Molecular Detection of Virulence Factors Genes in *Pseudomonas aeruginosa* Isolated from Different Infections Cases in Al-Diwaniya Hospital

Wafaa A. J. Al-Kaaby

College of Biotechnology/ AL-Qadisiya University

alkaaby-biotech@yahoo.com

Abstract:

Pseudomonas aeruginosa is the most important and dangerous organism in human infections due to production of several extracellular and cell-associated virulence factors that cause severe tissue damage, the most important virulence factors including (exoenzyme S, exotoxinA and lipoprotein), these factors was encoded by (exoS, toxA and oprI gene respectively). In this study we used polymerase chain traction technique (PCR) for detection virulence factor genes producing by *P. aeruginosa* that isolated from wound, burn and pulmonary tract infections patient swab samples. The PCR results was show that all *P. aeruginosa* isolates was carried virulence factors genes with difference in prevalence between them. The burn infection *P. areuginosa* isolates were show high prevalence of virulence factors genes more than wound and pulmonary tract infections isolates as well as the virulence factor gene (ToxA) was show high production in most isolates. In this study we concluded that the production of virulence factors genes in *P. aeruginosa* is important to human infection especially (ToxA) gene and the PCR technique is very specific and fast method in detection virulence factor genes in *P. areuginosa*.

Introduction:

P. aeruginosa is an opportunistic pathogen that pathogen capable of infecting the humans [1]. In human can be cause severe infections in virtually all tissues. Pulmonary tract infection with *P. aeruginosa* is a major cause of morbidity and mortality in patients with cystic fibrosis [2]. *P. aeruginosa* infections in hospitals mainly affect the patients in intensive care units with burn wound infections or chronic illnesses [3].

P. aeruginosa possesses a large number of cell-associated and extracellular virulence factors, which are tightly regulated by cell-to-cell signaling systems [4]. Most virulence factors produced by this bacterium which is important in its pathogenicity are: Exotoxin, Exoenzyme S, L lipoprotein, las B elastase and phospholipase C [5]. Among these virulence factors are a variety of secreted factors, such as proteases, phospholipases and the exotoxin A. *P. aeruginosa* strains also possess a type III secretion system that allows them to deliver toxins (effectors) directly into the cytoplasm of a host cell [6]. Exotoxin A which important causes tissue necrosis since it blocks protein synthesis [7]. Exotoxin A, encoded by the toxA gene, inhibits protein biosynthesis by transferring an ADP-ribosyl moiety to elongation factor 2 of eukaryotic cells. Exoenzyme S, encoded by the exoS gene, is also an ADP-ribosyltransferase that is secreted by a type-III secretion system in to the cytosol of epithelial cells [8]. L and I lipoproteins are two outer membrane proteins of *P. aeruginosa* responsible for inherent resistance of *P. aeruginosa* to antibiotics and antiseptics [9].

Key words: *Pseudomonas aeruginosa*, Virulence Factors Genes.

PCR has the potential for identifying microbial species rapidly by amplification of sequences unique to a particular organism [10]. In this study, we examined detection of most virulence factors genes in *P. aeruginosa* isolates that isolated from wound, burn and pulmonary tract by using polymerase chain reaction technique.

Materials and Methods:

Sample collection: 100 swab samples were collected from wound, burn and pulmonary tract infection Al-Diwaniya city. The samples placed in sterile transport media then transferred into microbiology laboratory College of Veterinary Medicine and store in refrigerator until bacterial isolation.

Bacterial isolation: *P. aeruginosa* was isolated from fecal samples by inoculation on brain heart infusion broth media at 37°C overnight for primary enrichment culture and then the bacterial growth were inoculated on chrome agar at 37°C overnight for selective isolation of pure culture *P. aeruginosa* isolates.

