Current Research in Microbiology and Biotechnology
Vol. 5, No. 1 (2017): 972-975
Research Article
Open Access

ISSN: 2320-2246

# Rapid Detection of *Pseudomonas aeruginosa* by using Molecular Methods

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Received: 19 January 2017 Accepted: 01 February 2017 Online: 03 February 2017

## ABSTRACT

*Pseudomonas aeruginosa is an aerobic Gram-negative bacterium, it is considered as one of the most nosocomial bacteria. In this study, One hundred and twenty clinical samples were collected during the period from December 2015 to April 2016 from different hospitals in Baghdad. Thirty isolates were identified as Pseudomonas aeruginosa by routine biochemical tests, Api 20 E; In attempting to the identification of P. aeruginosa strains at the DNA level, Polymerase chain reaction (PCR) is used based on specific primer (27F/1492R) for (16S rRNA) gene. The results showed that PCR has found to be rapid and more sensitive and specific in identification of P. aeruginosa.*

**Keywords:** *Pseudomonas aeruginosa*; biochemical tests; 16s rRNA.

## 1. INTRODUCTION

*Pseudomonas aeruginosa* is an opportunistic pathogen. Its infections in hospitals mainly affect the patients in intensive care units and those having catheterization, burn, and/or chronic illnesses [1]. It is the leading cause of wound infections, urinary tract, surgical wound and ear infection [2]. The ability of *P. aeruginosa* to survive on minimal nutritional requirements and to tolerate a variety of physical conditions has allowed this organism to persist in both community and hospital settings [3]. Though it infects healthy tissues rarely but when defenses are compromised, it can infect different tissues. This explains why most infections are nosocomial [4]. A rapid and accurate system for the identification of *Pseudomonas* is essential to isolate patients and prevent further spreading of the diseases. Culturing of bacteria is one of the most important techniques which still used in diagnostic microbiology, because of its ability to quantify the viable bacteria in a sample; as well as obtaining a pure sample for further testing [5]. Many studies satisfied by using the API 20E system or classical biochemical test for bacterial identification [6], however, *P. aeruginosa* adaptive ability causes complications for the sensitivity of these methods, Thus, it has become necessary to develop genotype-based characterization systems capable of precisely identifying these microorganisms despite any phenotypic alterations, the Polymerase Chain Reaction (PCR) is highly sensitive specific and rapid method which massively improved the detection of *P. aeruginosa* especially when using species-specific primer such as *16S*rRNA, *gyr*B, *tox*A, and *16S–23S*rRNA genes [7]. *16Sr*RNA genes are highly conserved among all organisms and they possess various unique species-specific regions that allow for bacterial identification. The aims of this study are: Isolation and identification of *P. aeruginosa* from the clinical sample, using phenotypic test, and polymerase chain reaction by using species-specific primers.

## 2. MATERIALS AND METHODS

### 2.1 Collection of samples:

One hundred and twenty clinical samples were collected from burns and infected wounds of patients attending AL-Yarmouk Hospital and Baghdad Teaching Hospital during the period from December 2015 to April 2016.

### 2.2 Phenotypic identification of *P. aeruginosa*

All collected swabs cultured on blood agar, MacConkey agar, and cetrimide agar and incubated aerobically at 37°C for 24 hr. bacterial characterization determined depending on the cultural, microscopic characterization and biochemical tests (8; 9). In addition to using API 20E system identification kit for confirmation.

### 2.3 DNA extraction

Bacterial genomic DNA was extracted and purified from *P. aeruginosa* by using the G-SPIN™ total DNA extraction Kit in accordance with the manufacturer's instructions (Intron biotechnology, Korea). Extracted DNA was stored at -20°C prior to PCR amplification.

### 2.4 Primers

Two PCR primers were used to amplify *16S* rRNA of *P. aeruginosa* supplied by Alpha DNA/Canada. To obtained DNA fragment was 1500 bp (10).

27- Forward primer: 5-AGA GTT TGA TCC TGG TCA GAA CGC T-3

1492- Reverse primer: 5-TAC GGC TAC CTT GTT ACG ACT TCA CCC C-3

### 2.5 Amplified 16S rRNA

Amplification was performed using automated thermal cycler (applied biosystem/ USA), using 10 µl Green Master Mix (Promega), 1 µl Of primers pair forward/ Reversward 10µM, were added into PCR mix tube , 2µL of *P. aeruginosa* chromosomal DNA was used as a template, and 6 µL of sterile deionized water was added to complete the final volume to 20 µL. PCR cycle was programmed as follows : One cycle of 94 ˚C for 4 min; 30 cycles of 94 ˚C for 30 sec , 5 2 ˚C fo r 30 sec ,7 2 ˚C for 3 min; and final extension at 72 ˚C for 5 min. They were added into PCR mix ture, 2µL of *P. aeruginosa* chromosomal DNA was used as a template, and 6 µL of sterile deionized water was added to complete the final volume to 20 µL. PCR cycle was programmed as follows: One cycle of 94 ˚C for 4 min; 30 cycles of 94 ˚C f or 0.45 sec., 58 ˚C for 1 min., 72 ˚C for 1.30 min; and a final extension at 72 ˚C for 10 min. PCR products were detected by electrophoresis on a 1.5 % agarose gel electrophoresis containing ethidium bromid run at a constant voltage (90 V) for 60 min and visualized by using a Gel documentation systems (USA).

