sepsis cystic fibrosis, and intensive care unit (Tille, 2014).

DNA sequencing is the process of determining the precise order of nucleotides within a DNA molecule. It includes any method or technology that is used to determine the order of the four bases—adenine, guanine, cytosine, and thymine—in a strand of DNA. The advent of rapid DNA sequencing methods has greatly accelerated biological and medical research and discovery. Knowledge of DNA sequences has become indispensable for basic biological research, And in numerous applied fields such as diagnostic, biotechnology, forensic biology, and biological systematics. (Clyde, 2007).

The rapid speed of sequencing attained with modern DNA sequencing technology has been instrumental in the sequencing of complete DNA sequences, or genomes of numerous types and species of life, including the human genome and other complete DNA sequences of many animal, plant, and microbial species (Pettersson *et al.*, 2009). Fifteen years elapsed between the discovery of the DNA double helix in 1953 and the first experimental determination of a DNA sequence (Reinert and Huson, 2007).

Whole genome sequencing, facilitated by the Advent of high-throughput approaches, brings the promise of single-base-pair resolution between isolates, making it the ultimate molecular typing method for bacteria. Several recent studies have shown that analysis of single nucleotide polymorphisms (SNPs) in bacterial genomes provides a means of determining relatedness between epidemiologically linked isolates and tracking bacterial evolution over periods of months to years(Eppinger *et al.*, 2011).

Snyder *et al.*, 2013 Studied of epidemiological investigation of *P. aeruginosa* isolate from a six-year-long hospital outbreak using

high –through put whole genom sequencing, In that study they demonstrated that the single base resolution of whole genome sequencing is a powerful tool in analysis of outbreak isolates that can not only show strain similarity, but also evolution over time and potential adaptation through gene sequence change.

In Iraq, there are several studies were conducted during the last five years on phenotypic and genotypic characterization of *P. aeruginosa* (Abdullah, 2012; Fadhel, 2013; Al Doory,2012; AL-Obaidi, 2013), but the present study focused on the causes of the continuous antibiotic resistance by *P. aeruginosa* that prevalent in hospitals especially that caused the nosocomial or hospitalized infections based on the identify the genetic variation between the isolates collected from different clinical samples by using the DNA sequencing and phylogeny analysis.

The study was aimed to identify some microbial factors (phenotypic and genotypic) that support the antibiotic resistance and prevalence of *Pseudomonas aeruginosa* that causes hospitalized infection. **2.2.**

Method

Five hundred specimens from urine, wounds, burns, sputum, ear swab and diabetic foot were collected from inpatients of many hospitals and Central Public Health Laboratory during November, 2015 to April, 2016 in Baghdad city. All Specimens were collected by clean sterilized cotton swabs or containers under supervision of a clinical consultant physicians. The time between samples collection and bacteriological exam never exceeded 1-2 hours. The swabs were primary cultivated on suitable incubation at 37C° for 18-24 hour under aerobic condition. Positive culture media represented by Blood agar and MacConkey agar, then culture samples re-cultured on selective media besides performing morphological characteristic, biochemical tests followed by confirmative diagnostic methods (phenotypic and genotypic method).

. Isolating Genomic DNA from *P. aeruginosa*

The following procedure was done according to the instructions of manufacturing company:

- A volume 1ml of an overnight culture was added to a 1.5ml microcentrifuge tube, Centrifuged at $16,000 \times g$ for 2 minutes to pellet the cells and Removed the supernatant.
- A volum 600 μ l of Nuclei Lysis Solution was added. Gently pipet until the cells are suspended, Incubated at 80°C for 5 minutes to lyse the cells and then cooled to room temperature.
- A volum 3 μ l of RNase solution was added to the cell lysate. Invert the tube 2–5 times to mix. Incubated at 37°C for 30 minutes. Cooled the sample to room temperature, A volume 200 μ l of Protein Precipitation Solution was added to the RNase-treated cell lysate Vortex vigorously at high speed for 20 seconds to mixed the Protein Precipitation Solution with the cell lysate.
- The sample was Incubated on ice for 5 minutes, Centrifuged at $13,000\text{--}16,000 \times g$ for 3 minutes and then transfered the supernatant containing the DNA to a clean 1.5ml microcentrifuge tube which contained on 600 μ l of isopropanol at room temperature then Gently mixed by inversion until the thread-like strands of DNA form a visible Mass then Centrifuge at $13,000\text{--}16,000 \times g$ for 2 minutes.
- Carefully the supernatant was poured off and drained the tube on clean absorbent paper, A volum 600 μ l of room temperature 70% ethanol was added and gently inverted the tube several times to wash the DNA pellet then Centrifuged at $13,000\text{--}16,000 \times g$ for 2 minutes and Drained the tube on clean absorbent paper and allow the pellet to air-dry for 10–15 minutes.
- A, volume 100 μ l of DNA rehydration solution was added to the tube and rehydrate the DNA by incubating at 65°C for 1 hour. Was

periodically mixed the solution by gently tapping the tube. Alternatively, rehydrate the DNA by incubating the solution overnight at room temperature or at 4°C and then stored the DNA at 2–8°C.

2.2.11.2. Isolation of Plasmid DNA from *p. aeruginosa*

The following procedure was performed at room temperature, to instructions according of Manufactory Company:

- A total of 600µl was transferred of bacterial culture grown in Lauria bertani broth medium to a 1.5ml micro centrifuge tube. A 100µl of Cell Lysis Buffer was added, and mixed by inverting the tube 6 times. The solutions should change from opaque to lysis clear blue, indicating complete
- A ,volum 350µl of cold (4–8°C) neutralization solution was used and mixed thoroughly by inverting the tube the sample will turn yellow when neutralization is completed, and will form a yellow precipitate, inverted the sample an additional 3 times to ensure completed neutralization, Centrifuged at maximum speed in a microcentrifuge for 3 minutes andTransfered the supernatant (~900µl) to a PureYield™ Minicolumn.Do not disturb the cell debris pellet. For maximum yield, transfer the supernatant with a pipette.
- The minicolumn was placed into a PureYield™ Collection tube, and centrifuged at maximum speed in a microcentrifuge for 15 seconds then discarded the flowthrough, and placed the minicolumn into the same PureYield™ Collection Tube and A volume 200µl of endotoxin removal washed to the minicolumn centrifuged at maximum speed in a microcentrifuge for 15 seconds. It is not necessary to empty the PureYield™ Collection Tube.
- A, volume of 400µl of column wash solution was added to the minicolumn, centrifuged at maximum speed in a micro centrifuge for

30 seconds then transferred the minicolumn to a clean 1.5ml micro centrifuge tube, then added 30µl of Elution buffer directly to the minicolumn matrix, let stand for 1 minute at room temperature. And then Centrifuged at maximum speed in a micro centrifuge for 15 seconds to elute the plasmid DNA. sealed the micro centrifuge tube, and stored eluted plasmid DNA at -20°C

2.2.12. Estimation of DNA yield and purity

The extracted genomic DNA was checked by using Nano drop spectrophotometer to estimate the concentration and extracted purity of DNA through reading the absorbance in at (260 /280 nm).

2.2.13. Detection of specific genes for *P. aeruginosa* by PCR method.

2.2.13.1. Preparing the Primers:

Oligonucleotide primers were prepared depending on manufacturer's instruction by dissolving the lyophilized product with Nuclease - free water after rotating down briefly. Working primer tube was prepared by diluting with Nuclease - free water. The final picomoles depended on the procedure of each primer.

Table (2-13): PCR amplification program of 16SrRNA gene used for confirmatory identification of *P. aeruginosa*.

Stage	Steps	Temperature (C°)	Time	No. of cycles
First	Initial Denaturation	95°C	120sec	1
Second	I Denaturation	95°C	20sec	25
	II Annealing	58°C	20sec	
	III Extension	72°C	40sec	
Third	Final Extension	72°C	60sec	1

Table (2-14): PCR amplification program of *16SrRNA* gene used for DNA sequencing in *P. aeruginosa*.

Stage	Steps	Temperature (C°)	Time (sec)	No. of cycles
First	Initial Denaturation	95°C	4min	1
Second	I Denaturation	95°C	30sec	30
	II Annealing	55°C	30sec	
	III Extension	72°C	90sec	
Third	Final Extension	72°C	9min	1

2.2.14. Agarose gel electrophoresis:

This agarose gel was prepared by dissolving 1 g of agarose powder in 100 ml of (1X) TBE buffer (pH 8) on hot plate with magnetic stirrer and magnetic capsule was added, left until boiling and becoming clear, allowed to cool to 50°C, and 5 µl Ethidium Bromide was added(Sambrook and Russell, 2001).

