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Evaluation of Virulence Genes *(PilB* and *LasB*) for *Pseudomonas aeruginosa* Isolated from Different Clinical Samples

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ABSTRACT
Background: *Pseudomonas aeruginosa* is the second most common pathogenic bacteria isolated from patients and a major cause of nosocomial infections. It can also cause both acute and chronic infectious illnesses. Being a virulent organism, *P. aeruginosa* has a propensity to acquire resistance to the majority of antibiotics used in therapy.

Objective: This study aimed to detect certain virulence genes in *P. aeruginosa* isolates from various clinical sources.

Patients and methods: A total of (200) clinical specimens were taken from the patients with various age groups (3-60) years, from (Al Yarmouk Teaching Hospitals) in Baghdad from beginning of December 2021 to the end of March 2022, different specimens included wounds, burns, ear and diabetic foot swabs. All specimens were cultured on (MacConkey agar and cetrimide agar). On the other hand, bacterial virulence factors have been studied and they included detection of biofilm production using microtiter plate method (MTP), which is considered most sensitive. Detection of some virulence genes was performed using specific primers for each gene (*PilB* and *LasB*).

Results: The culture results revealed 53 isolates belong to the *Pseudomonas* bacteria. Culture characteristic and Vitek2 system showed that all 53 isolates belonged to *P. aeruginosa.* The results showed 100 % from 53 isolated were adhesion and biofilm formation–positive by (MTP) on the OD 590 nm. The results showed that *LasB* gene was found in all bacterial isolates (100%) and *PilB* gene was detected in 56.6%.

Conclusions: Molecular analysis showed that *PilB* and *LasB* were associated with formation of biofilm.

Keywords: *P. aeruginosa,* Biofilm formation, Multi drug resistance.

INTRODUCTION

Among the Gram-negative pathogens reported to the National Nosocomial Infectious Surveillance (NNIS) system; *Pseudomonas aeruginosa* is the second most prevalent pathogen isolated from patients causing nosocomial infection [1]**.** The bacteria can cause a wide range of acute and chronic infectious diseases. The main symptoms of these infections are inflammation and sepsis. Particularly patients with burns whose skin's host defenses have been compromised and those with weakened immune systems, such as those who have HIV or cancer, which are immunosuppressed are more susceptible for infection[2]**.** *P. aeruginosa* was the third most causing of nosocomi al urinary tract infection, the second most frequent cause of nosocomial pneumonia and the seventh most f requent cause of nosocomial bacteremia[3].

Pseudomonas aeruginosa has a propensity to acquire resistance to the majority of antibiotics used in therapy. It is a major contributor to nosocomial infec tions that can be fatal. It poses a serious treatment chall enge for clinicians due to its inherent resistance to num erousantimicrobial drugs and the development of multi drug resistance [4].

Because of this bacteria's inherent and acquired resistance to many classes of antibiotics, *Pseudomonas aeruginosa* known as MDRPa (Multi-drugs resistance *Pseudomonas aeruginosa*) strain and their infections can be difficult to treatment and the main drugs that *P. aeruginosa* isolate intermediate or resistant to are β-lactams, fluoroquinolones, aminoglycosides and carbapenems. Limited permeability of the outer membrane, the Multidrug Efflux System and production of inducible β-lactamase are the causes of intrinsic MDRPa [5]. *Pseudomonas aeruginosa* tends to produce biofilms, which are complex bacterial communities that adhere to different surfaces, including medical implant materials, plastics, metals and tissues. Bacterial survival is enhanced by formation of biofilms and once a biofilm is formed; it becomes very challenging to remove [6].

P. aeruginosa has a survival advantage and a selective advantage. Biofilms are exopolysaccharide that surrounds bacterial or micro colonies that are forming on biotic or abiotic surfaces. They are generated from individual free-floating (planktonic) cells. Biofilms are common in nature and are linked to many bacterial infections and illnesses that reoccur repeatedly [7]**.** This study aimed to detect certain virulence genes in *P. aeruginosa* isolates from various clinical sources.