Bacterial genomic DNA extraction: Bacterial genomic DNA was extracted from P. *aeruginosa* isolates by using (PrestoTM Mini gDNA Bacteria Kit. Geneaid. USA). 1ml of overnight bacterial growth on BHI broth was placed in 1.5 ml micro centrifuge tubes and then transferred in centrifuge at 10000 rpm for 1 minute. After that, the supernatant was discarded and the bacterial cells pellets were used in genomic DNA extraction and the extraction was done according to company instruction. After that, the extracted gDNA was checked by Nano drop spectrophotometer, then store in -20ºC in refrigerator until perform PCR assay.

Polymerase chain reaction (PCR): PCR assay was performed by using specific primer for detection evaluates exoS toxA and oprI virulence factors genes according to method described by [11]. These primes were designed by using NCBI-Gene Bank and primer 3 plus design online. As show in the following table:

Primer		Sequence	Size	Gene Bank code
exoS gene	F	GCTTCAGCAGAGTCCGTCTT	684bp	L27629.1
	R	GCCGATACTCTGCTGACCTC		
ToxA gene	F	GGCTATGTGTTCGTCGGCTA	487	AF227424.1
	R	TGATCGCCTGTTCCTTGTCG		
oprI gene	F	CGGCTGGGAGATTGCTGTTA	202bp	X58714.1
	R	CCTTGCGATAGGCTTCGTCA		

These primers were provided by (Bioneer Company, Korea). Then PCR master mix was prepared by using (AccuPower® PCR PreMix kit. Bioneer. Korea). The PCR premix tube contains freeze-dried pellet of (Taq DNA polymerase 1U, dNTPs 250µM, Tris-HCl (pH 9.0) 10mM, KCl 30mM, $MgCl_2$ 1.5mM, stabilizer, and tracking dye) and the PCR master mix reaction was prepared according to kit instructions in 20µl total volume by added 5µl of purified genomic DNA and 1.5µl of 10pmole of forward primer and 1.5µl of 10 pmole of reverse primer, then complete the PCR premix tube by deionizer PCR water into 20µl and briefly mixed by Exispin vortex centrifuge (Bioneer. Korea). The reaction was performed in a thermocycler (Mygene Bioneer. Korea) by set up the following thermocycler conditions; initial denaturation temperature of 95 °C for 5 min; followed by 30 cycles at denaturation 95 °C for 30 s, annealing 58 °C for 30 s and extension 72 °C for 1min and then final extension at 72 °C for 10 min. The PCR products were examined by electrophoresis in a 1.5% agarose gel, stained with ethidium bromide and visualized under UV transilluminator.

Results and Discussion:

Bacterial isolation results of *P. aeruginosa* isolation results was show in the following table:

Sample type	Total samples	Positive isolate	Percent
Wound	35	18	51.4%
Burn	45	37	82.2%
Pulmonary tract	20	12	60%

PCR was appeared specific assay that used in detection of virulence factors genes (ToxA, exoS and OrpL) producing *P. aeruginosa* isolates human infections patients' samples. Where, The PCR results were show in following table:

Virulence gene	Clinical isolates		
	Wound (18)	Burn (37)	Pulmonary tract (12)
ToxA	13 (72.2%)	35(94.5%)	9 (75%)
exoA	7 (38.8%)	25 (67.5%)	5 (41.6%)
oprL	9 (50%)	13 (56.7%)	6 (50%)

Ch-square statistical significant at ($P < 0.05$)

