# Isolation and Analysis of Nucleotide Sequences of the *16S* rRNA Gene of *Pseudomonas aeruginosa* Isolated from Clinical Samples

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## Abstract

*Pseudomonas aeruginosa* consider one of the opportunistic pathogens is able to infection almost all body tissue as a result of having it a variety of virulence factors that contribute greatly to pathogenic events in the host this bacterium has been responsible for 30% of pneumonia, 19% of urinary tract infections and 10% of bloodstream infections. And leads to nosocomial pathogens causing infections that usually develop late during hospital stay. Consequently the aim of this research was to classify and characterize *P. aeruginosa* isolated from various clinical samples from Iraqi patients by sequencing the *16S* rRNA gene.

***Keywords:*** *Health; Clinical samples; RNA genes; toxicity; Pseudomonas aeruginosa*

## Introduction

*Pseudomonas aeruginosa* is a Gram-negative bacteria belong to the family Pseudomonaceae , motile with a polar flagellum, non-spore forming, obligate aerobes that grows in a wide range of temperatures (10-44 °C) and the optimum thermal temperature is 35°C, widespread in nature, this bacterium causing acute and chronic infections in patients who are in hospital, especially in patients with burns, its grows well when cultured on simple media , It has the ability to produce two kinds of pigment green- blue (pyocyanin) and yellowish green pigment (pyoverdin) [1-3]

This bacterium is a naturally common among humans. [4] .It has a relatively large genome, which is likely to promote survival in various environments, with a variety of gene-regulatory activities to facilitate adaptation to new environmental conditions [5]. Therefore, despite the development of new and improved antibiotics is still one of the key causes of death in critically impaired patients with immune systems [6]. *16S* rRNA sequencing has been instrumental in the successful detection of bacterial isolates and in the discovery of new isolates in diagnostic laboratories. this gene is especially important for bacterial detection with unique phenotypic profiles, rare bacteria, slow growing bacteria, uncultivable bacteria and culture-negative infections, it isn't only did it give information into the etiology of an infectious disease, it also allows physicians to pick antibiotics and assess the length of care and infection prevention procedures. [7] *16S* rRNA sequencing has provided important and valuable knowledge worldwide.

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## Materials and Methods

One hundred of specimens were collected (46 males and 54 females) from five hospitals in Baghdad, including: ghazi Al-hareri Hospital, Wound and Burn Hospital, Baghdad Medical City Hospital, Child Teaching Hospital, and Ibn Al-baladi Hospital, from September to December 2019. Collected from patients with various infections such as UTI s, burns, wounds, pus swab from ear infection and sputum . All specimens were cultured on MacConkey agar (selective and differential medium). Then used the biochemical tests for identify and recognize Gram negative bacteria, Also GN24 Kit had used to detect Pseudomonas species .

### DNA Extraction

For *16S* rRNA sequencing analysis, genomic

DNA has been extracted for (38) isolates belong to *P. aeruginosa* using ABIOpure Extraction Kit (USA).Then use Quantus Florometer to measure the concentration of collected DNA to measure the sampling goodness for downstream applications. All bacterial DNA has been used as a template for conventional PCR amplification bacterial *16S* rRNA gene were amplified by tow universal primers 27F (5`AGAGTTTGATCCTGGCTCAG-3`) and 1492R (5`TACGGTTACCTTGTTACGACTT-3`) [8] for forward and reverse primers (Macrogen, South Korea). Thermal cycling conditions were as follows: 5 min of 95 °C for initial denaturation (30 cycles of denaturation) annealing (60°C for 30 sec), extension (72°C for 30 sec): Final extension at 72 for 7 min (1 cycle). PCR was carried out on a (Bio-Rad, USA) Automatic Cycler Thermal. Duplicate PCR was performed for replication of each sample [9]. After amplification with PCR, the agarose gel electrophoresis was followed to validate the amplification existence.

**Bacterial *16S* rRNA sequences analysis**

*16S* rRNA gene were amplified by using universal primers and the PCR product undergo for sequence analysis in Macrogen Lab.(south Korea) the sequences were identified by alignment and comparing them with sequences deposited in genbank through the NCBI site.

**Phylogenetic analysis**

The sequences of *16s* rRNA compared with those in deposited in genbank database ( https://www.ncbi.nlm.nih.gov/blast) to find related species and creation Phylogenetic trees using geneious software version 11.1 [10]

## Results and Discussion

One hundred (100) clinical samples were collected from different infections include (wounds, burns, urine, ear infections and sputum), 38(38%) isolates belonging to *P. aeruginosa*, whereas the remaining 62 (62%) isolates belonged to other bacteria species as seen in the table (1).

