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Molecular Detection of Some Virulence Traits among *Pseudomonas aeruginosa* Isolates, Hilla-Iraq

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Wound infections regards one of the most common infections encountered in hospital records. *Pseudomonas aeruginosa* regard the 3rd common pathogen among healthcare-related infections. Their ability to adapt to different conditions and presence of pool of virulence factors may render their infections delay in healing. During a period of six months 114 wound swabs were collected and inoculated on Pseudomonas chromogenic agar and then Pseudomonas aeruginosa isolated confirmed by PCR using specific primer for 16S rDNA gene of Pseudomonas aeruginosa. Molecular investigation of some virulence factor like ExoA, OprL, OprI, LasI and LasB were performed using a sets of specific primer pairs. The results revealed that only 26 (22.8%) isolates were Pseudomonas aeruginosa and the coexistence of more than one virulence factors within the same isolates was also recorder. OprI and LasB were most common followed by LasI, ExoA and OprL. Occurrence of virulence factor genes were 12(46.15%) for exoA, oprL was 11(42.3%), oprI was 22(84.61%), lasI was 14(53.84%) and lasB was 18(69.23%). Results of this study can lead us to conclude that *P. aeruginosa* have an arrays of virulence traits via which can adapt to different conditions and so cause a wide-ranging of hard to cured infections and the delay in healing and worseness degree may be attributed to owning multivirulence factors.

Keywords: *P. aeruginosa*, ExoA, Virulence, LasI, LasB.

Pseudomonas aeruginosa is a momentous life-intimidating, hospital pathogen that play a noticeable role in wound infections of burned patients[1,2]. It is success can be attributed to wide arrays of virulence factors which leads to adaptation and withstand for different inconvenient niches. Burn injuries stays one of the utmost public forms of trauma that push a major community health problem internationally. Skin compile a physical barricade against invasive microbes and the infections resulted from burn is common when this barrier breached. It is the supreme cause of burn wound infections[3,4,5,6]. The notable aptitude of P. aeruginosa to acclimatize and flourish among varied environments due to its extensive inherited adaptability and its intrinsic and acquired resistance to different antibiotics, which contributes meaningfully to its possible pathogenicity[7,8]. Wound-associated infections

are frequently hard to treat due to various bacterial pathogens [9,10,11,12,13].

P. aeruginosa have an arrays of virulence factors that frustrate host defenses and make direct tissue's harm or rise bacterium's affordability. The most important virulence factors of P. aeruginosa includes Exotoxin A (ETA), outer membrane associated protein L and I and quorum-sensing determinant system[14]. Exotoxin A represent the main dangerous virulence factor produced by P. aeruginosa[15]. OprL and OprI are peptidoglycan-related outer membrane proteins that fetter the outer membrane to the principal peptidoglycan. Lipoprotein of outer membrane lipoprotein (OprL) concerned in purge transporting systems leading to disturbing cell penetrability[16]. They are accountable for intrinsic resistance to antiseptics and antibiotics. Quorum-sensing (QS) system permits bacteria to respond to their community mass by harmonized adaptable their gene expression patterns. It is vital for the countenance of a series of virulence traits in addition to biofilm development[17,18,19,20].

The current study aimed to investigate the occurrence of exoA, oprL, oprI, lasI and lasB gene among P. aeruginosa isolated from wound infection in Hlla-Iraq.