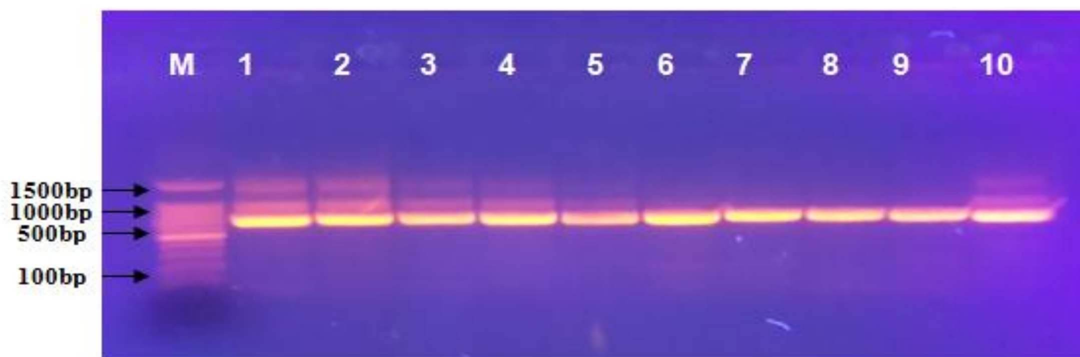

Figure (1): Agarose gel electrophoresis of PCR assay show the some positive results of exotoxinA toxA gene in P. aeruginosa. Where, Lane (M) DNA marker (100bp), Lane (1-3) positive samples at 684 bp for exoS gene in *P. aeruginosa* isolate.

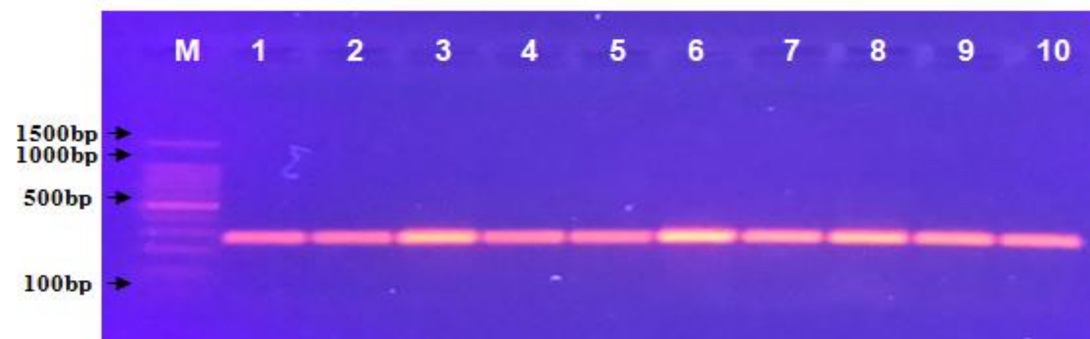

Figure (1): Agarose gel electrophoresis of PCR assay show the some positive results of exotoxin toxA gene in P. aeruginosa. Where, Lane (M) DNA marker (100bp), Lane (1-3) positive samples at 453bp for toxA gene in *P. aeruginosa* isolate.

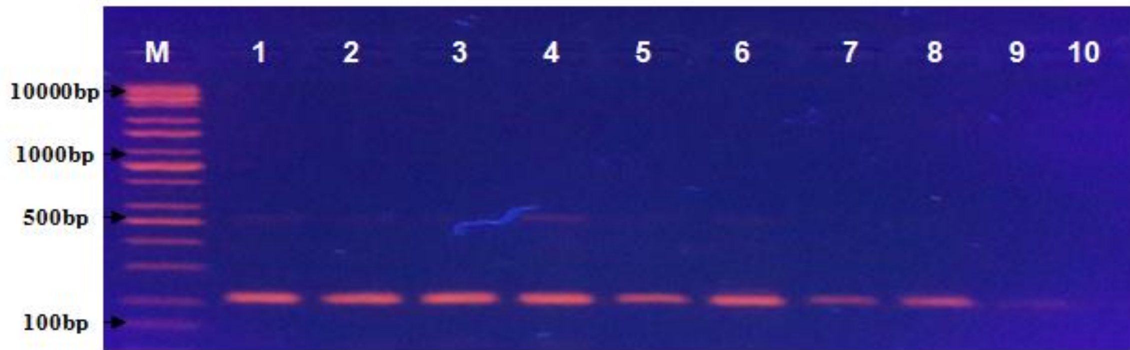

Figure (1): Agarose gel electrophoresis of PCR assay show the some positive results of Lipoprotein L gene in P. aeruginosa. Where, Lane (M) DNA marker (100bp), Lane (1-3) positive samples at 202bp for OrpL gene in *P. aeruginosa* isolate.

