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DETECTION NEW STRAINS AND MOLECULAR STUDY OF THE OPRL GENE OF PSEUDOMONAS AERUGINOSA ISOLATED FROM CLINICAL SOURCES IN THI-QAR PROVINCE

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ABSTRACT

The study was conducted for a period between March and September 2016.A total of 314 samples various clinical cases of different patients were randomly collected and examined for detection of Pseudomonas aeruginosa. These clinical samples included different sources. All collected samples were screened for the presence of Pseudomonas aeruginosa by culturing on appropriate media and 61(19.42%) isolates of Pseudomonas aeruginosa were identified via biochemical tests and confirmed by API 20NE system.

In attempting to the identification of P.aeruginosa strains at the DNA level, Polymerase chain reaction (PCR) was used based on specific primer for 16SrRNA.The results showed that PCR has found to be rapid and sensitive and specific for identification of P. aeruginosa. In addition, 16S rRNA was used as confirmation gene, while oprLused as virulence gene.

KEYWORDS: *Pseudomonas Aeruginosa, Oprl Gene, 16S rRNA Gene*

Article History

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INTRODUCTION

Pseudomonas is an extremely versatile Gram-negative bacterium capable of thriving in a broad spectrum of environments (Juhas,2015). *P. aeruginosa* is an opportunistic human pathogen that is a major causative agent of the hard to eradicate nosocomial infections. Immunocompromised, cancer, burn, cystic fibrosis and intensive care unit patients with mechanical ventilation are among those with the highest risk of being infected by *P. aeruginosa* (Cramer *et al*., 2012).

Burn injury is one of the most common and devastatingforms of trauma, and is a major public health problem worldwide.Infection of burns is common because the skin, whichacts as a physical barrier against microbes, has been compromised.*P. aeruginosa* are the most common source of burnwound infections. Severe burn injuries are part of the most devastating form of trauma, including lossof the skin barrier and tissue destruction, and require immediate and specializedmedical care to maintain homeostasis (Church *et al*., 2006).

In addition to body temperaturemaintenance, the prevention of fluid loss through supplementation of liquid andelectrolytes represents critical parameters for positive vital prognosis (Berger *et al*., 2007). Indeed,tissue damage at

burn wound sites causes the loss of the biological fluids defined asburn wound exudates (BWEs) (Cutting, 2003).

Pseudomonas aeruginosa possesses a variety of virulence factorsthat may contribute to its pathogenicity. *P. aeruginosa* also has a largenumber of virulence factors such as exotoxin A, exoenzyme S, nan 1and Las genes (Van Delden and Iglewski B, 1998).Bacterial lipoproteins are lipid anchored to the inner and outer membranes of gram-negative bacteria. Three fatty acidsare attached to the N-terminal cysteine residue that immediately follows the cleavage site of lipoprotein signal peptides (Lavenir *et al*., 2007). The lipids integrate into the periplasm-facing leaflet ofeither membrane, leaving most lipoproteins exposed to theperiplasm (Narita *et al*., 2004).

The aim of this study was detection new strains of Pseudomonas aeruginosa from clinical samples and detection oprL gene as virulence gene.

MATERIALS AND METHODS

The study was conducted through a period from March to September 2016.The samples were collected from outpatients and admitted patients to Al-Hussein Teaching Hospital and Public Health Laboratory in Thi-Qar province. A total of 314 samples from various clinical of different patients were randomly collected and examined for detection of *Pseudomonas aeruginosa*. These clinical samples included 141 burn swabs (44.90%), 30 wound swab (9.56%), 68 ear swab (21.66%), 43 sputum samples (13.69 %) and 32 urine samples (10.19%)

Isolation and Identification of Bacterial Isolates

All specimens were cultured on blood agar and MacConkey agar and incubated overnight at 37°C under aerobic conditions. Depending on morphological features of colonies and microscopically examination with Gram stain then biochemical tests were used to detection *Pseudomonas aeruginosa*bacteria. Diagnosis of species was confirmed by API 20NE system.