2.2.14.1. Casting of the horizontal Agarose gel:

The tape was placed across the end of the gel tray. The comb was fixed at one end of the tray for making wells used for loading DNA samples. The agarose was poured gently into the tray, and allowed to solidify at room temperature for 30 minutes. Then, the comb was removed gently from the tray and the tape was also removed from the

ends of the tray. The agarose gel was fixed in electrophoresis chamber which was filled with TBE buffer (1X) that had covered the surface of the gel (Sambrook and Russell, 2001).

2.2.14.2. Loading and running DNA in agarose gel:

Five μ l of each DNA template was transferred to eppendorf tube, 3 μ l of loading dye was added to the tube and the mixture was loaded into the wells in agarose gel with the addition of loading buffer and DNA Ladder (100bp) as standard in electrophoresis. The electric current was allowed at 100 volt for 10 minutes and then 80 volt for 1 hour to detect the 16srRNA gene (956 base pairs amplicon), DNA extraction and plasmid extraction while used 90 min at 100 volt/50 mA to detect, 16srRNA gene (1504 base pairs amplicon) and exotoxine A. After complete time read the results on UV transilluminator was used for the observation of DNA bands, and the gel was photographed.

2.2.14.6. Analysis of PCR results:

PCR product was analyzed by gel electrophoresis in 1-2% agarose gels containing Ethidium Bromide Staining Solution. 5 μ l from amplified sample was directly loaded in agarose gel with the addition of loading buffer and DNA Ladder (100 base pairs) as standard in electrophoresis and the gel was run at 100 Volt for 10 minutes and 80 volt for 1 hour. The products were visualized with UV illuminator and photographed.

2.2.15. DNA sequencing:

DNA sequencing method was performed for Phylogenetic tree analysis study of local *P. aeruginosa* isolates based on *16srRNA* gene 1504bp. DNA capillary sequencing was performed by using their

ABI 3730xl genetic analyzer (Applied Bio systems, US), (Larkin *etal.*, 2013).

As the following steps:

1. The specific PCR product was excised from the gel by clean, sharp scalpel. Then, transferred into a 1.5mL micro centrifuge tube.
2. 400µl Binding Buffer II was added to gel fragment. Then, incubated at 60°C for 10 minutes and shaken until the agarose gel is completely dissolved.
3. Add the above mixture to the EZ-10 column and let stand for 2 minutes. Centrifuge at 10,000rpm for 2 minutes and discard the flow-through in the tube.
4. 750µl Wash Solution was added to each tube and centrifuged at 10000rpm for one minute. Then, solution discarded.
5. After that, the step 4 was repeated. Then, centrifuged at 10000rpm for an additional minute to remove any residual wash Buffer.
6. The column was placed in a clean 1.5ml micro centrifuge tube and added 30µl of Elution Buffer to the center of the column and incubated at room temperature for 2 minutes. Then, the tube was centrifuged at 10000rpm for 2 minutes to elute PCR product and store at -20°C. (Tamura *etal.*, 2013)

Confirmative Detection of *P. aeruginosa* isolates by conventional PCR by using *16SrRNA* gene.

3.1.2.3.1. Extraction chromosomal DNA

The DNA from 60 isolates of *P.aeruginosa* was extracted and purified using genomic DNA kit. The results were detected by gel electrophoresis process using 1% agarose gel and then examined under UV. Light in which the DNA appeared as clear compact band in Figure (3-4).

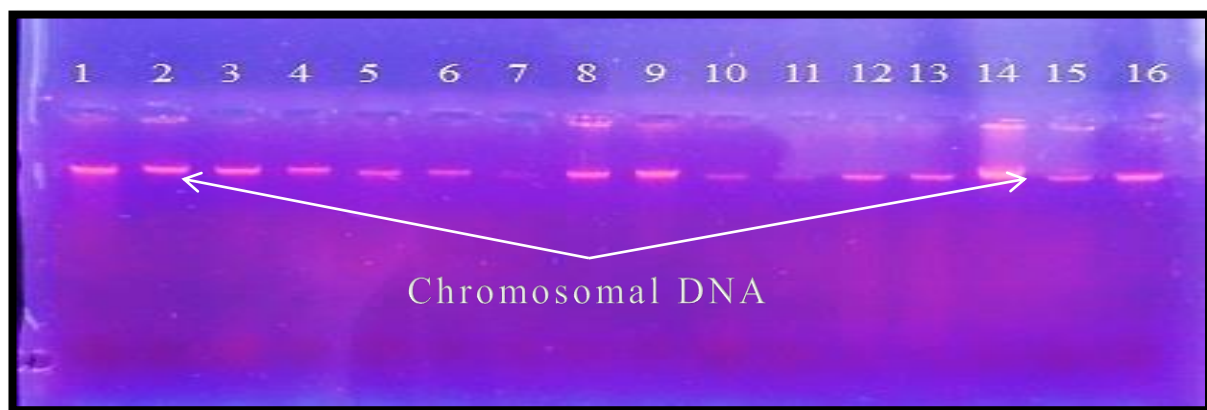


Figure (3-4): Ethidium bromide stained Agarose gel electrophoresis of extracted DNA from *P. aeruginosa* isolates using 1% Agarose, 90 min at 100 volt/50 mAmp.

3.1.2.3.2. Total DNA concentration and purity

Total **DNA** concentrations and purity that extracted from different clinical samples were measured by Nano drop spectrophotometer. Results (Mean) concentrations exhibited as ng/ μ l. The purity of the extracted **DNA** was estimated by measuring the ratio of A_{260}/A_{280} . It gave an optimal concentration of DNA for amplification process ranged from 35.3-39.8 ng/ μ l. While optimal purity amplification process ranged from 1.6-2.01 ng/ μ l. (Table 3-3).

Clinical samples	No. of isolates	Concentration of DNA (ng/ μ l) Mean	Purity A_{260}/A_{280} Mean
Diabetic foot	2	38.35	1.8

Sputum	8	35.3	1.8
Ear	13	38.5	2.01
Urines	5	35.6	1.6
Burns	16	38.4	1.96
Wounds	16	39.8	2.0

Table 3.3: Values of extracted DNA concentration and purity of selected sample of Extracted DNA.

Based on the standard values of DNA concentration for amplification, the values of the present study are considered an efficient values and suitable for the establishment of the DNA extracted with target primers or sequences amplification.

Sequencing and phylogeny analysis of *16SrRNA* gene

The sequencing *16srRNA* gene based on the PCR product of the nucleotide sequence with 1054 base pairs (fig.3-13) of 10 isolates *P. aeruginosa* were identify the genetic variation and phylogeny of these isolates after compared with their accession numbers and their definition of another global sequence data of two standard strains in NCBI-BLAST the 10 sequences were recognized as *P. aeruginosa* as follow with their accession number and their definition (fig3-13 to fig 3-22).

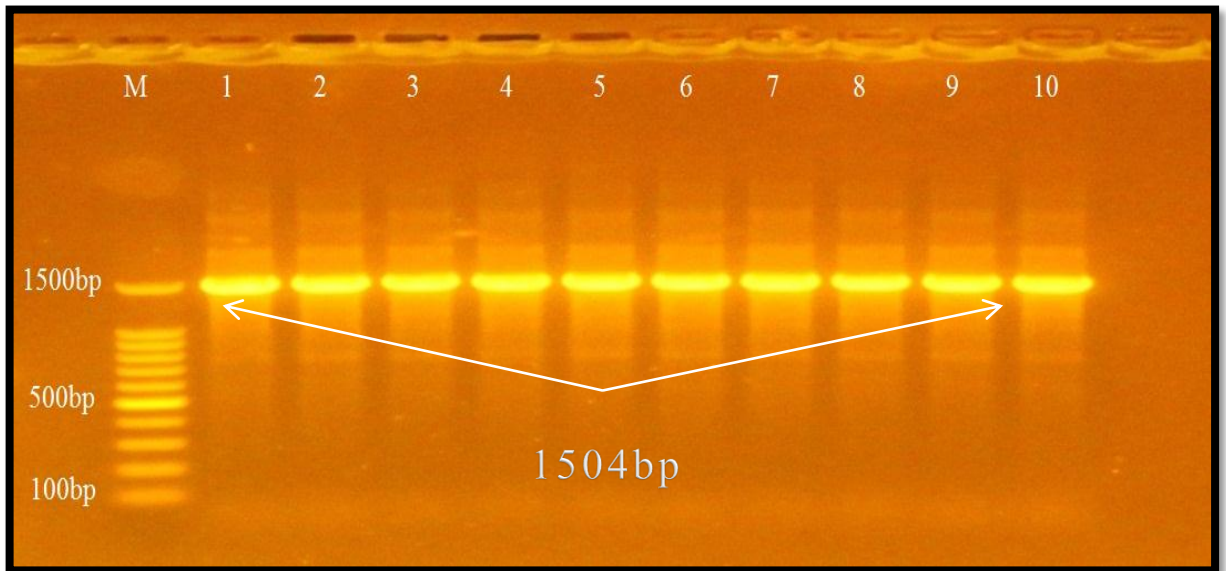


Figure (3-13): Ethidium bromide stained gel electrophoresis of PCR product~1504base pairs of *P. aeruginosa* isolates using (1%) Agarose for 90 minutes at 100 volt positive PCR product~1504b p size Lane (M) DNA marker 100bp. Lanes (1-10) show positive result to *16SrRNA* gene.