Molecular methods is better than the phenotypic methods that used in identification of *P. aeruginosa* more over biochemical testing takes long time to perform and requires extensive hands-on working the technologist [(12)]. The most important factor in the pathogenicity of P. aeruginosa is extracellular protein (exotoxin) that encoded by ToxA gene. This toxin can causes necrosis of liver, pulmonary edema, hemorrhage, and tubular necrosis of kidneys [(13)]. The percent study was designed singleplex PCR technique detection of virulence factor genes in *P. aeruginosa* isolated from different infection cases in Al-Diwaniya hospital.

The result show that burn infection *P. areuginosa* isolates were show high prevalence of virulence factors genes more than wound and pulmonary tract infections isolates as well as the virulence factor gene (ToxA) was show high production in most isolates. This finding was agreed with [(13)] who study different virulence genes as markers in *P. aeruginosa* that isolated from urinary tract infections and show high prevalence of virulence factor gene (exotoxin A) at 100%. Other Study [(14)] who developed PCR assay to detect *P. aeruginosa* by amplifying the toxA gene, they reported that of 130 *P. aeruginosa* isolates, 125 (96%) contained the toxA gene (sensitivity 96%) and (specificity 100%). These studies indicate that high specificity of toxA gene is unlike other virulence factors genes such as oprI and oprL genes that show a low sensitivity [(15)]. *P. aeruginosa* isolates generally producing cytotoxicity or invasion phenotypes which is correlated with presence of (exotoxin A) that encoding exoA gene. Our study by PCR technique was show difference in in production of virulence factors genes (ToxA, exoS and orpL) according to clinical source of *P. aeruginosa* isolates were the burn infection isolates were show more prevalence more than pulmonary tract and wound infection at statistically significant ($P < 0.05$) (Table 1). In this study, we examined detection of most virulence factors genes in *P. aeruginosa* isolates that isolated from wound, burn and pulmonary tract by using polymerase chain reaction technique.

Reference

1-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, PP: 201-229.

2-Fegan, M.; Francis, P.; Hayward, A.C.; Davis, G.H. and Furest, J. A. (1990). Phenotypic conversion of *Pseudomonas aeruginosa* in cystic fibrosis. J. Clin. Microbiol., 28: 1143-1146.

3-Yetkin, G.; Otlu, B.; Cicek, A.; Kuzucu, C. and Durmaz, R. (2006). Clinical, microbiologic and epidemiologic characteristics of *Pseudomonas aeruginosa* infections in a university hospital, Malatya, Turkey. Amer. J. Infect. Control, 34: 188-192.

4-Van Delden, C. and Iglewski, B.H. (1998). Cell-to-cell signaling and *Pseudomonas aeruginosa* infections. Emerg. Infect. Dis., 4: 551–560.

5-Yahr, T.L.; Hovey, A.K.; Kulich, S.M. and Frank, D.W. (1995). Transcriptional analysis of the *Pseudomonas aeruginosa* exoenzyme S structural gene. J. Bacteriol. 177: 1169–1178.

6-Rietisch, A.; Gely, V.I.; Dove, L.S. and Mekalane, J.J. (2005). Exs E a secreted regulator of type III sectior genes in *Pseudomonas aeruginosa*. J. Harvard Medical School, 102 (22): 8006-8011.

7-Al-Rubaiee, L. "The Role of *Pseudomonas aeruginosa* in chronic supportive otitis media infection". Thesis M.Sc., Medicine.

8-Van Delden, C. and Iglewski, B.H. (1998). Cell-to-cell signaling and *Pseudomonas aeruginosa* infections. Emerg. Infect. Dis., 4: 551-560.

9-deVos, D.; Lim, A.; Pirnay, J.P.; Struelens, M.; Vandenveld, C. and Duinslaeger, L. (1997). Direct detection and identification of *Pseudomonas aeruginosa* in clinical samples such as skin biopsy specimens and expectorations by multiplex PCR based on two outer membrane genes, oprI and oprL. J. Clin. Microbiol., 35: 1295-1299.