MATERIALS AND METHODS

Samples

Wounds of 114 burned patients were swabbed from patients admitted to Al-Hilla teaching hospitals, Hilla-Iraq during a period of 6 months. All swabs were firstly cultivated on Pseudomonas chromogenic agar plate (Condalab/

Table 1. Show primer pairs sequence and amplicon size

Primer Name	Sequence (5[-3[)	Amplicon(bp)	References
PA-SS-F	GGGGGATCTTCGGACCTCA	956	[21]
PA-SS-R	TCCTTAGAGTGCCCACCCG		
ETA-F	GACAACGCCCTCAGCATCACCA	397	[22]
ETA-R	CGCTGGCCCATTCGCTCCAGCG		
oprL-F	ATG GAAATGCTGAAATTCGGC	504	[23]
oprL-R	CTTCTTCAGCTCGACGCGACG		
oprI-F	ATGAACAACGTTCTGAAATTCTCTGCT	249	[23]
oprI-R	CTTGCGGCTGGCTTTTTCCAG		
LasI-F	CGTGCTCAAGTGTTCAAGG	295	[24]
LasI-R	TACAGTCGGAAAAGCCCAG		
lasB-F	TTCTACCCGAAGGACTGATAC	153	[24]
lasB-R	AACACCCATGATCGCAAC		

Table 2. Show the PCR. conditions

Gene	Ini. Denat.	Denat.	Anneal.	Exten.	Final. Exten.	Reference
16S rDNA	95°C.2 min.	95°C.30 sec.	61°C.30 sec.	72°C.100 sec.	72°C.5 min.	Current study
# of Cycles	1	30	1			
exoA	95°C.2 min.	95°C.30 sec.	64.6°C.30 sec.	72°C.40 sec.	72°C.5 min.	Current study
# of Cycles	1	30	1			
oprL	95°C.2 min.	95°C.30 sec.	60.7°C.30 sec.	72°C.30 sec.	72°C.5 min.	Current study
# of Cycles	1	30	1			
oprI	95°C.2min.	95°C.30 sec.	60.7°C.30 sec.	72°C.30 sec.	72°C.5 min.	Current study
# of Cycles	1	30	1			
lasI	95°C.2 min.	95°C.30 sec.	56.7°C.30 sec.	72°C.30 sec.	72°C.5 min.	Current study
# of Cycles	1	30	1			
lasB	95°C.2 min.	95°C.30 sec.	54.6°C.30 sec.	72°C.30 sec.	72°C.5 min.	Current study
# of Cycles	1	30	1			

Spain) and then confirmed as Pseudomonas aeruginosa by amplification of 16S rDNA gene using specific primer pairs.

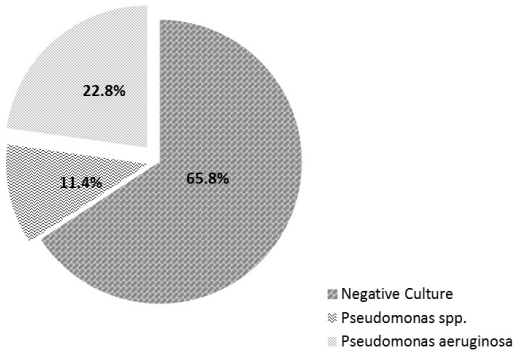

Fig. 1. Percentage of isolate recovery of wound swabs

DNA Extraction

Preparation of bacterial samples were performed according to Al-Dahmoshi (2017)[6] and then the steps of protocol of Mini DNA extraction kit (Favorgen/Taiwan) were followed. The extracted DNA checked using gel electrophoresis (0.7%) and then stained with safe DNA stain, RedSafe (IntronBio/Korea) and then visualized using Gel Documentation (Vilber/France).

Preparation of Primer and PCR

A working solution of primer pair were prepared by addition of nuclease free water (New England Biolabs/UK) to the lyophilized primer pairs (Realgene/China) to get 10pmole/¼l concentration. A reaction mixture of 25 µl were prepared using OneTaq Quick-Load 2X Master