## 3. RESULTS AND DISCUSSION

### 3.1 Sampling

Out of the 120 specimens that were collected from the two hospitals, 30 isolates successfully were diagnosed as *P. aeruginosa*, representing 30 (25%) of total isolates and the highest percentage of *P. aeruginosa* was obtained from burn samples 26 (31.7%) whereas the lowest percentage were obtained from wounds samples 4 (10.5%) as shown in table(1). Rahman, (11) agrees with our result of burn infections as indicated only 28.1% of the total, also Kolmos *et al*., (12) established that burn represents 25-29%. This may be due to the high distribution of this organism around the hospital environment hence exposure risk to the burn wounds (13). Results from this study indicated that (10.5%) of wounds infection which is near to the results of Delden and Iglwesky (14) reported that *P. aeruginosa* is responsible for 8% of surgical wound infections. In a study in Jakarta, Lucky *et al.* (15) reported that *P. aeruginosa* was in the big five most frequent Gram-negative bacteria found in clinical samples.

**Table 1:** Frequency of *P. aeruginosa* in different clinical sample.

| Source of samples | Total No. of samples | No.(%) of *P. aeruginosa* isolates | No.(%) of Gram positive and negative isolates |
|---|---|---|---|
| **Burns** | 82 | 26 (31.7%) | 56 (68.3%) |
| **wounds** | 38 | 4 (10.5%) | 34 (89.5%) |
| **Total** | 120 | 30 (25%) | 90 (75%) |

### 3.2 Phenotypic test of *P. aeruginosa*

This study included thirty *P. aeruginosa* isolates identified using a variety of techniques, which are morphological and biochemical characterization. The result showed that *P. aeruginosa* produces green pigment and characteristic dour on cetrimide agar. *P. aeruginosa* reacted positively to catalase and oxidase tests and Simmon Citrate test, while it was negative for methyl red, Voges Proskauer and indole. These characteristics of the isolates were consistent with the description of typical *P. aeruginosa* according to (8; 16). Further biochemical tests were carried out to confirm characterization by using the API 20E system as in figure (1), which revealed that all 30 tested isolates belonged to the *P.aeruginosa*.

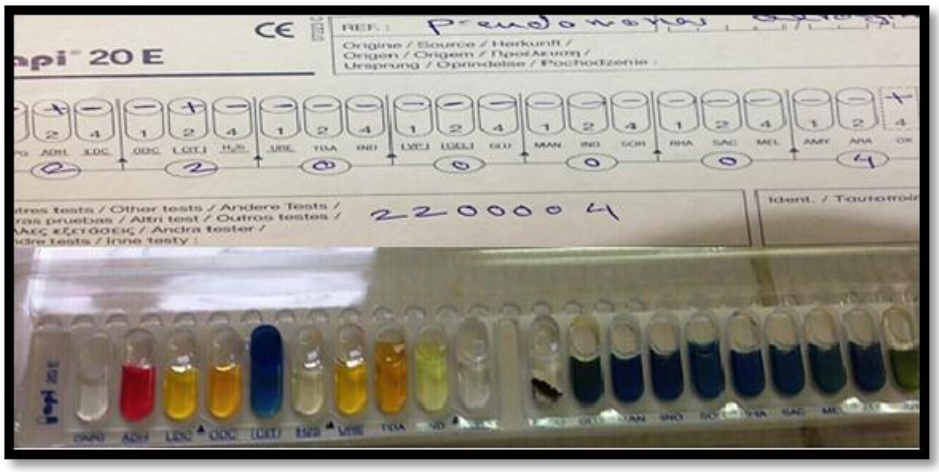


**Figure 1:** API 20E tests for identification of *P. aeruginosa*

### 3.3 PCR Analysis

Molecular technique 16S rRNA species-specific primers for *P. aeruginosa* was used. Thirty strains identified as *P. aeruginosa* after amplification of 16S rRNA genes using PCR technique. Results indicated in the figure (2) showed the amplified 16S rRNA fragment with a molecular size of about 1500 bp in length. This result is agreement with which mentioned by Jereny A. Frank *et al.*(10).