Sequencing1: Results of 16S *Ribosomal* RNA gene, partial sequences forward of *P. aeruginosa* isolates from Diabetic foot, showed the identities percent 74% with world strains when examined in Gene bank of NCBI (fig.3-12).

Sequencing2: Results of 16S *Ribosomal* RNA gene, partial sequences forward of *P. aeruginosa* isolates from burns, showed the identities percent 77% with world strains when examined in Gene bank of NCBI (fig.3-).

Sequencing 3: The results of 16S *Ribosomal* RNA gene, partial sequences forward of *P. aeruginosa* isolates from urine, showed the identities percent 99% with world strains when examined in gene bank of NCBI in (fig.3-).

Sequencing4: The results of 16S *Ribosomal* RNA gene, partial sequences forward, *P. aeruginosa* isolates from sputum, showed the

identities percent 95% with world strains when examined in Gene bank of NCBI (fig.3.19).

Sequencing 5: The results of *16S Ribosomal* RNA gene, partial sequences forward, *P. aeruginosa* isolates burns, showed the identities percent 99% with world strains when examined in Gene bank of NCBI fig 3-20.

Sequencing 6: Results of *16S Ribosomal* RNA gene, partial sequences forward, *P. aeruginosa* isolates sputum, showed the identities percent 99% with world strains when examined Gene bank of NCBI (fig 3-21).

Sequencing7: The results of *16S Ribosomal* RNA gene, partial sequences forward, *P. aeruginosa* isolates wound showed the identities percent 81% with world strains when examined in Gene bank of NCBI (fig.3-22).

Sequencing 8: The results of *16S Ribosomal* RNA gene, partial sequences forward, *P. aeruginosa* isolates ear, showed the identities percent 89% with world strains when examined in Gene bank of NCBI (fig.3-23).

Sequencing 9: Results of *16S Ribosomal* RNA gene, partial sequences forward, *P. aeruginosa* isolates wound, showed the identities percent 99% with world strains when examined in Gene bank of NCBI fig. (3-24).

Sequencing 10: The results of *16S Ribosomal* RNA gene, partial sequences forward, *P. aeruginosa* isolates wound showed the

identities percent 98% with world strains when examined in Gene bank of NCBI (fig.3-25).

Pseudomonas aeruginosa Iraq.PA-1 isolate 16S ribosomal RNA gene, partial sequence						
Score	Expect	Identities	Gaps	Strand	Plus/Plus	
609 bits (674)	6e-178	768/1032 (74%)	25/1032 (2%)			
Query	19	TGCAGTCGAGCGG-TACAGGGAGAATCTTGTCTCTTTGACGAGCGACGGATGGGTGAG				77
Sbjct	1	TGCAGTCGAGCGGATGAAGGGAG---CTTGCTCCTGGATT--AGCGGCGGACGGGTGAG				55
Query	78	TAATGTGTGGGGATCTGCCCCGAGAGGGAGGGATAACTACAGCGGACGCTGGCTCAGACCG				137
Sbjct	56	TAATGCCTGGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCTAATACCG				115
Query	138	CATAATCTCTTAGGAGCAGAGCAGACGAACCTCCGTCCTTTTCGCTATCGAATGAACCCCT				197
Sbjct	116	CATACGTCCTGAGGGAGAAAGTGGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAG				175
Query	198	ATGGGATTAGCTAGTAGGTGGGGTGTACTCAGTAAGCAACCATACTAGCTGGACTG				257
Sbjct	176	GTCGGATTAGCTAGTTGGTGGGGTAAAGGCCACCAAGGCGACGATCCGTAACCTGGTCTG				235
Query	258	AAAGAATAATCACCCCTCACTGTGACAGAGACACGGCACACACACCTACGGGAGGCATCAG				317
Sbjct	236	AGAGGATGATCAGTCACACTGGAACCTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAG				295
Query	318	TGGGGAAATATTGCAGAATGAGCGCAAGCCTGAGGCATGCCTGCCGCGTGTATAAAGAAAG				377
Sbjct	296	TGGGGAAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGG				355
Query	378	CGCAATGGTTGTAAGTACTTTTAGTCGGGAGGAAAGCGTTGATGCTAATATCATCTTCG				437
Sbjct	356	TCTTCGATTGTAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGT				415
Query	438	ATTGTCGTTACCGACTGAACAAGCGCCGGCTAACTCCGTGCCACCACCCGCGGTAATACG				497
Sbjct	416	TTTGACGTTACCAACAGAATAAGCACCCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACG				475
Query	498	GAGGGTGCAGCGTTAATCTTAATTACTGTGCGTATAGCGCACGAGCGGTTGATTAGG				557
Sbjct	476	AAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCTAGGTGGTTTCAGCAAG				535
Query	558	TTGTATGTGAAATCGCCGGGCTCAGCCTGGGGATGGCATCTCAAACCTGGTCAGCTAGAGT				617
Sbjct	536	TTGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACACTGAGCTAGAGT				595
Query	618	CTTGTGGAgggggggTATAATTCCATGTGTATCGCTGAAATGCGTATAGATGTGGAGGAA				677
Sbjct	596	ACGGTAGA-GGGTGGTGGAAATTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAA				654
Query	678	TATCGGTGGCGAAAGCGCCCCCTGGACACAGACAGACACTCACGTGCGAAAGCGTGGG				737
Sbjct	655	CACCACTGGCGAAGGC-GACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGG				713
Query	738	GAGCACACATGATTAATACCCTGGTAATCCACGCTGTAAACGATATCTATTTGTAGGTT				797
Sbjct	714	GAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAACGATGTCGACTAGCCGTTG				773
Query	798	GTGTCCTTAGACGTGGCTTCCGGAACCTCACGCGTTAAATCGACCCTGGGGAGTACCG				857
Sbjct	774	GGATCCTTGAGATCTTAGTGCCGACGCTAACGCCGATAAGTCGACCCTGGGGAGTA-CG				832
Query	858	CCCGCAAGGTTAAAACCTAATTAATTGACGGGGGGCCCCACAAACGGAGGGAGCATG				917
Sbjct	833	GCCGCAAGGTTAAAACCTC-AAATGAATTGAC-GGGGGCCCCGACAAGCGG-TGGAGCATG				889
Query	918	TGGATTTAATTCCATGCCACCGCaAAAAAACCTTACCTACTCCTTGACATCCCGAGTAAC				977
Sbjct	890	TGG-TTTAATTCGAAG-CAACGC-GAAGAACCTTACCT-GGCCTTGACATGCTGAG-AAC				944
Query	978	TTATCAGAAAATTCTTTTGTCTTTCACGAAAAATCTCA-AAACACGTGCTGGCATGGC				1036
Sbjct	945	TTTCCAGAGA--TGGATTGGTG-CCTTCGGGAA--CTCAGACACAGGTGCT-GCATGGT				997
Query	1037	TGTCCCCACCTC 1048				
Sbjct	998	TGTCTTCAGCTC 1009				

Fig. (3-13): Partial sequences of *16S* RNA gene of *P. aeruginosa* isolates from diabetic foot.

