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Original Research Article

Detection of Repetitive DNA Sequence and Outer Membrane Lipoprotein in Local Isolates of *Pseudomonas aeruginosa*

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Abstract
A total of 100 clinical samples were collected during this study which were obtained from patients suffering from different infections such as UTI, skins, burns, wounds and from sputum in patient with respiratory diseases, only (12) isolates of *P.aeruginosa* isolated.
At first molecular detection of *P.aeruginosa* (*OprI*) was done by using specific PCR primer. It was found that *OprI* marker was observed in all bacterial isolates (100%) with molecular size (250bp).The results showed that Box-element at a molecular size (500 bp) gave positive in only 9 (75%) isolates. This will reflect on the distribution of some genes among different isolates of *P.aeruginosa* isolated from different sources.

Key Words: *Pseudomonas aeruginosa* , Box-element , Lipoprotein I.

التحري عن التتابعات المتكررة والبروتين الدهني في الغشاء الخارجي لعزلات محلية من بكتريا *Pseudomonas aeruginosa*

الخلاصة
تم في هذه الدراسة جمع 100مسحة من المرضى الذين يعانون من التهابات مختلفة مثل التهاب المسالك البولية والحروق والجروح كذلك اخذت عينات من القشع من المرضى الذين يعانون من امراض الجهاز التنفسي .تم استخدام بادئات نوعية (primers)في تقنية تفاعل البلمرة المتسلسل (PCR) للتحري عن الجينات الخاصة بالتحري عن البروتين السطحي *P.aeruginosa* (*OprI*)باستخدام بادئ متخصص , اذ لوحظ ان كافة العزلات كانت موجبة لهذا الجين.(100%) وكذلك في هذه الدراسة استخدمنا تقنية تفاعل البلمرة المتسلسل (PCR) للكشف عن تقنية–Box element حيث لوحظ تسعة عينات فقط اعطت نتيجة موجبة(75%).

1. Introduction

P.aeruginosa is consider one of common gram negative bacteria ,have rod-shaped, *P.aeruginos a*adapted in numerous environmental conditions and also role in biodegradation of the compounds present in the environment [1].
This organism has many mechanisms of resistant to the antibiotics, so the treatment of *Pseudomonas* infections is very difficult when compared to other bacterial infections caused by other bacteria [2].*Pseudomonas* have outside proteins called Lipoproteins "I and L" are forming membrane of *P.aeruginosa* which is blamable for resistance of *Pseudomonas* to antibiotics . Due to these proteins are originate just in this bacteria , it can be useful and responsible aspect for fast identification of *P.aeruginosa* [3].
Both outer membrane proteins *OprI* and *OprL* consider very important for *P.aeruginosa* because these proteins play important roles in interaction of this bacteria with environment also help bacteria to resistance antibiotics because present these proteins (specific outer membrane proteins) play important role in

efflux transport systems that effect cell permeability[4].

It was detected *OprI* and *OprL* by using molecular method and has seen that *OprI* and *OprL* considers dependable factor for *P.aeruginosa* and we can distinguish the *P. aeruginosa* from different organisms by detect *OprI* for the genus and *OprL* for species of this organism [5].

However, the presence of Box-elements which consist of repetitive DNA sequences may express on *Pseudomonas* genome length and evolution so, these elements can be used to distinguish *Pseudomonas* strains isolated from different sources particularly those from soil sources and also to study the phylogenic groups [6].

Indeed, the BOX-PCR protocols were favorable for the rapid molecular characterization of phosphate solubilizing for *P.aeruginosa*, especially at the strains level . The BOX - PCR technique used to distinguished between isolates,especially the isolates that were not easily distinguished by dependent on other phenotypic and phylogenetic techniques such as 16S rDNA[7].

The aim of this study is to isolate *Pseudomonas aeruginosa* from different clinical samples and to invasion are some cytotoxic factors in the local isolates.