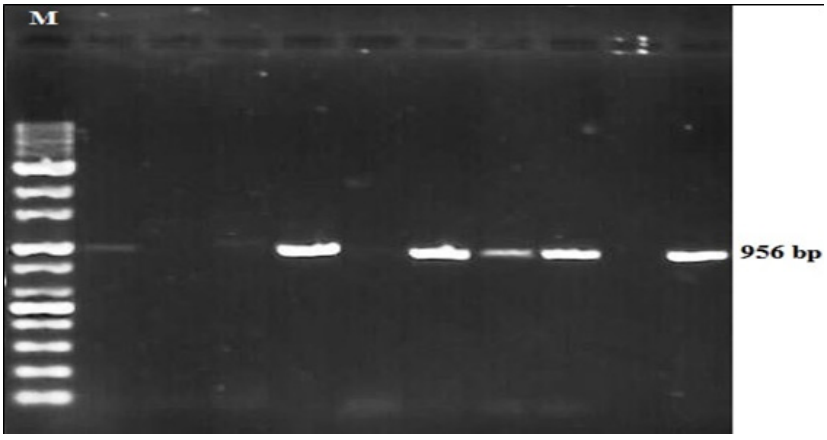

Fig. 2. Agarose electrophoresis (0.7%) of amplicon (956 bp) of 16S rDNA gene of Pseudomonas aeruginosa. M lane represent 100 bp DNA Marker while the rest lanes represents samples

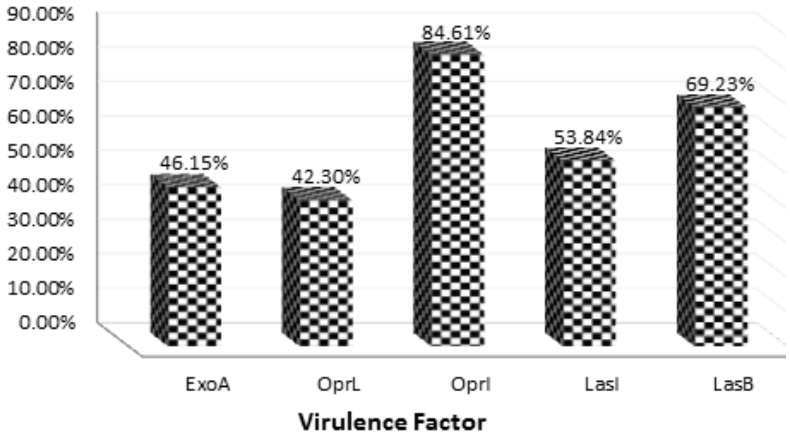

Fig. 3. Percentage of *P. aeruginosa* isolation among wound swabs

Mix. The primer sequence and PCR conditions were mentioned in the table 1 and 2 respectively.

RESULTS AND DISCUSSION

Results of isolation revealed that 39 isolate were Pseudomonas spp. while amplification

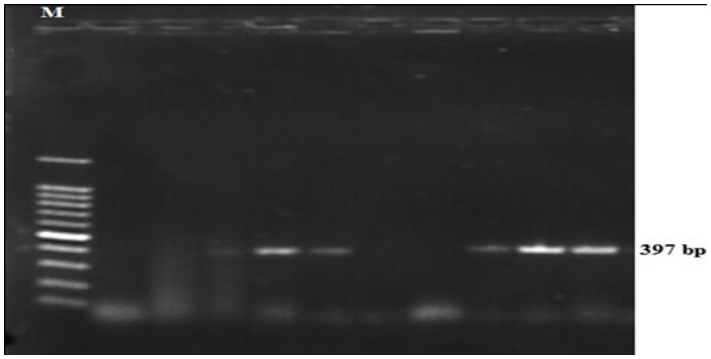

Fig. 4. Agarose electrophoresis (0.7%) of amplicon (397 bp) of exoA gene of Pseudomonas aeruginosa. M lane represent 100 bp DNA Marker while the rest lanes represents samples