Pseudomonas aeruginosa Iraq.PA-2 isolate 16S ribosomal RNA gene, partial sequence					
Score	Expect	Identities	Gaps	Strand	Plus/Plus
576 bits(638)	4e-168	618/803(77%)		12/803(1%)	
Query	20	TGCAGTCGAACGCAATTTATCGGTCCTTGCTCCTGGATTCAACCGGCGGAAGGGAGAGTCA			79
Sbjct	1	TGCAGTCGAGCGGA--TGAAGGGAGCTTGCTCCTGGATTCAAGCGCGGACGGGTGAGTAA			58
Query	80	TGCCGGTGAATCTGCCTGGTAGTGGCTGACCATCTCAGGAAACAGGACGCTTGACCGCA			139
Sbjct	59	TGCCTGGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGG-CGCTAATACCGCA			117
Query	140	GTGCTCCTGCCGGAGAAAGTGGTGTACCTTCGGACCTCACGCTGTGAGATGATCCTACGT			199
Sbjct	118	TACGTCTGAGGGAGAAAGTGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGT			177
Query	200	CGGATTAGC-AGTTGGCGCGGTGAAGGCCACTACCATGGCGACGATCCGTATCTGGTCTGAG			258
Sbjct	178	CGGATTAGCTAGTTGGTGGGTAAAGGCCACTACCAAGGCCGACGATCCGTAACCTGGTCTGAG			237
Query	259	ACGATGATCAGTCACACTGGAAGTGAAGACACGGCCACACTCCTACAGAAAGCAGCCCTA			318
Sbjct	238	AGGATGATCAGTCACACTGGAAGTGAAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTG			297
Query	319	GC-AATATTGGACAATGGGCGAAGGCCCGATCCCGCCGTGCCACGTGCGTAAAGAACGTC			377
Sbjct	298	GGGAATATTGGACAATGGGCGAAGGCCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTC			357
Query	378	TTCGCATTGGTGAGCACTTTATTCTGAGAGGAAGGGTCTTTTGTAAAACTTGCTGTTA			437
Sbjct	358	TTCGGATTGTAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTT			417
Query	438	TGACATTACCACCTTAATACCCTCCGGGTAACCTCGCGCCAACACCCGCGGTAATACGAG			497
Sbjct	418	TGACGTTACCAACAGAATAAGCACCAGGCTAATTCGTGCCAGCAGCCGCGGTAATACGAA			477
Query	498	GGGTGCACCCGTGAATCGCAATTACTGGGCGTATTGCGCGCGTTTGTGGGTCAGCAAGTT			557
Sbjct	478	GGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCAGCAAGTT			537
Query	558	GGATGTGGAATCTTCGGGATCCTCATGGGAACTGCCTCCGATCCTACTGAGATGGACTAT			617
Sbjct	538	GGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAACTACTGAGCTAGAGTAC			597
Query	618	GGTAAATGGGGGATGTTATTTCTGTATAACGGAGTGGTGTCTCCATGTTAGCGGGAACA			677
Sbjct	598	GGTAGA-GGGTGGTGGAAATTTCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACA			656
Query	678	TGCGTGGATATATGCGACGAACTGCACTGGATACAGACGGCTCTCTGGGCTAGCACTGAG			737
Sbjct	657	CCAGTGGCGA-AGGCGACCACTGGACT-GATACTGACACTGAGGTGCGAAAGC-GTGGG			713
Query	738	GAGCTAAGCTCGAATAGATGCGCTAGCTAATCCAGGTTATATACCCTGGTCGACTCACCC			797
Sbjct	714	GAGC-AAACAGGATTAGATACCCT-GGTAGTCCACGCCGTAAACGAT-GTCGACTAGCCG			770
Query	798	CTGAAATCCTTGAGATCTTATTG			820
Sbjct	771	TTGGGATCCTTGAGATCTTAGTG			793

Fig. (3-14): Partial sequences of *16S* RNA gene of *P. aeruginosa* isolates from burns.

Pseudomonas aeruginosa Iraq.PA-3 isolate 16S ribosomal RNA gene, partial sequence						
Score	Expect	Identities	Gaps	Strand	Plus/Plus	
1793 bits (1988)	0.0	1021/1032 (99%)	5/1032 (0%)			
Query	21	TGCAGTCGAGCGGATGAAGGGAGCTTGCTCCTGGATTACAGCGCGGACGGGTGAGTAATG				80
Sbjct	1	TGCAGTCGAGCGGATGAAGGGAGCTTGCTCCTGGATTACAGCGCGGACGGGTGAGTAATG				60
Query	81	CCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCTAATACCGCATA				140
Sbjct	61	CCTGGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCTAATACCGCATA				120
Query	141	GTCCTGAGGGAGAAAAGTGGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGTCGG				200
Sbjct	121	GTCCTGAGGGAGAAAAGTGGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGTCGG				180
Query	201	ATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCCGTAACCTGGTCTGAGAGG				260
Sbjct	181	ATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCCGTAACCTGGTCTGAGAGG				240
Query	261	ATGATCAGTCACACTGGAAGTGAAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGG				320
Sbjct	241	ATGATCAGTCACACTGGAAGTGAAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGG				300
Query	321	AATATTGGACAATGGGCGAAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTC				380
Sbjct	301	AATATTGGACAATGGGCGAAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTC				360
Query	381	GGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGA				440
Sbjct	361	GGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGA				420
Query	441	CGTTACCAACAGAATAAGCACCCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGG				500
Sbjct	421	CGTTACCAACAGAATAAGCACCCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGG				480
Query	501	TGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCAGCAAGTTGGA				560
Sbjct	481	TGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCAGCAAGTTGGA				540
Query	561	TGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACACTACTGAGCTAGAGTACGGT				620
Sbjct	541	TGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACACTACTGAGCTAGAGTACGGT				600
Query	621	AGAGGGTGGTGGAAATTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAG				680
Sbjct	601	AGAGGGTGGTGGAAATTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAG				660
Query	681	TGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAAGCGTGGGGAGCAAA				740
Sbjct	661	TGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAAGCGTGGGGAGCAAA				720
Query	741	CAGGATTAGATACCCTGGTAGTCCACGCCGTAACGATGTCGACTAGCCGTTGGGATCCT				800
Sbjct	721	CAGGATTAGATACCCTGGTAGTCCACGCCGTAACGATGTCGACTAGCCGTTGGGATCCT				780
Query	801	TGAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGACCGCCTGGGGGAGTACGGCCGCAA				860
Sbjct	781	TGAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGACCGCCTGGGGGAGTACGGCCGCAA				839
Query	861	GGTTAAAACCAAATGAATTGACGGGGGCCCGCACAAAGCGGTGGAGCATGTGGGTTAAT				920
Sbjct	840	GGTTAAAACCAAATGAATTGACGGGGGCCCGCACAAAGCGGTGGAGCATGTGGGTTAAT				898
Query	921	TCGAAGCAACGCGAAGAACCCTTACCTGGCCTTGACATGCTGAGAACTTTCCAGAAAATGG				980
Sbjct	899	TCGAAGCAACGCGAAGAACCCTTACCTGGCCTTGACATGCTGAGAACTTTCCAG-AGATGG				957
Query	981	ATTGGTGGCCTTCGGGAACTCAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGCCT				1040
Sbjct	958	ATTGGT-GCCTTCGGGAACTCAGACACAGGTGCTGCATGGTTGCTTCAGCTCGTGCCT				1016
Query	1041	GAAAATGTTTGG 1052				
Sbjct	1017	G-AGATGTTGGG 1027				

Fig. (3-15): Partial sequences of 16S RNA gene of *P. aeruginosa* isolates from urine.

Pseudomonas aeruginosa Iraq.PA-4 isolate 16S ribosomal RNA gene, partial sequence							
Score	Expect	Identities	Gaps	Strand	Plus/Plus		
1361 bits	(1508)	0.0	852/901 (95%)	12/901 (1%)			
Query	23	TGCAGTCGAGCGGATGAAGGGAGCTTGCTCCTGGATTCAGCGGCGGACGGGTGAGTAATG					82
Sbjct	1	TGCAGTCGAGCGGATGAAGGGAGCTTGCTCCTGGATTCAGCGGCGGACGGGTGAGTAATG					60
Query	83	CCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCTAATACCGCATAAC					142
Sbjct	61	CCTGGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCTAATACCGCATAAC					120
Query	143	GTCCTGAGGGAGAAAAGTGGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGTCCGG					202
Sbjct	121	GTCCTGAGGGAGAAAAGTGGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGTCCGG					180
Query	203	ATTAGCTAGTTGGTGGGGTAAAGGCCACCAAGGCGACGATCCGTAACCTGGTCTGAGAGG					262
Sbjct	181	ATTAGCTAGTTGGTGGGGTAAAGGCCACCAAGGCGACGATCCGTAACCTGGTCTGAGAGG					240
Query	263	ATGATCAGTCACACTGGAACCTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGG					322
Sbjct	241	ATGATCAGTCACACTGGAACCTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGG					300
Query	323	AATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTC					382
Sbjct	301	AATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTC					360
Query	383	GGATTGTAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGA					442
Sbjct	361	GGATTGTAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGA					420
Query	443	CGTTACCAACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGG					502
Sbjct	421	CGTTACCAACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGG					480
Query	503	TGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCTAGGTGGTTCAGCAAGTTGGA					562
Sbjct	481	TGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCTAGGTGGTTCAGCAAGTTGGA					540
Query	563	TGTGAAATCCCCGGGCTCAACCTAGGAACTGCATCCAAAACACTACTGAGCTAGAGTACGGT					622
Sbjct	541	TGTGAAATCCCCGGGCTCAACCTAGGAACTGCATCCAAAACACTACTGAGCTAGAGTACGGT					600
Query	623	AGAGGGTGGTGGAAATTCCTGTGTAGCGGTGAAAATGCGTAGATATAGGAAAGGAACACC					682
Sbjct	601	AGAGGGTGGTGGAAATTCCTGTGTAGCGGTG-AAATGCGTAGATATAGG-AAGGAACACC					658
Query	683	AGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGATGTGCGAAAGCGTGGGGGAGC					742
Sbjct	659	AGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGT-GGGGAGC					717
Query	743	AAACAACGAATAGATACCCTGGCAGTGCAGGCCCTACACCATGTCAACTAACCCCTTGGG					802
Sbjct	718	AAAC-AGGATTAGATACCCTGGTAGTCCACG-CCGTAACGATGTCGACTAGCCGTTGGG					775
Query	803	ATCCGTGAGATTTTA-TTGCGC-GCT-ACACGATAA-TCAACAGCTGGGGGAGTACGGCC					858
Sbjct	776	ATCCTTGAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGACCGCCTGGGGAGTACGGCC					835
Query	859	-CCCGGGTGAACCTC-AATGTATTGTCAATGGCCCTGCACAAACACACGAGCAGGTGGTT					916
Sbjct	836	GCAAGGTAAAACCTCAAATGAATTGACGGGGGCC- GCACAAGCGGTGGAGCATGTGGTT					894
Query	917	T					917
Sbjct	895	T					895