16S rRNA gene sequence offered a useful method for the identification of bacteria. It had long been used as a taxonomic method in determining the phylogenies of bacterial species (17).

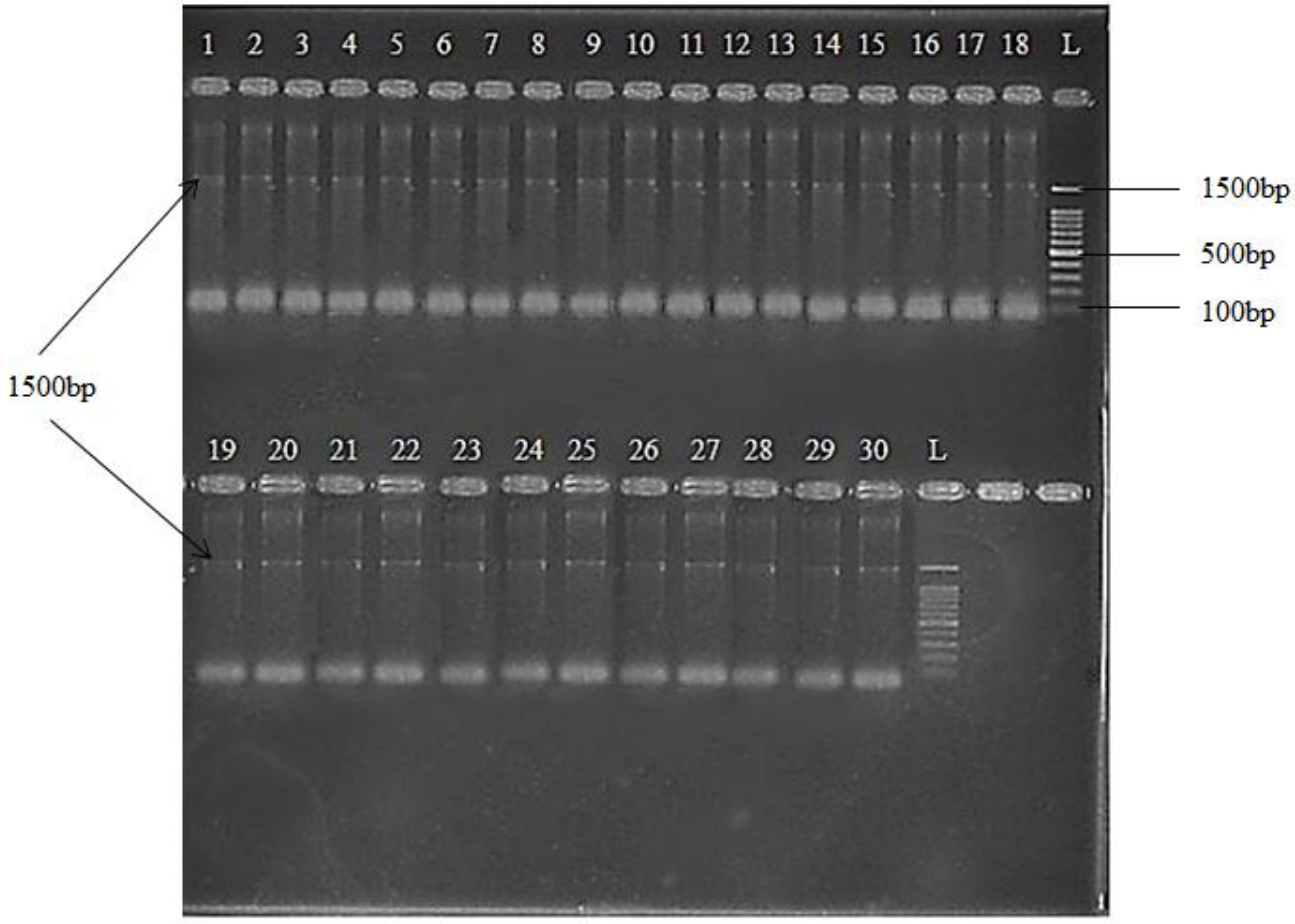


**Figure 2:** Gel electrophoresis for *P. aeruginosa* 16s rRNA gene with product size 1500 bp, Lane (L), DNA molecular marker (1500-100bp ladder), Lanes (1-30) the PCR products on agarose gel (1.5%) at (90 vol for 60 min.).

## 4. CONCLUSION

The molecular method (16SrRNA gene amplification) offered a useful method for the detection and identification of bacterial strain used for genus - species - level identification.

## 5. ACKNOWLEDGEMENTS

Authors would like to thank all workers in the Biotechnology Lab at the Department of Biotechnology, College of Science, the University of Baghdad for their assistance to make this work possible by providing the place, materials, and instruments.

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