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MOLECULAR CHARACTERIZATION OF 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 of seven months 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 wound swabs, burn swabs , ear swabs, urine,and sputum samples.All collected samples were screened for the presence of Pseudomonas aeruginosa by culturing on appropriate media and 61 isolates of Pseudomonas aeruginosa were identified via biochemical tests and confirmed by the 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 toxA used as virulence gene.

***KEYWORDS*:** *Pseudomonas Aeruginosa,Virulencegene,Toxa Gene*

INTRODUCTION

P. aeruginosa is gram-negative, obligate anaerobic and ubiquities organisms widely distributed in soil, water, and living hosts (Akanji*et al*.,2011)*P. aeruginosa* grows well at 37–42°C, its growth at 42°C helps differentiate it from other Pseudomonas species that produce fluorescent pigments,it is oxidase positive it does not ferment carbohydrates, but many strains oxidize glucose identification is usually based on colonial morphology, oxidase positivity, the presence of characteristic pigments (Jawetz*et al*.,2013).*P.aeruginosa* can cause disease through their ability to enter the host, but pathogenic and non-pathogenic terms explain the virulence of the organism or its ability to induce disease under certain conditions. *P.eruginosa* include leading infection of urinary tract infection, ear infection, infection Eye, skin infection, central nervous system, bone and joint infection(Trautmann*et al*., 2008).

Exotoxin A (ExoA, *toxA*) is a 66 kDa protein acts as the main virulence factor of *P.aeruginosa*, it is a similar action to diphtheria toxin. ExoA is a strongly virulent protein, it is clear that is *toxA* mutants are less virulent than wildtype strains, and that Immunization against Exo gives partial immunity to *P.aeruginosa* infection in animals (Engel *et al*.,2003). Injection of purified ExoA results in leucopenia, hepatic necrosis, hypotension and shock, when injected into test animals.On the microscopic level, collagen is disrupted, proteoglycan ground substance is lost and widespread endothelial

and epithelial cell death is observed (Pitt *et al*.,1998).

P.aeruginosa produces two different ADP-ribosyltransferase toxins: ETA and Exoenzyme S (Wolfgang *et al*.,2003). Exoenzyme S Cause to tissue damage in the lung, burn, and wound infections (Wolfgang *et al*.,2003). ETA consists of two subunits; fragment A is catalytic, and fragment B is responsible for interaction with eukaryotic cell receptors. ETA is cytotoxic to many mammalian cells (Middlebrook *et al.*,1977).

The aim of this study was to isolate *Pseudomonas aeruginosa* from clinical samples and to confirm the isolates using*16SrRNA* diagnostic gene with the detection *toxA* 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 the blood agar and MacConkey agar and incubated overnight at 37°C under aerobic conditions. Depending on morphological features of colonies and microscopical examination with Gram stain then biochemical tests were used to detection of *Pseudomonas aeruginosa* bacteria. Diagnosis of species was confirmed by the API 20NE system.

PCR Technique

pure colonies of the clinical isolates were used for DNA extraction (Geneaid, England).Primers of the *16SrRNA* gene (956 bp) and *toxA* gene (270 bp) genes,which designed by Theodore *et al*.,(2004) and Stover *et al.*, (2000) respectively were used, Table (4).PCR amplification of targeted DNA for both genes were carried out in 20µl reaction volumes,each of them containing 2mM MgCl 2,50 mMTris(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 the Rapid Cyclert erm 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 *toxA* gene the DNA was amplified using the following protocol 94°C for 3 min, 30 cycles of 94°C for 30 seconds,57°C for 1 min and,72°C for 1 min and 30 seconds, and 72 °C for 5 min.DNA fragments were analyzed 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

Out of 14144.90%) burn swabs, 30(9.56%) wound swabs, 32(10.19%) urine sample,43(13.69%) sputum sample and 68(21.66%) ear swabs gave *p. aeruginosa* as showed in the table (1).The results of the study showed that the higher percentage of *P.aeruginosa* was isolated from burn swabs 42 (29.78%).all collected samples were screened for the

presence of *Pseudomonas aeruginosa* by culturing on appropriate media and (61) *Pseudomonas aeruginosa* were identified via biochemical tests and confirmed by the API 20NE system. The amplification products were identified *16S rRNA* and *toxA* positive from their sizes in agarose gels. Overall, *16SrRNA* gene (956 bp) were identified in 61/61 (100%) of all clinical isolates. The results of screening for *toxA* gene gave positive results 54/61 (88.52%) that equal to target (270 bp) product size as found in Table(2).Of the 314 isolates, 200 (63.69%) were females and 141 (36.30%) from males. From the study population, 221 (70.38%) patients were aged between 21-40 years, while 93 (29.61%) were below 20 years. (Table 3).