Fig. (3-16): Partial sequences of 16S RNA gene of *P. aeruginosa* isolates from sputum.

```

Pseudomonas aeruginosa Iraq.PA-5 isolate 16S ribosomal RNA gene, partial sequence
Score Expect Identities Gaps Strand Plus/Plus
1828 bits (2026) 0.0 1023/1028 (99%) 1/1028 (0%)
Query 23 TGCAGTCGAGCGGATGAAGGGAGCTTGCTCCTGAGATTGAGCGGCGGACGGGTGAGTAAT 82
Sbjct 1 TGCAGTCGAGCGGATGAAGGGAGCTTGCTCCTG-GATTGAGCGGCGGACGGGTGAGTAAT 59
Query 83 GCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCTAATACCGCATA 142
Sbjct 60 GCCTGGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCTAATACCGCATA 119
Query 143 CGTCCTGAGGGAGAAAGTGGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGTCG 202
Sbjct 120 CGTCCTGAGGGAGAAAGTGGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGTCG 179
Query 203 GATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCCGTAAGTGGTCTGAGAG 262
Sbjct 180 GATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCCGTAAGTGGTCTGAGAG 239
Query 263 GATGATCAGTCACACTGGAAGTGAAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGG 322
Sbjct 240 GATGATCAGTCACACTGGAAGTGAAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGG 299
Query 323 GAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTT 382
Sbjct 300 GAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTT 359
Query 383 CGGATTGTAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTG 442
Sbjct 360 CGGATTGTAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTG 419
Query 443 ACGTTACCAACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGG 502
Sbjct 420 ACGTTACCAACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGG 479
Query 503 GTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCAGCAAGTTGG 562
Sbjct 480 GTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCAGCAAGTTGG 539
Query 563 ATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACACTGAGCTAGAGTACGG 622
Sbjct 540 ATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACACTGAGCTAGAGTACGG 599
Query 623 TAGAGGGTGGTGGAAATTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAAACACCA 682
Sbjct 600 TAGAGGGTGGTGGAAATTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAAACACCA 659
Query 683 GTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAA 742
Sbjct 660 GTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAA 719
Query 743 ACAGGATTAGATACCCTGGTAGTCCACGCCGTAACGATGTCGACTAGCCGTTGGGATCC 802
Sbjct 720 ACAGGATTAGATACCCTGGTAGTCCACGCCGTAACGATGTCGACTAGCCGTTGGGATCC 779
Query 803 TTGAGATCTTAGTGGCGCAGCTAACGCGATAAGTGCACCGCCTGGGGAGTACGGCCGCAA 862
Sbjct 780 TTGAGATCTTAGTGGCGCAGCTAACGCGATAAGTGCACCGCCTGGGGAGTACGGCCGCAA 839
Query 863 GGTAAAACCTCAAATGAATTGACGGGGGCCCCGACAAGCGGTGGAGCATGTGGTTTAATT 922
Sbjct 840 GGTAAAACCTCAAATGAATTGACGGGGGCCCCGACAAGCGGTGGAGCATGTGGTTTAATT 899
Query 923 CGAAGCAACGCGAAGAACCCTTACCTGGCCTTGACATGCTGAGAACCTTCCAGAGATGGAT 982
Sbjct 900 CGAAGCAACGCGAAGAACCCTTACCTGGCCTTGACATGCTGAGAACCTTCCAGAGATGGAT 959
Query 983 TGGTGCCTTCGGGAACTCAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCTGAG 1042
Sbjct 960 TGGTGCCTTCGGGAACTCAGACACAGGTGCTGCATGGTTGTCTTCAGCTCGTGTCTGAG 1019
Query 1043 ATGTTGGG 1050

```

Fig. (3-17): Partial sequences of 16S RNA gene of *P. aeruginosa* isolates from sputum.

Pseudomonas aeruginosa Iraq.PA-6 isolate 16S ribosomal RNA gene, partial sequence						
Score	Expect	Identities	Gaps	Strand	Plus/Plus	
1777 bits (1970)	0.0	1020/1034 (99%)		7/1034 (0%)		
Query	25	TGCAGTCGAGCGGATGAAGGGGAGCTTGCTCCTGGATTTCAGCGGCGGACGGGTGAGTAATG				84
Sbjct	1	TGCAGTCGAGCGGATGAAGGGGAGCTTGCTCCTGGATTTCAGCGGCGGACGGGTGAGTAATG				60
Query	85	CCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCTAATACCGCATA				144
Sbjct	61	CCTGGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCTAATACCGCATA				120
Query	145	GTCCTGAGGGGGAAAGTGGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGTCGG				204
Sbjct	121	GTCCTGAGGGGAGAAAGTGGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGTCGG				180
Query	205	ATTAGCTAGTTGGTGGGGTAAAGGCCACCAAGGCGACGATCCGTAAGTGGTCTGAGAGG				264
Sbjct	181	ATTAGCTAGTTGGTGGGGTAAAGGCCACCAAGGCGACGATCCGTAAGTGGTCTGAGAGG				240
Query	265	ATGATCAGTCACACTGGAAGTGAAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGG				324
Sbjct	241	ATGATCAGTCACACTGGAAGTGAAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGG				300
Query	325	AATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTC				384
Sbjct	301	AATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTC				360
Query	385	GGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGA				444
Sbjct	361	GGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGA				420
Query	445	CGTTACCAACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGG				504
Sbjct	421	CGTTACCAACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGG				480
Query	505	TGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCTAGGTGGTTCAGCAAGTTGGA				564
Sbjct	481	TGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCTAGGTGGTTCAGCAAGTTGGA				540
Query	565	TGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACACTACTGAGCTAGAGTACGGT				624
Sbjct	541	TGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACACTACTGAGCTAGAGTACGGT				600
Query	625	AGAGGGTGGGTGGAATTTCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCA				684
Sbjct	601	AGAGGGT-GGTGGAATTTCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCA				659
Query	685	GTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAA				744
Sbjct	660	GTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAA				719
Query	745	ACAGGATTAGATACCCTGGTAGTCCACGCCGTAACGATGTCGACTAGCCGTTGGGATCC				804
Sbjct	720	ACAGGATTAGATACCCTGGTAGTCCACGCCGTAACGATGTCGACTAGCCGTTGGGATCC				779
Query	805	TTGAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGACCCTGGGGGAGTACGGCCGCA				864
Sbjct	780	TTGAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGACCCT-GGGGAGTACGGCCGCA				838
Query	865	AGGTTAAAACCTCAAATGAATTGACGGGGGCCCGCACAAAGCGGTGGAGCATGTGTTTAA				924
Sbjct	839	AGGTTAAAACCTCAAATGAATTGACGGGGGCCCGCACAAAGCGGTGGAGCATGTGTTTAA				898
Query	925	TCGAAGCAACGCGAAGAACCCTTACCTGGCCTTGACATGCTGAGAACTTCCAGAGAATGG				984
Sbjct	899	TCGAAGCAACGCGAAGAACCCTTACCTGGCCTTGACATGCTGAGAACTTCCAGAG-ATGG				957
Query	985	ATTGGTGGCCTTCGGGAACTCAGACACAGGTGCTGCATGGGCTGTCGTCCACCTCGTGT				1044
Sbjct	958	ATTGGT-GCCTTCGGGAACTCAGACACAGGTGCTGCAT-GGTTGTC-TTCAGCTCGTGT				1014
Query	1045	GTGGAGATGTTTGG 1058				
Sbjct	1015	CT-GAGATGTTGGG 1027				

Fig. (3-18): Partial sequences of 16S RNA gene of *P. aeruginosa* isolates from sputum.