PCR Technique: Pure colonies of the clinical isolates were used for DNA extraction (Geneaid, Korea).Primers of the *16SrRNA* gene (956 bp) and *oprL* gene (504 bp) genes, were used.PCR amplification of targeted DNA for both genes were carried out in 20µl reaction volumes,each of them containing 2mM MgCl 2,50 mM Tris(pH 8.3; Sigma, St.Louis, Mo.),250 µM (each) deoxynucleoside triphosphates (Promega, Madison, Wis.),0.4 µM(each) primer,1U of *Taq* polymerase(Invitrogen,Carlsbad, Calif.), and 2 µl of whole-cell bacterial lysate, and adjusted to 20 µl by the addition of high-performance liquid chromatography-grade H_2O. Amplification of *16SrRNA* gene was performed by RapidCycler term controller.After an initial denaturation for 2 min at 95 °C, 25 cycles were completed, each consisting of 20 s at 94°C, 20 s at the appropriate annealing temperature and 40 s at 72°C. A final extension of 1 min at 72°C was applied.In the *oprL*gene the DNA was amplified using the following protocol 96°C for 5 min, 40 cycles of 96°C for 1min,55°C for 1 min and,72°C for 1 min, and 72 °C for 10 min.DNA fragments were analysed by electrophoresis in a 1.2% agarose gel at 85 V for 1 h in 1X TBE [40 mMTris–HCl (pH8.3), 2 mM acetate and 1 mM EDTA] containing 0.05 mg/L ethidium bromide.

RESULTS

The microscopic examination showed that the bacterial isolates appeared as Gram negative, small bacilli, and Non-spore-forming bacteria. All isolates of *P.aeruginosa* on MacConkey agar were circular colonies, flat; smell like of apples damaged and showed pigment bluish-green on Muller-Hinton agar.

A total of 314 samples from clinical of different patients were randomly selected and examined for detection of *P. aeruginosa*bacteria, clinical samples included burn swab (n=141, 44.90%),wound swab (n=30,9.56%),ear swab (n=68, 21.66%),sputum (n=43,13.69%), urine of patients with U.T.I (n= 32, 10.19%)

All collected samples were screened for thepresence of *P.aeruginosa*by culturing on appropriate media and (N=61, 19.42%)*P.aeruginosa*were identified via biochemical tests and confirmed by API 20NE system.

The results of the study showed that the higher percentage of *P.aeruginosa* was isolated from burn swabs 42 (29.78%) and then sputum swabs 6 (13.95%), wound swabs 4(13.33%), urine sample 4 (12.50%) and ear swabs 5(7.35%) as intable (1).

Detection of the presence of genomic DNA from the *P.aeruginosa* samples by electrophoresis on 0.8% agarose gel using method the Sambrook and Russell. (2001) as figure (1).

The amplification products were identified *16S rRNA* geneas figure (2) and *oprL* genepositive from their sizes in agarose gelsas figure (3). Overall, *16Sr RNA* gene (956 bp) were identified in 61/61(100%) of all clinical isolates. The results of screened for *oprL* gene gave positive results 59/61(96.72 %) that equal to target (504 bp).The isolates were 42/42(100 %) in burn and 4/4 (100%) in wound and 4/4 (100 %) in urine and 5/6 (83.33 %) in sputumand 4/5 (80 %) in ear as in table (2).

After DNA extraction of *P.aeruginosa* 10 isolates were sent to the Korean company Macrogen to determine the sequence of the *16S rRNA* gene found through site BLAST at the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/) that there was a match in the sequence of bases with the rest of the *P. aeruginosa* strains at rate 99% where the information was sent to the gene bank, which confirmed the existence of new strains it was recorded and gave them accession numbers for nucleotide sequences(**KY582729,KY582730, KY582731, KY582732KY582733, KY582734, KY582735, KY582736, KY582737, KY582738**) and posting them on the NBCI webs.