Table 1:The Number and Percentage of Bacterial Isolates from Clinical Specimens

Source of Samples	Cases	%	Positive Cases	%
Burn swab	141	44.90	42	29.78
Wound swab	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: Distribution of *16sr Rna* and *Toxa* Genes in *Pseudomonas Aeruginosa* Isolates

Source of Samples	*16S rRNA gene*			*toxA gene*		
	No	*Positive*	*%*	*No*	*Positive*	*%*
Burn	42	42	100	42	40	95.23
Wound	4	4	100	4	3	75
Urine	4	4	100	4	4	100
Sputum	6	6	100	6	4	66.66
Ear	5	5	100	5	3	60
total	61	61	100	61	54	88.52

Table 3: Age Distribution of Cases

Sample	Age (years)				Total	%
	1-10 %	11-20 %	21-30 %	31-40 %		
Burn	6 (4.25%)	33 (23.40%)	59 (41.84%)	43 (30.49%)	141	44.90
Wound	1 (3.33%)	3 (10%)	6 (20%)	20 (66.66%)	30	9.56
Urine	0 (0%)	2 (6.25%)	3 (9.37%)	27 (84.37%)	32	10.19
Sputum	3 (6.97%)	6 (13.95%)	11 (25.58%)	23 (53.48%)	43	13.69
Ear	28 (41.17%)	11 (16.17%)	4 (5.88%)	25 (36.76%)	68	21.66
Total	**38 (12.10%)**	**55 (17.51%)**	**83 (26.43%)**	**138 (43.94%)**	**314**	**100%**

Table 4:Sequences and Product Size of Each Primer to *P.Aeruginosa*

Primer Name	DNA Sequences (5'-3')		Product Size bp	Reference
16SrRNA	F	GGG GGA TCT TCG GAC CTC A	956	Theodore et al.,(2004)
	R	TCC TTA GAG TGC CCA CCC G		
toxA	F	C TG CGC GGG TCT ATG TGC C	270	Stover et al.,(2000)
	R	GAT GCT GGA CGG GTC GAG		

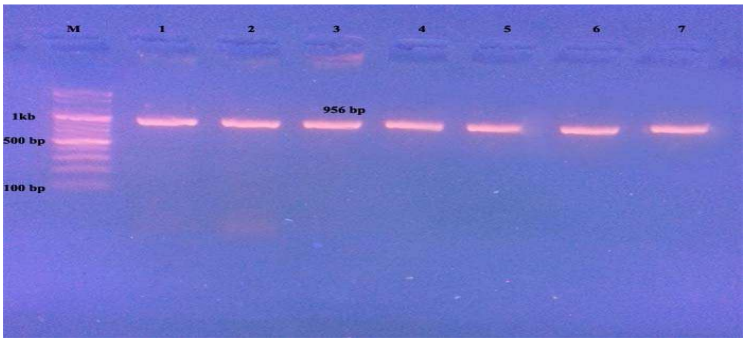

Figure 1: 956 bp PCR Products of *16S rRNA* Which Was Specific for *P. Aeruginosa* Were Identified in all Samples in 1.2% Agarose Gel Electrophoresis. M 1kb DNA ladder, 1 -7 were Various Samples of P. Aeruginosa Isolates

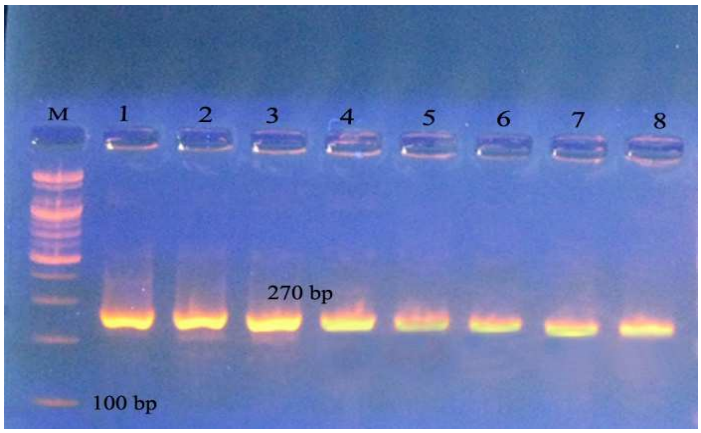

Figure 2: Ethidium Bromide-Stained Agarose Gel. of PCR Amplified Products from Extracted *P.aeruginosa* isolates DNA Amplified With Primer for.*toxA* gene). lane (M), DNA Molecular Size. Marker (2Kb ladder); lane (1, 2, 3, 4, 5, 6, 7, 8) Shows Positive. Results with the *toxA* gene (270 bp)