Pseudomonas aeruginosa Iraq.PA-7 isolate 16S ribosomal RNA gene, partial sequence						
Score	Expect	Identities	Gaps	Strand	Plus/Plus	
902 bits (1000)	0.0	783/965 (81%)		15/965 (1%)		
Query	67	AGCGGCGGACGGGTGACTAATGTATGGGGATGTGCCCGATAGAGGGGGATAACTACTGGA				126
Sbjct	39	AGCGGCGGACGGGTGAGTAATGCCTGGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGA				98
Query	127	AACGGTGGCTAATACCGCATAATGTCTACAGACCAAAGCAGGGGCTCTTCCGACCTTGCA				186
Sbjct	99	AACGGGCGCTAATACCGCATACTGCTGAGGGAGAAAGTGGGGGATCTTCGGACCTCACG				158
Query	187	CTAGACCATCAACCGATATGGGATTAATCTACTAGCTGGGCTAAAGCCTGGGCTACGGGAC				246

Fig. (3-19): Partial sequences of *16S* RNA gene of *P. aeruginosa* isolates from wound.


```

Pseudomonas aeruginosa Iraq.PA-8 isolate 16S ribosomal RNA gene, partial sequence
Score Expect Identities Gaps Strand Plus/Plus
1229 bits (1362) 0.0 906/1013(89%) 36/1013(3%)
Query 22 TGCAGTCGAGCGGATGA-GGGAGCTTGCTCCTGGATTACGCGGCGGACGGGTGAGTAATG 80
Sbjct 1 TGCAGTCGAGCGGATGAAGGGAGCTTGCTCCTGGATTACGCGGCGGACGGGTGAGTAATG 60
Query 81 CCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCTAGTACCGCAGAG 140
Sbjct 61 CCTGGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCTAATACCGCATAAC 120
Query 141 CTCCTGAGGGAGAAAAGTGGGGGATCTTCGGACCTCACGCTATCAGATGATCCTAGGTCGG 200
Sbjct 121 GTCCTGAGGGAGAAAAGTGGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGTCGG 180
Query 201 ATTAAGTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCCGTAACCTGGTCTGAGAGG 260
Sbjct 181 ATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCCGTAACCTGGTCTGAGAGG 240
Query 261 ATGATCAGTCACACTGGAAGTGGAGACACGGTCCACACTCCTACGGGAGGCAGCAGTGGGG 320
Sbjct 241 ATGATCAGTCACACTGGAAGTGGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGG 300
Query 321 AATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTC 380
Sbjct 301 AATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTC 360
Query 381 GGATTGTAAGCACTTTAAGTTGGGAGGAAGGGCATTAAAGTTAATACCTTGCTGTTTTGA 440
Sbjct 361 GGATTGTAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGA 420
Query 441 CGTTACCAACGGAATAAGCACC GGCTAACTTCGTGCCAGCACCCGCGGTAATACCAAGGG 500
Sbjct 421 CGTTACCAACAGAATAAGCACC GGCTAACTTCGTGCCAGCACCCGCGGTAATACGAAGGG 480
Query 501 TGCAAGCGTTAATCGGAATTACTGGGCGTAGAGCGCGCGTATGTGGGTCAGCAAGTTGGA 560
Sbjct 481 TGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTACAGCAAGTTGGA 540
Query 561 TGTGAGATCCCCGGGCTCAACCTGGGAACTGCATCCGGAACACTGAGCTAGAGTACGGT 620
Sbjct 541 TGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACACTGAGCTAGAGTACGGT 600
Query 621 AGAGGGGGGATGGAATTCGTGTGTAACGATAATGTGCTTACATGTGGGAAGGAGCACTG 680
Sbjct 601 AGAGGGTGG-TGGAATTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAAACCA 659
Query 681 CTGACAAATGCCAACGACTGGAATGATACTGACACTGACGTGGGAAAGCGTGTGAAGCTA 740
Sbjct 660 GTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAA 719
Query 741 ACGCGATTAATACGCTGGATAATCCACGCCGTAATCCATGGGCTACTACCCCGTTGGG 800
Sbjct 720 ACAGGATTAGATACCCTGG-TAGTCCACGCCGTAATA-CGAT-GTCGACTA-GCCGTTGGG 775
Query 801 AATCCATGAGATCTTAGTGGTGGCAGCTTATCCCGATCATTTCGAGCGGCCTGGGGAAGT 860
Sbjct 776 -ATCCTTGAGATCTTAGTGG-CGCAGC-TAACGCGAT-AAGTCGA-CCGCCTGGGG-AGT 829
Query 861 ACGGACC GCAATGGTTAAAGGCTCAGATGAAATTGACGGGGGGCCCGCACAAAGCGAATT 920
Sbjct 830 ACGG-CCGCAA-GGTAAA-ACTCAAATG-AATTGAC-GGGGGCCCGCAC-AAGCG--GT 881
Query 921 GAACCATGTGGCTTTAATTTCCGAAAGCGAACGCGTAAGAACCCTTACCTGGACCTTGAC 980
Sbjct 882 GGAGCATGTGG-TTTAA-TTCG-AAGC-AACGCG-AAGAACC-TTACCTGG-CCTTG-AC 933
Query 981 ATGACTGAACATCTTTTCCGGCAGATGGGATTGGTTGCCCTTCGGGAAACTCA 1033
Sbjct 934 ATG-CTG-AGAACTTCCAG--AGAT-GGATTGGT--GCCTTCGGG-AACTCA 978

```

Fig. (3-20): Partial sequences of 16S RNA gene of *P. aeruginosa* isolates from ear.

```

Pseudomonas aeruginosa Iraq.PA-9 isolate 16S ribosomal RNA gene, partial sequence
Score Expect Identities Gaps Strand Plus/Plus
1838 bits (2038) 0.0 1024/1027(99%) 0/1027(0%)
Query 25 TGCAGTCGAGCGGATGAAGGGAGCTTGCTCCTGGATTGAGCGGCGGACGGGTGAGTAATG 84
Sbjct 1 TGCAGTCGAGCGGATGAAGGGAGCTTGCTCCTGGATTGAGCGGCGGACGGGTGAGTAATG 60
Query 85 CCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCGCGAAACGGGCCTAATACCGCATAAC 144
Sbjct 61 CCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCGCGAAACGGGCCTAATACCGCATAAC 120
Query 145 GTCCTGAGGGAGAAAAGTGGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGTCGG 204
Sbjct 121 GTCCTGAGGGAGAAAAGTGGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGTCGG 180
Query 205 ATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCCGTAACCTGGTCTGAGAGG 264
Sbjct 181 ATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCCGTAACCTGGTCTGAGAGG 240
Query 265 ATGATCAGTCACACTGGAAGTGGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGG 324
Sbjct 241 ATGATCAGTCACACTGGAAGTGGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGG 300
Query 325 AATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTC 384
Sbjct 301 AATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTC 360
Query 385 GGATTGTAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGA 444
Sbjct 361 GGATTGTAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGA 420
Query 445 CGTTACCAACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGG 504
Sbjct 421 CGTTACCAACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGG 480
Query 505 TGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCAGCAAGTTGGA 564
Sbjct 481 TGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCAGCAAGTTGGA 540
Query 565 TGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACACTACTGAGCTAGAGTACGGT 624
Sbjct 541 TGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACACTACTGAGCTAGAGTACGGT 600
Query 625 AGAGGGTGGTGGAAATTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAG 684
Sbjct 601 AGAGGGTGGTGGAAATTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAG 660
Query 685 TGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAA 744
Sbjct 661 TGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAA 720
Query 745 CAGGATTAGATACCCTGGTAGTCCACGCCGTAACGATGTCGACTAGCCGTTGGGATCCT 804
Sbjct 721 CAGGATTAGATACCCTGGTAGTCCACGCCGTAACGATGTCGACTAGCCGTTGGGATCCT 780
Query 805 TGAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGACCGCCTGGGGAGTACGGCCGCAAG 864
Sbjct 781 TGAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGACCGCCTGGGGAGTACGGCCGCAAG 840
Query 865 GTTAAAACCTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTC 924
Sbjct 841 GTTAAAACCTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTC 900
Query 925 GAAGCAACGCGAAGAACCTTACCTGGCCTTGACATGCTGAGAACCTTCCAGAGATGGATT 984
Sbjct 901 GAAGCAACGCGAAGAACCTTACCTGGCCTTGACATGCTGAGAACCTTCCAGAGATGGATT 960
Query 985 GGTGCCCTTCGGGAACTCAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGCCTGAGA 1044
Sbjct 961 GGTGCCCTTCGGGAACTCAGACACAGGTGCTGCATGGTTGCTTCAGCTCGTGCCTGAGA 1020
Query 1045 TGTTGGG 1051
Sbjct 1021 TGTTGGG 1027

```

Fig. (3-21): Partial sequences of 16S RNA gene of *P. aeruginosa* isolates from wound.