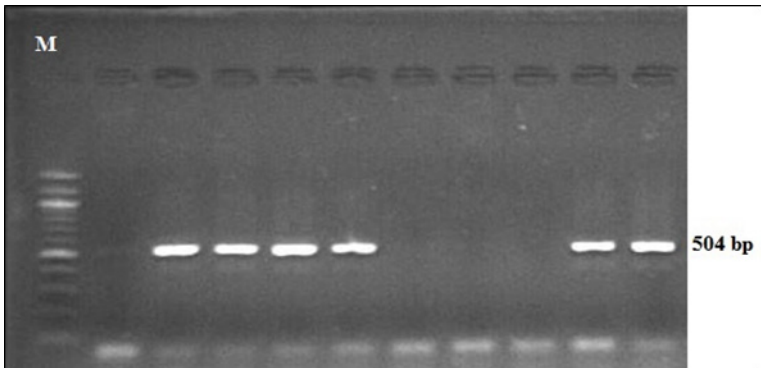

Fig. 5. Agarose electrophoresis (0.7%) of amplicon (504 bp) of oprL gene of *P. aeruginosa*. M lane represent 100 bp DNA Marker while the rest lanes represents samples

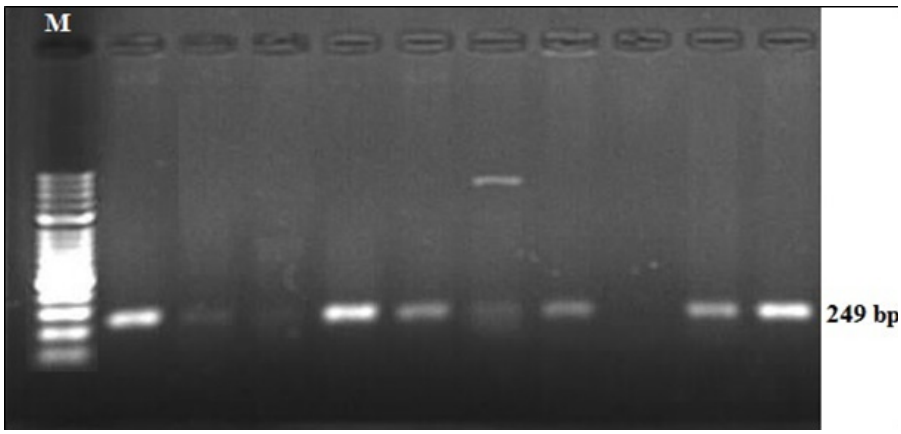

Fig. 6. Agarose electrophoresis (0.7%) of amplicon (249 bp) of oprI gene of *P. aeruginosa*. M lane represent 100 bp DNA Marker while the rest lanes represents samples

of 16S rDNA gene showed that only 26 (22.8%) isolates were Pseudomonas aeruginosa (figure 1). The PCR product was 956 bp (figure 2) and this results was in accordance with many local and international studies who found that the isolation percentage of *P. aeruginosa* were 22-32%[1,8,12,25,26].

Table 3. Show the coexisted virulence factors among isolates

Virulence Factor	No. of Isolates
ExoA, OprL, OprI, lasI, LasB	8
ExoA, OprI, lasI, LasB	2
OprL, OprI, lasI, LasB	2
OprI, LasI, LasB	1
OprI, LasB	2
OprI, LasI	2
OprL, OprI	1
Total	18

The highest isolation percentage may be due to the fact that it is the 3rd popular pathogen related with hospital-acquired. infections[25,26].

The PCR results of virulence factor occurrence showed that exoA was present among 12(46.15%), oprL was 11(42.3%), oprI was 22(84.61%), lasI was 14(53.84%) and lasB was 18(69.23%) (figure 3). Amplicon of exoA was 397 bp, 504 bp for oprL, 249 bp for oprI, 295 bp for lasI and 153 bp for lasB (figure 4,5,6,7,8)

Coexistence of more than one virulence factor within the same isolate were recorder and the results displayed that 8/26 have all five virulence factors, 4/26 have four virulence factors, 1/26 have three virulence factors and 5/26 have only two virulence factors (table 3).