10-Khan, A.A. and Cerniglia, C.E. (1994). Detection of *Pseudomonas aeruginosa* from clinical and environmental samples by amplification of the exotoxin A gene using PCR. Appl. Environ. Microbiol., 60: 3739-3745.

11-Nikbin, V.S.; Aslani, M.M.; Sharafi, Z.; Hashemipour, M.; Shahcheraghi, F. and Ebrahimipour, G.H. (2012). Molecular identification and detection of virulence genes among *Pseudomonas aeruginosa* isolated from different infectious origins. 4(3): 118-123.

12-Qin, X.; Emerson, J.; Stapp, J.; Stapp, L.; Abe, P. and Burns, L. (2003). Use of real-time PCR with multiple targets to identify *Pseudomonas aeruginosa* and other non-fermenting gram-negative bacilli from patients with cystic fibrosis. J. Clin. Microbiol., 4: 4312-4317.

13-Sabharwal, N.; Dhall, S.; Chhibber, S. and Harjai, K. (2014). Molecular detection of virulence genes as markers in *Pseudomonas aeruginosa* isolated from urinary tract infections. Int. J. Mol. Epidemiol. Gen., 5(3): 125-134.

14-Khan, A.A. and Cerniglia, C.E. (1994). Detection of *Pseudomonas aeruginosa* from clinical and environmental samples by amplification of the exotoxin A gene using PCR. Appl. Environ. Microbiol., 60: 3739-3745.

15-Lavenir, R.; Jocktane, D.; Laurent, F.; Nazaret, S. and Cournoyer, B. (2007). Improved reliability of *Pseudomonas aeruginosa* PCR detection by the use of the specific ecfx gene target. J. Microbiol. Methods, 70: 20-29.

الكشف الجزيئي عن جينات عوامل الضراوة في الزائفة الزنجارية المعزولة من حالات إصابات مختلفة في مستشفى الديوانية

وفاء عبد الواحد جحيل الكعبي

كلية التقانات الإحيائية/ جامعة القادسية

alkaaby-biotech@yahoo.com

الخلاصة:

الزائفة الزنجارية هي الكائن الأكثر أهمية وخطورة في الإصابات البشرية نتيجةً لإنتاجها عوامل ضراوة عِدة خارج الخلية والخلايا المرتبطة التي تسبب التلف الشديد للأنسجة، ومن أهم تلك العوامل المتضمنة (exoenzyme S و exotoxinA والبروتين الدهني)، وهذه العوامل تكون مشفرة بواسطة جينات (exoS و toxA و oprI على التوالي). إستخدمت في هذه الدراسة تقنية سلسلة إنزيم البلمرة (PCR) للكشف عن جينات عوامل الضراوة المنتجة بواسطة بكتريا الزائفة الزنجارية *P. aeruginosa* التي عزلت بواسطة مُسحات أخذت من مرضى الجروح والحروق وإصابات المسالك الرئوية. وبينت نتائج ألـ PCR أن جميع عزلات *P. aeruginosa* كانت حاملة لجينات عوامل الضراوة مع وجود فرق في الإنتشار فيما بينهما.

عزلات *P. aeruginosa* من إصابات الحروق أظهرت إرتفاع معدل الإنتشار لجينات عوامل الضراوة أكثر من الجرح وعزلات إصابات المسالك الرئوية وكذلك جين عامل الضراوة (ToxA) الذي أظهر أعلى إنتاج في غالبية العزلات. وإستنتج من هذه الدراسة أن إنتاج جينات عوامل الضراوة في *P. aeruginosa* هو مهم بالنسبة للإصابات البشرية خاصةً جين (ToxA)، وتقنية PCR هي طريقة سريعة جداً ومحددة في الكشف عن جينات عامل الضراوة في *P. aeruginosa*.

كلمات مفتاحية: الزائفة الزنجارية، جينات عوامل الضراوة.