**Table (1) bacterial isolates which obtain from different infection sources**

| Bacterial isolates | No. of isolate | percentage |
|---|---|---|
| Escherichia coli | 39 | 39% |
| Pseudomonas aeruginosa | 38 | 38% |
| Klebseilla spp. | 12 | 12% |
| Proteus spp. | 7 | 7% |
| Enterobacter spp. | 4 | 4% |
| total | 100 | 100% |

Thirty eight (38) isolates from the total clinical isolate were classified by traditional methods (characteristics, morphology) as *P. aeruginosa*. In addition to use Identification system for Gram negative bacteria (GN24 Kit) the results are shown in figure (1,2) :

**Figure (1): GN24 colorimetric identification card**

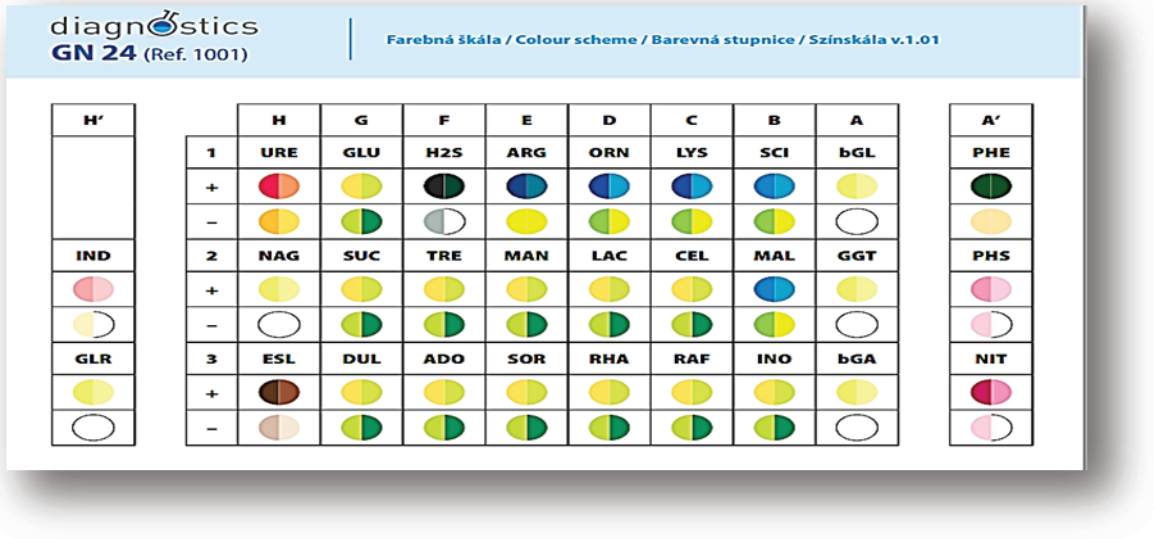


**Figure (2): Color Scheme of GN24**

Kit GN 24 consists of 24 wells Microtitration plate strip in the classic 96 well format containing dehydrated substrates Reconstitution of substrates is performed by bacterial suspension inoculation. During incubation, color changes occur in wells due to microorganisms ' metabolic activity. Test results may be measured either by automated readers or visually depending on the color scheme, or by the color definition displayed in the flyer. The results of recognition can be collected from the evaluation table or by using evaluation tools located at ( www.diagnostics.sk/idmicro).

**Conventional polymerase chain reaction PCR**

The results of Conventional polymerase chain reaction followed electrophoresis to detect of *P. aeruginosa* using *16S* rRNA that previously identification by GN24 Kit showed all isolates were *P. aeruginosa* and gave a good confirmative identification as shown in the figure (3). That gives the band size (1500 bp) which was the product size of primers used for identification

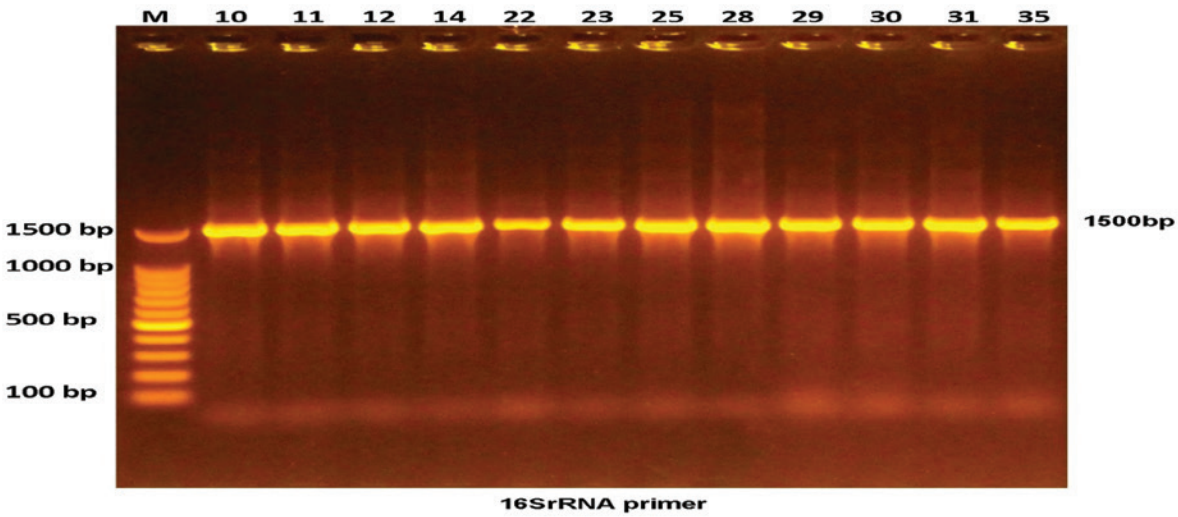


**Figure (3) Results of the amplification of 16S rRNA gene of *Pseudomonas aeruginosa* samples fractionated on 1% agaros gel electrophoresis stained by Eth.Br. Lane1:100bp DNA marker.**

*16S* rRNA gene sequences contain hyper variable regions with high conservation which have potential to identify species -specific signature sequences that are helpful to the classification of bacteria [11,12]. Analysis sequencing of this gene consider as an important method to assess the phylogenetic relationship between strains. The properties of this molecular target make it important and beneficial for the detection and diagnosis of bacteria in the clinical laboratory.