Pseudomonas aeruginosa Iraq.PA-10 isolate 16S ribosomal RNA gene, partial sequence					
Score	Expect	Identities	Gaps	Strand	
1741 bits (1930)	0.0	1021/1041 (98%)	14/1041 (1%)	Plus/Plus	
Query	21	TGCAGTCGAGCGGATGAAGGGAGCTTGCTCCTGGATTACAGCGCGGACGGGTGAGTAATG			80
Sbjct	1	TGCAGTCGAGCGGATGAAGGGAGCTTGCTCCTGGATTACAGCGCGGACGGGTGAGTAATG			60
Query	81	CCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGAAACGGGCGCTAATACCGCATA			140
Sbjct	61	CCTGGGAATCTGCCTGGTAGTGGGGGATAACGTCCGAAACGGGCGCTAATACCGCATA			120
Query	141	GTCCTGAGGGAGAAAGTGGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGTCCG			200
Sbjct	121	GTCCTGAGGGAGAAAGTGGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGTCCG			180
Query	201	ATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCCGTAACCTGGTCTGAGAGG			260
Sbjct	181	ATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCCGTAACCTGGTCTGAGAGG			240
Query	261	ATGATCAGTCACACTGGAAGTGAAGACCGGTCCAGACTCCTACGGGAGGCAGCAGTGGGG			320
Sbjct	241	ATGATCAGTCACACTGGAAGTGAAGACCGGTCCAGACTCCTACGGGAGGCAGCAGTGGGG			300
Query	321	AATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTC			380
Sbjct	301	AATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTC			360
Query	381	GGATTGTAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGA			440
Sbjct	361	GGATTGTAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGA			420
Query	441	CGTTACCAACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGTAATACGAAGGG			500
Sbjct	421	CGTTACCAACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGTAATACGAAGGG			480
Query	501	TGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCTAGGTGGTTTCAGCAAGTTGGA			560
Sbjct	481	TGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCTAGGTGGTTTCAGCAAGTTGGA			540
Query	561	TGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACCTACTGAGCTAGAGTACGGT			620
Sbjct	541	TGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACCTACTGAGCTAGAGTACGGT			600
Query	621	AGAGGGTGGGTGGAATTTCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCA			680
Sbjct	601	AGAGGGT-GGTGGAATTTCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCA			659
Query	681	GTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAA			740
Sbjct	660	GTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAA			719
Query	741	ACAGGATTAGATACCCTGGTAGTCCACGCCGTAACGATGTCGACTAGCCGTTGGGATCC			800
Sbjct	720	ACAGGATTAGATACCCTGGTAGTCCACGCCGTAACGATGTCGACTAGCCGTTGGGATCC			779
Query	801	TTGAGATCTTAGTGCCGAGCTAACGCGATAAGTCGACCGCCTGGGGAGTACGGCCGCA			860
Sbjct	780	TTGAGATCTTAGTGCCGAGCTAACGCGATAAGTCGACCGCCT-GGGGAGTACGGCCGCA			838
Query	861	AGGTTAAAACCTCAAATGAATTGACGGGGGCCCCGACAAGCGGTGGAAGCATGTGGGTTTA			920
Sbjct	839	AGGTTAAAACCTCAAATGAATTGACGGGGGCCCCGACAAGCGGTGG-AGCATGT-GGTTTA			896
Query	921	ATTCGAAGCAACGCGAAGAACCCTTACCTGGCCTTGACATGCTGAGAACTTTCCAGAGATG			980
Sbjct	897	ATTCGAAGCAACGCGAAGAACCCTTACCTGGCCTTGACATGCTGAGAACTTTCCAGAGAT-			955
Query	981	GGATTGGTGCCTTCGGGGAACTCAGAACACAGGGTGCCTTGCATGGGCTGTCGTTACCT			1040
Sbjct	956	GGATTGGTGCCTTCGGGGAACTCAGAACACAGGGTGCCTTGCATGGGCTGTCGTTACCT			1008
Query	1041	CCTGTCCTTGAAGATGTTGG	1061		
Sbjct	1009	CGTGCCT--GAGATGTTGGG	1027		

Fig. (3-22): Partial sequences of 16S RNA gene of *P. aeruginosa* isolates from wound.

The results of DNA sequencing should be firstly examined to confirm the nucleotide sequences and closed relationships with others world strain, test used to confirm was through NCBI-BLAST-query-

nucleotide –online, it was perfect program and gave the exact results of identity percentage with other world strains and they were ranged from (97%-100%).

It has been demonstrated that *16srRNA* gene sequence data on an individual strain with a nearest neighbor exhibiting a similarity score <97% represent a new species, the meaning of similarity scores of >97% is not as clear (Petti, 2007).

Table. (3-8), shows the Evolutionary Divergence between studied sequences of *P. aeruginosa* based on the number of base substitutions per site between sequences. The differences in comparison among bases sequences were considered in evolutionary. The analysis involved 11 nucleotide sequences 10 local isolates and one stranded strain: KR815840.1. All ambiguous positions were removed for each sequence pair. There were a total of 1606 base positions in the final dataset. The results revealed a genetic variation as base substitutions in the nucleotide sequences of involved local isolates when compared with standard world. The evolutionary divergence between involved sequences ranged from 0.005between local isolates No.5 burns sample) and Standard world isolates to 0.332 between local isolates No.1 and standard World strain (KR815840.1) based on the sequences analysis of *16srRNA* gene.

No. of isolate in Sequence and source	NCBI Accession Number	NCBI-BLAST Homology sequence identity
---------------------------------------	-----------------------	---------------------------------------

		Sequence Identity (%)	Genetic variation (base substitutions)
1.Diabetic foot	KX963356	74%	0.332
2.burn	KX963357	77%	0.350
3.urine	KX963358	99%	0.006
4.sputum	KX963359	95%	0.117
5.burn	KX963360	99%	0.005
6.treach sputum	KX963361	99%	0.013
7.wound	KX963361	81%	0.255
8.ear	KX963362	89%	0.148
9.wound	KX963363	99%	0.003
10.wound	KX963365	98%	0.017

Table (3-8): Homology sequence identity of *P. aeruginosa* compared with *P. aeruginosa* Standard strain by using NCBI-Blast Gen bank Database:

	1	2	3	4	5	6	7	8	9	10	11
1. <i>P.aeruginosa</i> iraq-1 isolate 16S ribosomal RNA gene											
2. <i>P.aeruginosa</i> iraq-2 isolate 16S ribosomal RNA gene	0.688										
3. <i>P.aeruginosa</i> iraq-3 isolate 16S ribosomal RNA gene	0.387	0.406									
4. <i>P.aeruginosa</i> iraq-4 isolate 16S ribosomal RNA gene	0.422	0.417	0.121								
5. <i>P.aeruginosa</i> iraq-5 isolate 16S ribosomal RNA gene	0.397	0.413	0.037	0.121							
6. <i>P.aeruginosa</i> iraq-6 isolate 16S ribosomal RNA gene	0.373	0.419	0.079	0.128	0.073						
7. <i>P.aeruginosa</i> iraq-7 isolate 16S ribosomal RNA gene	0.270	0.621	0.290	0.339	0.289	0.292					
8. <i>P.aeruginosa</i> iraq-8 isolate 16S ribosomal RNA gene	0.398	0.394	0.197	0.218	0.189	0.204	0.382				
9. <i>P.aeruginosa</i> iraq-9 isolate 16S ribosomal RNA gene	0.405	0.421	0.041	0.127	0.054	0.087	0.294	0.209			
10. <i>P.aeruginosa</i> iraq-10 isolate 16S ribosomal RNA gene	0.347	0.382	0.051	0.121	0.049	0.065	0.294	0.184	0.053		
11. <i>Pseudomonas aeruginosa</i> 16S ribosomal RNA gene (KR815840.1)	0.332	0.350	0.006	0.117	0.005	0.013	0.255	0.148	0.003	0.017	

The present study showed that showed that sequences identity (%) of *P. aeruginosa* (10 isolates) when using DNA sequencing of *16srRNA* gene ranged from 74% -99% according to type of isolates.(Table 3-8) when compred with world strain (KR815840.1). There are 5/10 of local isolates of *P. aeruginosa* gave more than 97% of sequence identity which mean these isolates belong to same species or strain while the remaining isolates (n=5) had a similarity score less than 97%, which mean these isolates belong to other new strains of *P. aeruginosa*.

Open reading frame (ORF) program was a perfect tool which used for reading each nucleotide sequences to 4-6 segments of genetic codes with its translation to amino acids within six ORF three ORF in the direction 5-3 and other 3 ORF in the direction 3-5, one genetic code or more consider proper code which start by methionine and end by stop codon. Also, ORF provide high information about amino acid translations for each sequence. The translated protein by ORF program then submitted to NCBI-Blast by using this protein query for searching on translated database, and results of the ORF were important for searching on other sequences on NCBI, these sequences were collected and download, then added with local sequences of this

study, then submitted for MEGA6 software for alignment and phylogeny.

ORF also provide information used in the submission data of this study to gene bank database information for recording and publishing isolates of this study. The Molecular Evolutionary Genetics Analysis (MEGA) software is a desktop application designed for either from multi gene families or from different species with a special emphasis on different evolutionary relationship and patterns of DNA and protein evolution.