ExoA responsible for toxigenesis trait of *P. aeruginosa* while invasiveness achieved by LasB and so the coexistence of ExoA, LasI and LasB

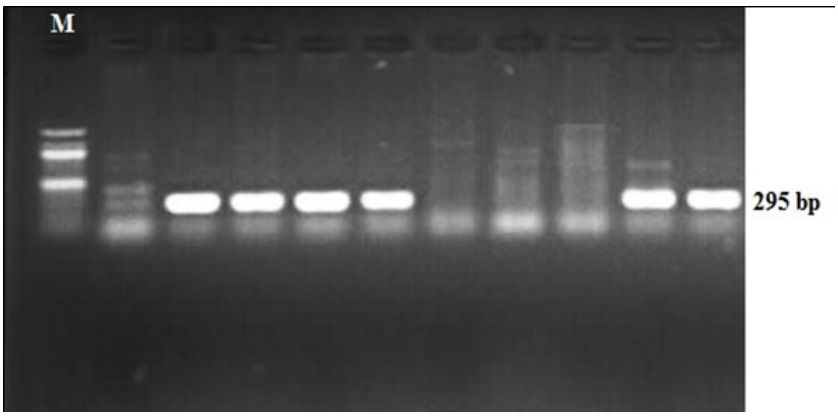

Fig. 7. Agarose electrophoresis (0.7%) of amplicon (295 bp) of lasI gene of *P. aeruginosa*. M lane represent 100 bp DNA Marker while the rest lanes represents samples

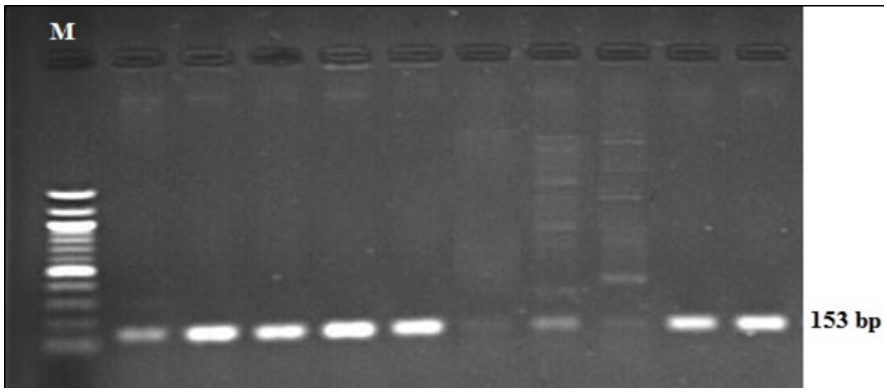

Fig. 8. Agarose electrophoresis (0.7%) of amplicon (153 bp) of lasB gene of *P. aeruginosa*. M lane represent 100 bp DNA Marker while the rest lanes represents samples

let both of mechanism of infection available and increase the degree of wound worseness[27,28,29,30]. ExoA had distinct role in hindrance of wound contraction and remedial[15]. Both of OprL and OprI have a role in antibiotic resistance via efflux mechanism and alterations of membrane permeability. OprL, OprI and LasI were engaged in antibiotic resistance and biofilm creation leads to many problems concerning treatment of P. aeruginosa infections and make the infection hard to cured[31,32].Bacteria attachment and immune system disruption can be facilitated by LasB. It is a protease (Elastase) that splits collagen, immunoglobulin G and A and complement moreover to destruction of fibronectin to uncover ligands for bacterial adhesion[27]. Nikbin et al., (2012) found that all isolates carried *oprI*, *oprL* and *lasB* genes[16]. The presence of ExoA, OprL, OprI, LasI and LasB among *P. aeruginosa* isolates suppose their linking with different levels of intrinsic virulence and pathogenicity[33, 34]. The presence of toxins and enzymes genes make them important clinically and environmtally[35].

Results of this study can lead us to conclude that *P. aeruginosa* have an arrays of virulence traits via which can adapt to different conditions and so cause a wide range of hard to cured infections and the delay in healing and worseness degree may be attributed to owning multivirulence factors.

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