Materials and Methods

1- Specimens Collections:

The specimens are obtained from different sites of infections (burns , wounds and sputum's), Each swabs were taken carefully from the sites of infections and placed in tubes containing ready-made media to maintain the swab wet until taken to laboratory. While urine samples were generally collected from catheter tube in sterilized screw-cap containers and then each samples were inoculated on culture media and incubated aerobically at 37Cº for 24 hours.

2-Identification of *P.aeruginosa* isolates:

A total of 100 clinical samples were collected during this study being obtained from patients suffering from different infections such as UTI, skins, burns, wounds and from sputum in patient with respiratory diseases. The patient ages ranged from (15-60) years old who admitted to two main hospitals in Hilla city: Al-Hilla General Teaching Hospital, and Mergan Teaching Hospital during a period of three months lasting from (October 2015 to January 2016). It was found that (12) *P.aeruginosa* isolates were recovered where Six isolates (50%) were isolated from burns patient and three isolates (25%) from catheters patient who have UTI or renal failure and Only two isolates (16.6%) from wound and one isolate (8.3%) from sputum as shown in Table (1).

Table 1: Numbers of *Pseudomonas aeruginosa* Isolates From Different Sources

Source of samples	Total No. of samples	No. of *P.aeruginosa*isolates	Percentage (%)
Burns	45	6	50
Catheterized urine	27	3	25
Wounds	17	2	16.6
Sputum	11	1	8.3
Total	100	12	100

3-Detection of primers used in this study:
DNA extraction from bacterial cell was used as template in PCR for detection of some virulence genes of *P.aeruginosa*. DNA was purified from bacterial cell by using Favor Prep. The primers used for the amplification of fragment gene were listed in Table (2).

Table 2: Primers sequences and PCR conditions

Genes name	Primer sequence (5´- 3´)	Size pb	PCR conditions	Ref.
OprI	Sense – ATG AAC AAC GTT CTG AAA TTC TCT GCT Antisense – CTT GCG GCT GGC TTT TTC CAG	250	94Cº 4min 1X 94Cº20Sec 55 Cº 1min 30X 72Cº 30Sec 72Cº 1min 1X	Khattab*et al*.,2015 [5]
Box primer	Sense – CTA CGG CAAGGC GAC GCT GAC G	500	94Cº 5min 1X 94Cº 1min 48Cº 2min 35X 72Cº 2min 72Cº 5min 1X	Wolska*et al* .,2011 [10]

Results and Discussions

Molecular Detection for Virulence Factors:

4 -1 Detection of Outer Membrane Lipoprotein *OprI*:

Molecular detection of *P.aeruginosa* (*OprI*) was done by using specific PCR primer. It was found that *OprI* marker was observed in all bacterial isolates (100%) with molecule size (250bp) as shown in figure(1).

This result was agreement with result obtained by[5]and [8]who detected *OprI* gene by PCR and they found the ability *P.aeruginosa* to produce *OprI* gene for all isolate .

Also, wessel *et al* [9]said that *OprI* is highly abundant in the outer membrane and can exist in a free and peptidoglycan bound form.