PATIENTS AND METHODS

Ethical consent:

The research was conducted after approval from the Scientific Research Ethics Commission, Baghdad University Institute of Genetic Engineering and Biotechnology for Post Graduate Studies.

Informed consent was taken from the patient's relatives or the patient himself when he was still conscious with keeping the patients` records confidential in all stages of the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association

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(Declaration of Helsinki) for studies involving humans.

1. Collection of samples

Clinical specimens (200 swabs) were taken from patients with various age groups (3-60) years, from (Al Yarmouk Teaching Hospitals) in Baghdad, from beginning of December 2021 to the end of March 2022, from different specimens that included burns, wounds, diabetic foot and ear swabs. Taken from two hindered patients.

2. Isolation a nd identification of *Pseudomonas aeruginosa*

All specimen were cultured on (MacConkey agar and cetrimide agar). The culture results revealed 53 isolates of the *Pseudomonas* bacteria depending on culture characteristic, while the conformation by Vitek2 system showed that the 53 isolates belonged to the genus *P. aeruginosa.*

Molecular methods

1. DNA extraction

DNA was extracted from bacterial isolates using Purification depending on instruction of manufacturing company (Intron / Korea).

2. Conventional PCR technique

Specific primers were used for detecting the *P. aeruginosa* bacteria and some virulence genes. They were prepared according to information of supplying company (Table 1).

Table (1): The primers sequence and sizes used for detection of the genes

		Primer sequence	Size of Product (bp
pilB	F	**TCGAACTGATGATCGTGG**	
	R	**CTTTCGGAGTGAACATCG**	**408** [8]
lasB	F	**GGAATGAACGAAGCGTTCTC**	
	R	**GGTCCAGTAGTAGCGGTTGG**	**300** [9]
oprL	F	***ATGGAAATGCTGAAATTCGGC***	
	R	***CTTCTTCAGCTCGACGCGACG***	**504** [9]

Statistical Analysis

Data were presented as number and percentage.

RESULTS AND DISCUSSION

A total of 200 clinical specimens were cultured on different medium including (MacConkey and cetrimide) agar. The suspected colonies were identified depending on culture characteristics and biochemical properties.

1.Microtiter plate methods (MTP) for detection of biofilm formation by *P. aeruginosa*

In Microtiter plate methods, polystyrene plate of 96 wells was used to detect formation of biofilm. The results in table (2) showed 13 (24.5%) isolates of *P. aeruginosa* produced strong biofilm, 30 (56.6%) produced intermediate and 10 (18.9 %) produced weak biofilm.

This is nearly in agreement with the results reported by other studies that found 9 (25.7 %) strong, 14 (40%) intermediate and 9 (33.3) weak[10, 11].

Table (2): Percentage of *P. aeruginosa* biofilm producing isolate depending on MTP method

***Pseudomonas* Species**	**Number of isolates**	**Biofilm formation**		
		Strong	**moderate**	**weak**
P. aeruginosa	**53**	**13 (24.5 %)**	**30 (56.6%)**	**10 (18.9 %)**

Molecular methods

1. Extraction of genomic DNA

DNA of 53 bacterial isolates was extracted from that previously diagnosed by vitek2 system and molecular method using *oprL* gene. which give the same results when compared with traditional methods and vitek2 system. Product of conventional PCR for 53 isolates detected by using gel electrophoresis is showing in figure (1).