The *16srRNA* gene sequence analysis is the most commonly used method for identifying bacteria or for constructing bacterial phylogenetic relationships (Wang *et al.*, 2008).

In addition to the tools for statistical analysis of data MEGA6 provides many convenient facilities for the assembly of sequence data sets from files or web based repositories, and it includes tools for visual presentation of the results obtained in the form of interactive phylogenetic trees and evolutionary distance matrices (Kumar *et al.*, 2008).

First step of analysis by alignment of all sequences of this study with other world selected reference by using (clustal W) program step in MEGA6. This program demonstrated had accurate degree of identity with all world sequences including sequences of this study.

These results with (clustal W) were important because they used directly in the phylogeny tree construction.

The present used study nucleotide maximum likelihood method for detection the close relationship of world and local sequences which was better method of the nucleotide sequences in MEGA6. Also the phylogenetic tree was constructed using unweight pair group method with arithmetic mean (UPGMA Tree) in MEGA6 version. In the *16srRNA* gene phylogeny of present study introduced for sequencing.

The *16srRNA* gene sequences in formation have an expanding role in the identification of bacteria in clinical or public health settings. This may due to several reasons include (i) its presence in almost all bacteria often existing as a multi gene family, or operons; (ii) the function of *16srRNA* gene over time has not change, suggestion the random sequences change are a more accurate measure of time (evolution); and (iii) the *16srRNA* gene (1500bp) is large enough for informatics purpose (Janda and Abbott, 2007).

3.5. Phylogenetic analysis of local strains:

The present study used the *16srRNA* gene for the phylogeny whem submitted (10 sequences belong to the local isolates and 5 sequences belong to global strains downloaded from NCBI to analysis by using MAGA6 software programe to find nucleotide maximum likelihood between sequencing and obtaining Phylogenetic relationship among local and global strains sequences.

The results showed that the local isolates of these bacteria were closed related to NCBI-Blast *P. aeruginosa* (KR815840.1) except the Iraq-2 isolate (Burn sources) and Iraq-8 isolate (ear sources) showed genetically difference as unique isolates. Whereas the NCBI-Blast *P.aeruginosa* isolates showed different and out of tree. These results means that isolates is considered as a new strains record of *P. eruginosa* in Iraq and world fig. (3-23).

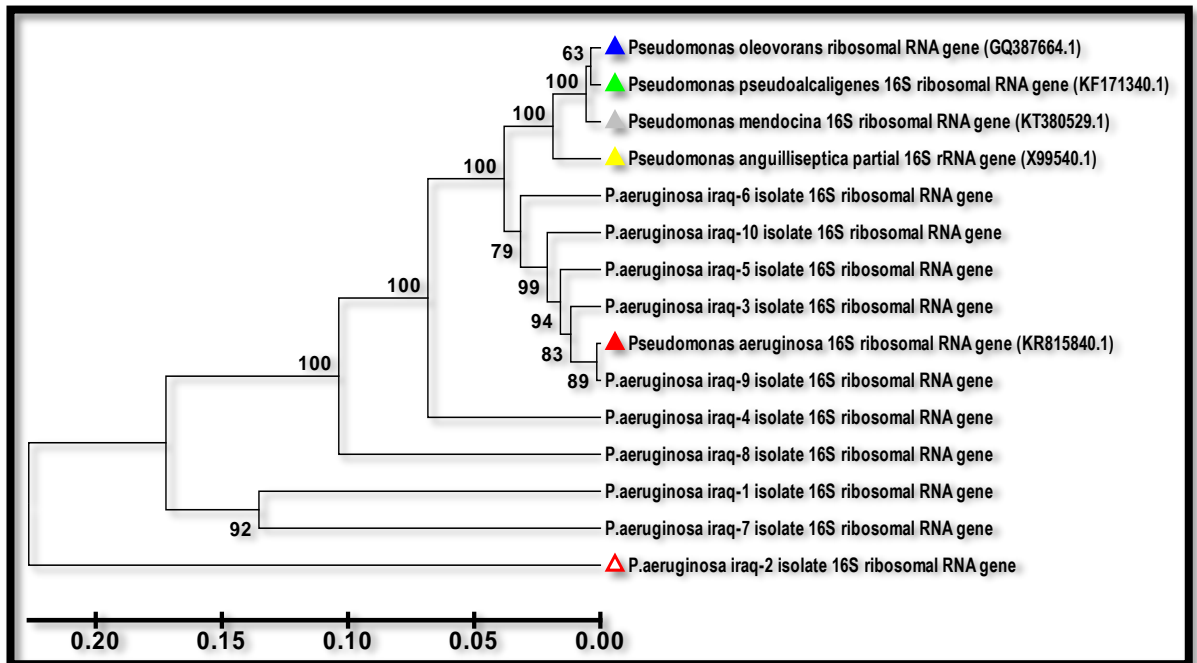


Figure (3- 23): Shows the Phylogenetic tree analysis based on *16S ribosomal RNA* gene partial sequence of 10 *P. aeruginosa* isolates.

P. aeruginosa according to clinical samples that collected from hospitals the results indicated that the sources of 9 isolate with the same origin which No.3 represent (urine), No.7, 9, 10(wounds) No. 5, (burns), No.6 (Trachea) No.4 (sputum), No.1 (Diabetic foot) while the isolate No.8 from ear and isolate No. 2 from burns that collected show different origin or sources (unique isolate) fig 3-

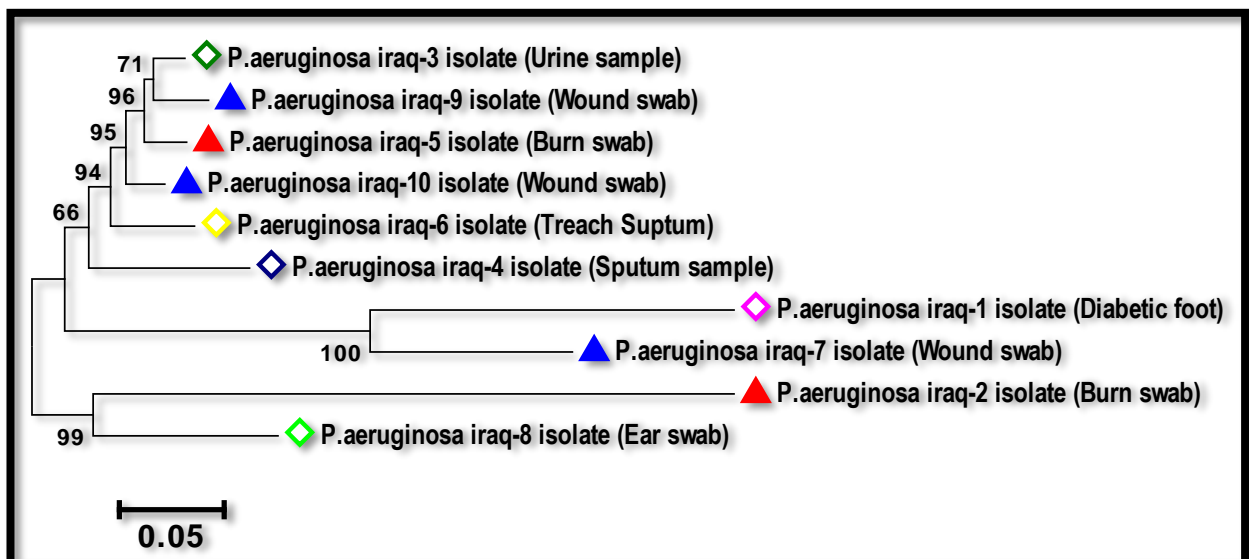


Figure (3-24): Shows the Phylogenetic tree analysis based on 16S ribosomal RNA gene partial sequence 10 isolates.

3.6. Recording Iraqi *P. aeruginosa* isolates in gene bank-NCBI:

Ten sequences of *P. aeruginosa* were isolated from human sources in Baghdad city and each sequence has a symbol code (Iraq.PA-1isolate, Iraq.PA-2isolate,Iraq.PA-3isolate, Iraq.PA-1isolate4, Iraq. PA-5isolate, Iraq.PA-6isolate, Iraq.PA-7isolate, Iraq.PA-8isolate, Iraq.PA-9isolate, Iraq.PA-10isolate. *16srRNA* gene sequences submitted to Gen Bank- Ban kit under submission code BankIt11959593.The results of these sequences were analyzed and examined by professional staff in gene bank in two working days. All these sequences accepted in gene bank and each sequence take accession number (KX963356, KX963357, KX963358, KX963359, KX963360, KX963361, KX963362 KX963363, and KX963364 KX963365).(appendices. -10) Version number codes after two months (KP420229.1, KP420230.1, KP420231.1, KP420232.1, KP420233.1, KP420234.1, KP420235.1, and KP420236.1) in the pobset number: 798546704. Appendix (III -) Accession number of Iraqi isolates of *P. aeruginosa* in Gene bank.