DISCUSSIONS

The results of the study showed that burns represented the highest percentage of *P.aeruginosa* (29.78%).*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 (Floret et al., 2009; Leseva et al.,2013). The clinical importance of *P. aeruginosa* it is often associated with its high resistance to antimicrobial drugs, in addition to its self-resistance to antibiotics, and becomes resistant to drugs during treatment (Tsutsui*et al.*,2011).

The study also showed that there is a significant increase in the presence of P.aeruginosa bacteria in both respiratory tract infection and wounds infection which was (13.95%), (13.33%) respectively.

P. aeruginosa is well known for its ability to find constant residency in the airways of cystic fibrosis (CF) patients, resulting in the return of chronic lung infections, gradual in lung function and increased morbidity and mortality rates (Nixon *et al*., 2001).

Wounds infection continues to be a challenge and a major global problem, resulting in many complications and

increases morbidity and mortality among hospital patients(Raza et al., 2013).Urinary tract infections (UTI) occasion by *P.aeruginosa* usually occurs secondary to catheterization, instrumentation or surgery, catheterization of the urinary tract is the main cause of nosocomial acquired-UTI by P. aeruginosa (Mittal *et al*.,2009).

Exotoxin A, encoded by the toxA gene,inhibits protein biosynthesis by transferring an ADP-ribosyl moiety to elongation factor 2 of eukaryotic cells.(Rumbaugh*et al*., 1999).

ExoA is a strongly virulent protein, it is clear that is toxA mutants are less virulent than wild type strains, and that Immunization against Exo gives partial immunity to P.aeruginosa infection in animals (Engel *et al*.,2003). Injection of purified ExoA results in leucopenia, hepatic necrosis, hypotension and shock when injected into test animals.On the microscopic level, collagen is disrupted, proteoglycan ground substance is lost and wide spread endothelial and epithelial cell death is observed (Pitt *et al*.,1998).

REFERENCES

1. ***Akanji,B.O.; Onasanya, A. and Oyelakin, O.*** *(2011). Genetic Fingerprinting of Pseudomonas aeuroginosa Involved in Nosocomial Infection as Revealed by RAPD- PCR Markers.Biotech.; 10(1):70-77*
2. ***Engel, J. N.*** *(2003). Molecular pathogenesis of acute Pseudomonas aeruginosa infections. Severe Infections Caused by Pseudomonas aeruginosa. A. R. Hauser and J. Rello. Dordrecht, Kluwer Academic Publishers, 201-229.*
3. ***Floret,N.; Bertrand,X.; Thouverez,M.; Talon,D****.(2009). Nosocomial infections caused by Pseudomonas aeruginosa: theexogenous or endogenous origin of this bacterium? Pathol Biol. 57(1):9–12. PMID: 18848405.*
4. ***Jawetz, E.;Karen, C. Carroll.;Jeffery,A. Hobden. ; Steve, Miller.; Stephen,A. Morse.;Timothy,A. Mietzner.; Barbara, Detrick.;Thomas,G,Mitchell. ; James, H. McKerrow. ; Judy, A. Sakanari.****(2013). Jawetz, Melnick, &Adelberg's Medical Microbiology Twenty-Seventh Edition.pp245.*
5. ***Leseva,M.;Arguirova,M.;Nashev,D.; Zamfirova,E.; Hadzhyiski, O****. (2013). Nosocomial infections in burn patients: etiology, antimicrobial resistance, means to control. Ann Burns Fire Disasters. 2013;26(1):5-11. PMID: 23966892.*
6. ***Anwar Kadhim Al-Saffar, Kadhim CH. Hasan & Maha Jadooa Noshi****, The Effect of Mixture (Sidr Infusion with Hydrogen Peroxide) on Bacteria Pseudomonas aeruginosa, IMPACT: International Journal of Research in Applied,Natural and Social Sciences (IMPACT: IJRANSS), Volume 2, Issue 4, April 2014, pp.73-78*
7. ***Middlebrook, J. L, and R. B. Dorland.*** *(1977).The response of cultured mammalian cells to the exotoxins of Pseudomonas aeruginosa and Corynebacteriumdiphtheria: differential cytotoxicity. Can. J. Microbiol. Vol.23: pp.183-189.*
8. ***Mittal,R.; Aggarwal, S,.;Sharma, S.; Chhibber, S.; Harjai,K****.(2009). Urinary tract infections caused by Pseudomonas aeruginosa: a mini review. J Infect Public Health. 2(3):101-11*
9. ***Nixon,GM.; Armstrong, D.S.; Carzino, R.; Carlin, J.B.; Olinsky,A.; Robertson,C.F.; et al.*** *(2001).Clinical outcome after early Pseudomonas aeruginosa infection in cystic fibrosis. J Paediatr. 138(5):699-704.*