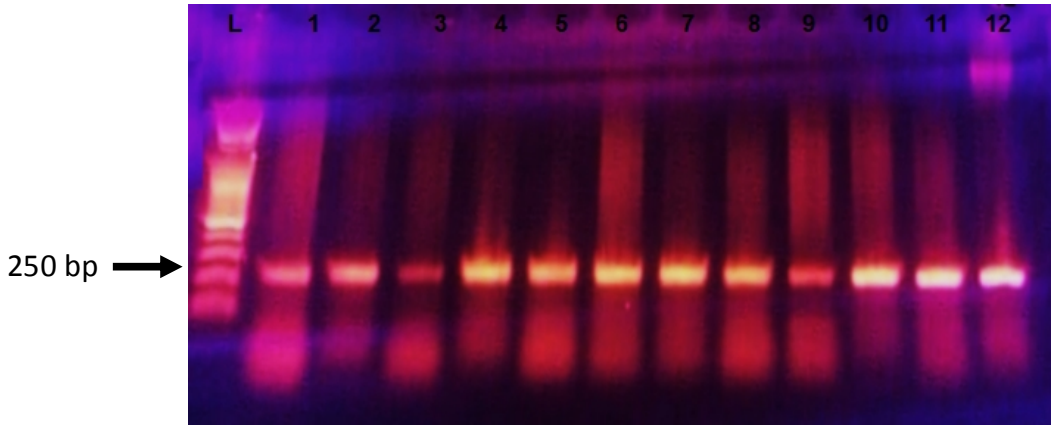

Figure 1 : Gel electrophoresis of PCR product of *OprI* at a locus 250bp among *P.aeruginosa* isolates,L: ladder molecular weight marker of ladder(100bp),1-12samples obtain from different sources

4-2 Detection of Box-element

Box-element is considered a repetitive DNA sequences present in *P. aeruginosa* that is used to distinguish between strains and evolution strains of *Pseudomonas*, So this technique may make discrimination between pathogenic and environmental isolates come from soil.

The results showed that in this technique for Box-element at a locus (500 bp) gave positive in only 9 (75%) isolates, as shown in figure (2), So this result mean that all the nine isolates strains transmitted through the hospital environment and not from the soil environment as mentioned by Nassir [6]who reported that highly similarity in binding patterns of clinical and environment isolate which about 45-99% and 61-100%.

The Box primer sequence was pointed used in PCR technique to detected Varity in the number and division of this bacterial repetitive sequence in the clinical sample of *P.aeruginosa* genomes[10].

As well as Box-PCR technique has been used to classify and distinguished different strain of many bacteria. The ensure of the stability of the typing test of Box-PCR is lead to the fact that high distinguished power with reproducibility, stability fast turnaround times and cost-effective alternatives for typing bacteria [11].

BOX-PCR pattern does not depend on the culture age of the strain to be analyzed[12] and fingerprinting output can be easily analyzed by computer assisted methods[13].In a study carried out by [14] it was indicated that genotyping of *P.aeruginosa* strains could not be associated to the phenotypic characteristics studies.

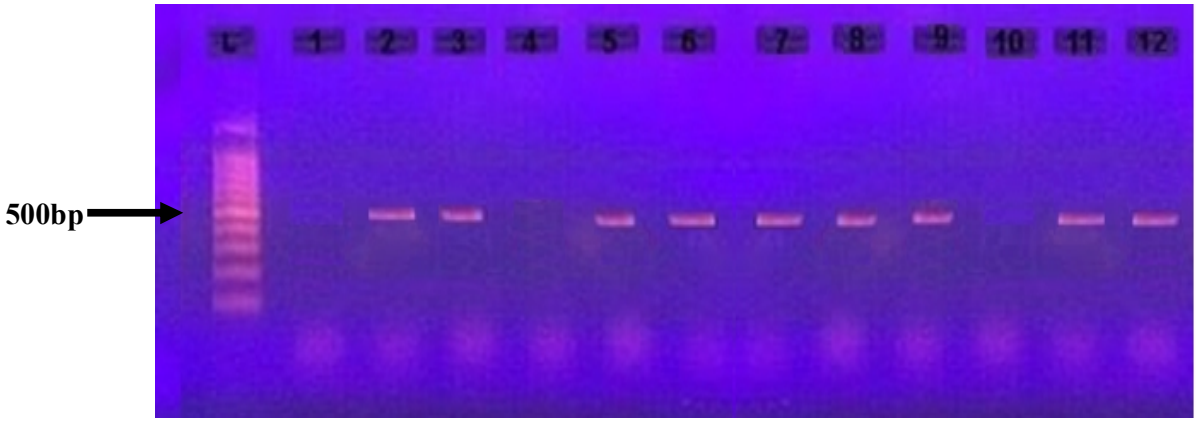

Figure 2: Gel electrophoresis of PCR product of BOX-element at a locus (500bp) for *P.aeruginosa* isolates,L:ladder molecular weight marker of ladder(100bp),1-12samples obtain from different sources**.**

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