Table 1: The Number and Percentage of a Bacterial Isolate from Clinical Samples

Source of Samples	Number of Cases	%	Number of Positive Cases	%
Burn swabs	141	44.90	42	29.78
Wound swabs	30	9.56	4	13.33
Urine	32	10.19	4	12.50
Sputum	43	13.69	6	13.95
Ear swabs	68	21.66	5	7.35
Total	**314**	**100**	**61**	**19.42**

Table 2: Prevalence of Virulence Genes of *P. Aeruginosa*

Source of samples	Cases	positive Cases	%	*16sRNA* gene	%	*oprL* Gene	%
Burn	141	42	29.78	42	100	42	100
Wound	30	4	13.33	4	100	4	100
Urine	32	4	12.50	4	100	4	100
Sputum	43	6	13.95	6	100	5	83.33
Ear swaps	68	5	7.35	5	100	4	80
Total	**314**	**61**	**19.42**	**61**	**100**	**59**	**96.72**

Table 3: Sequences and Product Size of Each Primer to *P. Aeruginosa*

Primer Name	DNA Sequences (5'-3')		Product Size bp
16SrRNA	F	GGG GGA TCT TCG GAC CTC A	956
	R	TCC TTA GAG TGC CCA CCC G	
oprL	F	ATG GAA ATG CTG AAA TTC GGC	504
	R	CTT CTT CAG CTC GAC GCG ACG	

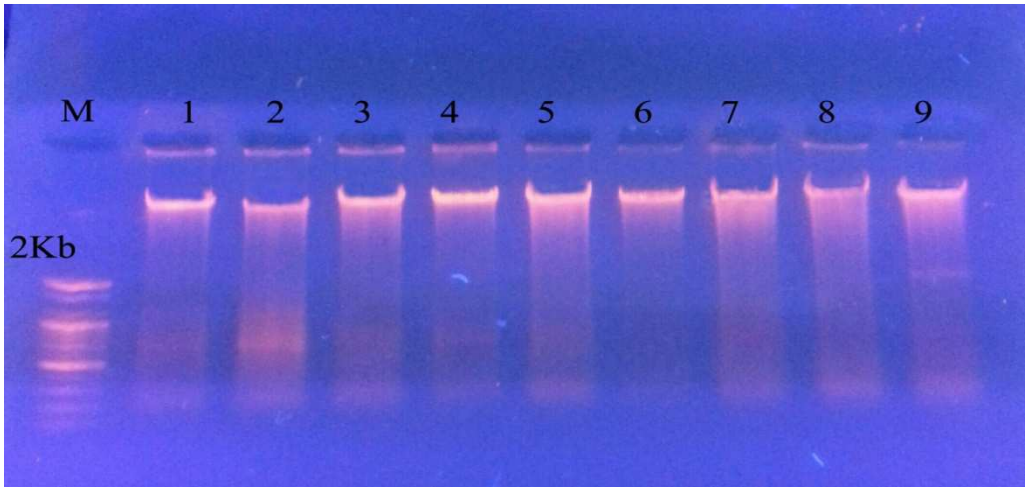

Figure 1: Electrophoresis of Genomic DNA on Agarose gel 0.8% (M) DNA Marker (100bp ladder). Lane (1, 2, 3, 4, 5, 6, 7, 8, 9) No. of Genomic DNA of *P. Aeruginosa* Isolates