10. ***Pitt, T. L****.(1998). Pseudomonas, Burkholderia,and related genera.Topley& Wilson's Microbiology and Microbial Infections. L. Collier, A. Balows& M. Sussman. London, Arnold. Vol.2: pp.1109-1126.*

11. ***Raza, M.; Chander, A.,Ranabhat, A.*** *(2013). Antimicrobial susceptibility patterns of the bacterial isolates in postoperative wound infections in a tertiary care hospital, Kathmandu, Nepal. OJMM, 3: 159–163.*

12. *Rumbaugh, K.P.; B.S. Abdul; N. Hamood and J.A. Griswold. (l999). Analysis of Pseudomonas aeruginosa clinical isolates for possible variations within the virulence genes exotoxin A and exoenzyme S. J. Surg. Research 82: 95–l05.*

13. ***Doukani Koula & Tabak Souhila,*** *Antibacterial Effect of Arbutus Unedo L. Fruit and Its Essential Oils on Salmonella Typhi (Atcc 14028) and Pseudomonas Aeruginosa (Atcc 27853), International Journal of Applied and Natural Sciences (IJANS), Volume 3, Issue 6, October-November 2014, pp. 25-30*

14. ***Stover, C. K.; Pham, X.Q.; Erwin, A.L.; Mizoguchi, S.D.; Warrener, P.; Hickey,M.J.; Brinkman, F.S.L.; Hufnagle, W.O.; Kowalik, D.J.; Lagrou, M.; Garber, R.L.; Goltry, L.; Tolentino, E.; Westbrook- Wadman,S.; Yuan, Y.; Brody, L.L.; Coulter, S.N.; Folger,K.R.; Kas, A.; Larbig,K.; Lim,R.; Smith, K.; Spencer,D.; Wong, G.K.; Wu, Z.; Paulsen, I.T.; Reiser, J.; Saier, M.H.; Hancock, R.E.W.; Lory, S. and Olson, M.V.*** *(2000). Complete genome sequence of Pseudomonas aeruginosa PAO1, an opportunistic pathogen. Nature. 406: 959–964. http://dx.doi.org/10.1038/35023079.*

15. ***Theodore,S.; Tom,C.; Peter, V.; and John,J. L.(****2004). PCR-Based Assay for Differentiation of Pseudomonas aeruginosa from Other Pseudomonas Species Recovered from Cystic Fibrosis Patients. JOURNAL OF CLINICAL MICROBIOLOGY, May 2004, p. 2074–2079 Vol. 42, No. 50095-1137/04/$08.00 0 DOI: 10.1128/JCM.42.5.2074–2079.2004Copyright © 2004, American Society for Microbiology*

16. ***Trautmann, M. and Halder, S.****(2008).Point-of-use filtration reduces endemic Pseudomonas aeruginosa infections on a surgical intensive care unit. American Journal of Infection Control: 36:421-429.*

17. ***Tsutsui, A.; Suzuki,S.; Yamane, K.; Matsui, M.; Konda, T., and Marui E****.(2011) Genotypes and infection sites in an outbreak of multidrug resistant Pseudomonas aeruginosa. J Hosp Infect. 78:317-22. PMID: 21689862.*

18. ***Wolfgang, M.C.; Kulasekara, B.R.; Liang, X.; Boyd, D.; Wu, K.; Yang, Q. et al.(****2003)"Conservation of genome content and virulence determinants among clinical and environmental isolates of pseudomonas aeruginosa". ProcNatlAcadSci USA. Vol.100: pp.8484-9.*