**Bioinformatics analysis**

The *16S* rRNA nucleotide sequences scanned for similarity sequences using online BLASTn. And use geneious software version 11.1 .To create phylogenetic tree for 12 isolates selected randomly, the result tend to have a strong identical between them and identical with those strains deposited in genbank under accession numbers China KF894970; Iran MG016493; China MK825339; Bangladesh MN256396; India KM675975; India KX268504; China KT759043 .The phylogenetic tree for this gene appears all of the isolates have a common ancestor. (Figure 4)

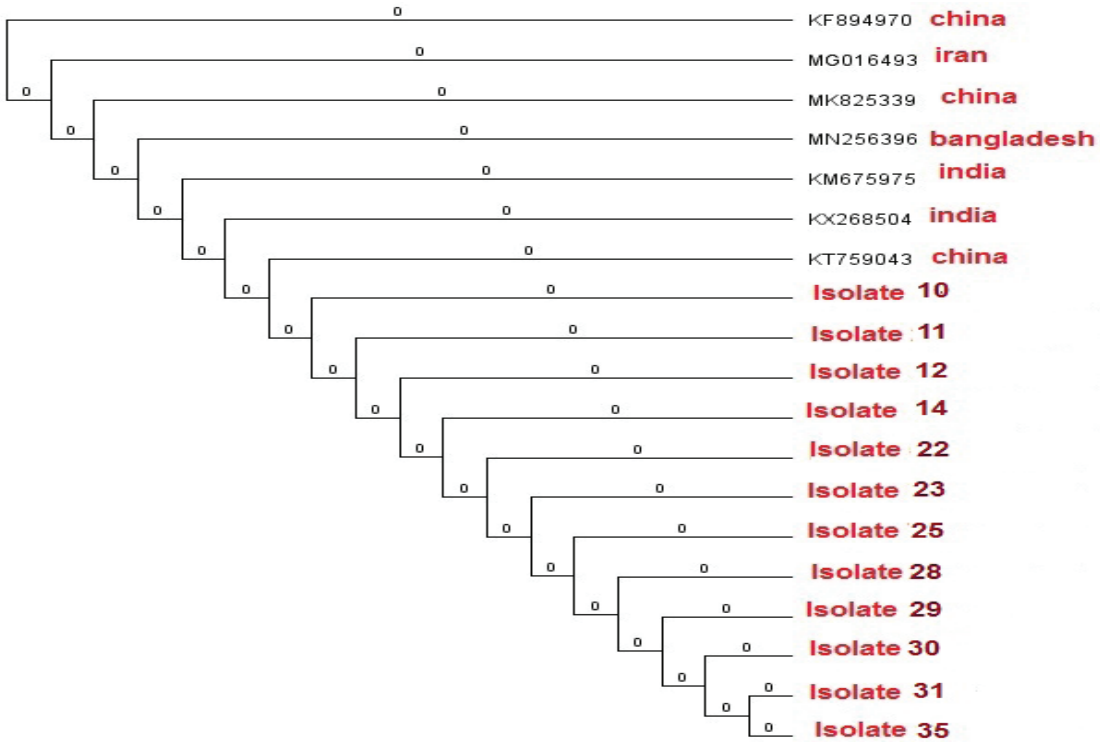


**Figure 4: Construction of phylogeny tree for twelve isolates of *P. aeruginosa* using geneious software, appear all of the isolates has a common ancestor.**

*16S* rRNA sequence has long been used as a "gold standard" taxonomy in the determination of the phylogeny of bacterial species. In the last decade, the Pseudomonas classification has gained further attention and has been reclassified by Brosch *et al* 1996, Kersters *et al* 1996, Palleroni 1992. [13- 15]. Identification of Pseudomonas is causing a lot of difficulties [16, 17]. Morphologically related organisms have a like biochemical properties. Sequence of highly conserved gene area *16S* rRNA data allows us to predict accurate taxonomy. Our present research was performed on the *16S* rRNA sequence based on PCR amplification for recognition and genetic level identification of *Pseudomonas aeruginosa.*

## Conclusion

In conclusion, PCR assay based on *16S* rRNA sequencing is highly precise, responsive and useful in the detection of bacteria.. And use for recognition of

those closely connected ones genotypical species of Pseudomonas. DNA sequencing of the *16S* rRNA gene was used as an important method for studying Bacterial phylogeny and taxonomy relationships between strains.

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