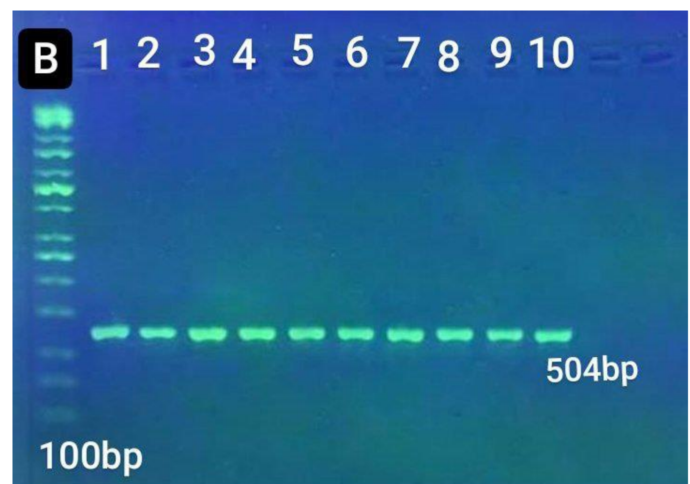

Figure (1): Agarose gel electrophoresis (75 Vol / 1.30 hour, 1.5% agarose) of conventional PCR for amplification of *Opr*L gene (504bp) to identify *Pseudomonas aeruginosa*, M: marker (100bp ladder), lanes (1, 2, 3, 4, 5, 6, 7,8, 9, 10)

Detection of some virulence genes (*AlgD* and *PilA*) in *Pseudomonas.*

Conventional PCR for *P. aeruginosa* was performed in order to prove the presence of *PilB* and *LasB* genes by using specific primers for each gene, the product of conventional PCR detect by using gel electrophoresis is show in table (3) and figures (2 and 3) respectively.

Table (3): The tota.l number and percentage of *PilB* and *LasB* genes in *P.* aeruginosa

Genes	Total number	Percentage
PilB	30	56.6%
LasB	53	100%

Pseudomonas aeruginosa strain also has the ability to produce virulence factors that play an important role in human infection including *PilB* gene, which encodes bacterial pill found on the surface of a wide variety of microorganisms that involved in the bacterial adhesion that play a role in early airway colonization and can trigger an inflammatory response and also can play role in movement of microorganisms as well as biofilm formation. The result of *PilB* gene was 56.6%, which was nearly similar to study in Iran that found *PilB* gene was 50% [12].

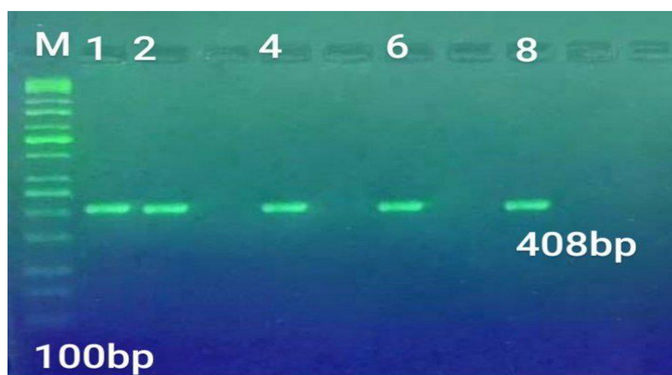

Figure (2): Agarose gel electrophoresis of conventional PCR amplification products of *PilB* gene (408bp) for *P. aeruginosa* (1.5% agarose, 75 Vol / 1.30 hour). M: marker (100 bp ladder) lanes (1, 2, 4, 6, 8).

Furthermore, the investigation showed the presence of another important virulence gene *LasB* that encoded for elastase, which is one of extracellular enzymes. It is active on elastin as well as it inactivates human immunoglobulin and degrades collagen and also plays essential role in biofilm formation, which was found in all 53 isolates, which matches study in Iran that recorded 100% *LasB* gene [13].

Figure (3): Agarose gel electrophoresis (1.5% agarose, 75 Vol / 1.30 hour) of conventional PCR amplification products of *P. aeruginosa lasB* gene (408bp). M: marker (100 bp ladder) lanes (1, 2, 3, 4, 5, 6, 7, 8, 9, 10).

CONCLUSION

Depending on the results of the present study, the following conclusions can be elucidated:

Conventional PCR technique for detection *opr*L gene of the species gave good and rapid results. And the molecular analysis showed that the *PilB* and *LasB* genes are associated with formation of biofilm.

Conflict of interest: The authors declare no conflict of interest.

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