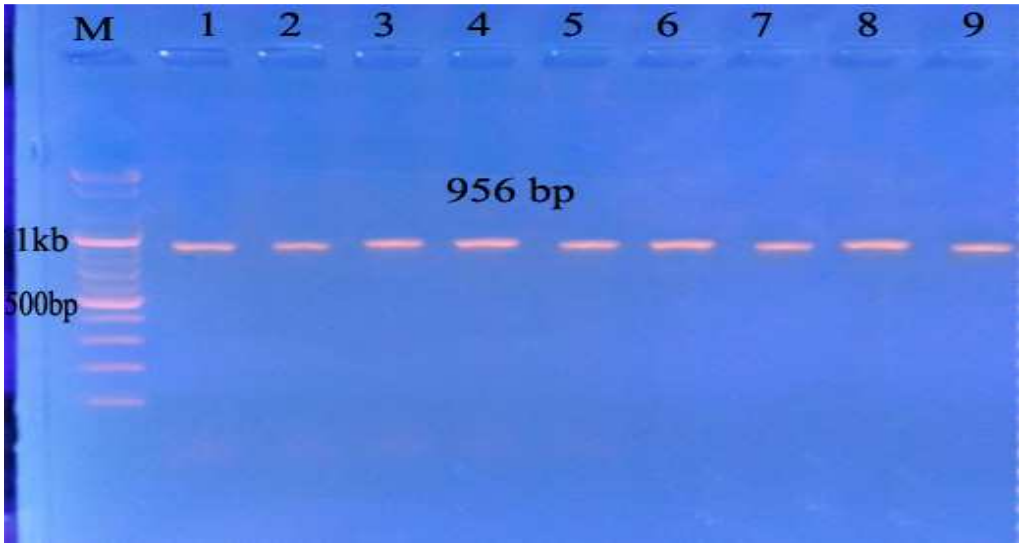

Figure 2: PCR Amplified Products of *16S RRNA* Gene of the *P. Aeruginosa* using the Designed Primers with Expected Size 956bp. (M) DNA Marker (100bpladder). Lane (1, 2, 3, 4, 5, 6, 7, 8, 9) No. of Amplify of *16S rRNA* Gene in Isolates of *P.Aeruginosa*

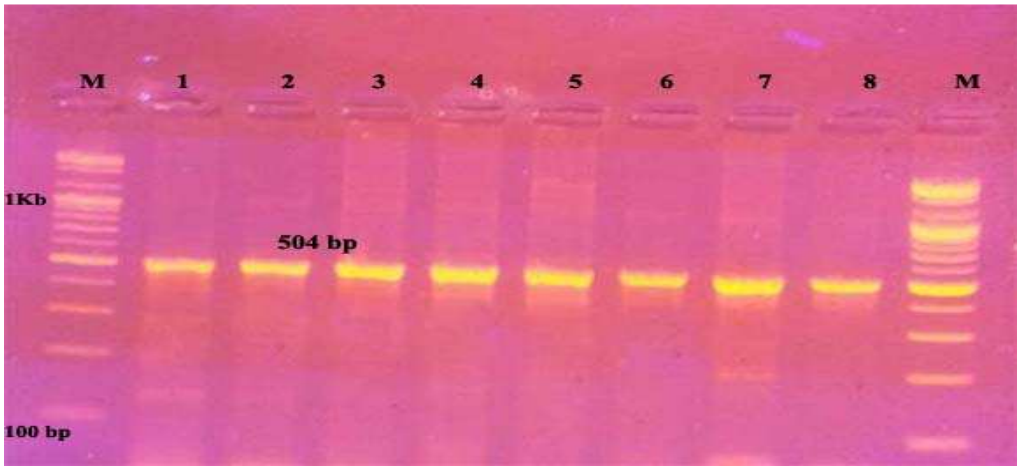

Figure 3: Ethidium Bromide-Stained Agarose gel of PCR Amplified Products from Extracted *P.Aeruginosa*isolates DNA Amplified with Primer for the *Oprl*gene). Lane (M), DNA Molecular Size. Marker (2Kb Ladder); Lane (1, 2, 3, 4, 5, 6, 7, 8) Shows Positive. Results with the *Oprl*gene (504 bp)

Discuss

The results of the study showed that burn infection the highest percentage of *P.aeruginosa* by 42/141(29.78%) and the *oprL* gene present by100%.The presence of a high percentage of *P.aeruginosa* indicates a high percentage of pollution as well as the presence of new strains resistant to antibiotics, which contributed to the spread of it and infected the patients from the population of Thi-Qar.

P.aeruginosa is one of the most common causes of nosocomial infections which mainly affects patients with immunodeficiency in hospitals, in particular, it is the leading cause of life-threatening infections in patients with burns(Church *et al*.,2006).

The current study showed the presence of the *oprL* gene at 96.72% in the *Pseudomonas aeruginosa* isolates of the clinical samples (Lavenir *et al*., 2007) also noted that all of *P. aeruginosa* strains contained the *oprI* and *oprL* genes (sensitivity=100%, specificity=80%).The outer membrane proteins of *P.aeruginosa* OprI and OprL play important roles in the interaction of the bacterium with the environment as well as the inherent resistance of *P. aeruginosa* to antibiotics where the consequence of the presence of these specific outer membrane proteins that have been implicated in efflux transport systems that affect cell permeability (Nikaido,1994) As these proteins are found only in this organism, they could be a reliable factor for rapid identification of *P. aeruginosa* in clinical samples (De Vos*et al*.,1997).

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