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Molecular Detection of *oprI* and *oprL* Virulence Genes of *Pseudomonas aeruginosa* Isolated from Burns and Wounds

Mohammed A. Gawad [1], Wasan A. Gharbi[2]

[1] Muthanna Health Department, Ministry of Health
[2]Institute of Genetic Engineering and Biotechnology for Post Graduate Studies/ Baghdad University

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Abstract: Objectives: *Pseudomonas aeruginosa* bacteria are opportunistic pathogens capable of infecting almost all tissues of the body due to their possession of a variety of virulence factors that significantly contribute to pathogenicity in the host. 125 swabs were collected from burns and wounds patients to investigate the spread of *Pseudomonas aeruginosa* bacteria in these sources and to study some of the virulence factors possessed by this bacteria. The developing isolates were diagnosed after cultivation on different culture media through their phenotypic and microscopic characteristics, in addition to genetic diagnosis as a final diagnosis for the isolates that gave a positive result as *pseudomonas aeruginosa* during the previous tests and this was done by relying on the 16srDNA diagnostic gene with the sequence of P. aeruginosa bacteria. Diagnostic results are displayed that only 69 isolates (55.2%) of which were identified as *P. aeruginosa* divided on 53 isolates 60.9% of cases of burns infections and 16 isolates 42.1% of cases of wound. The prevalence of the genes encoding for lipoproteins Opr I, opr L, was investigated using PCR technology, and the results showed the presence of the opr I gene with a percentage of 100%, while the opr L gene was at a rate of (85.5%). Within the genetic structure of P. aeruginosa isolates under study, and as a result of their high presence in most of the studied isolated, these genes can be considered a successful and rapid alternative for the diagnosis of *p. aeruginosa* in a molecular way based on the polymerase chain reaction (PCR) technique.

Keywords: *Pseudomonas aeruginosa* , opr L ,opr I, 16SrDNA burns and wounds.

Corresponding author: (Email: mohammed.ali1200@ige.uobaghdad.edu.iq).

Introduction

Pseudomonas aeruginosa is a common bacterium that is a pathogenic germ that can cause significant opportunistic infections, especially in immunocompromised people. The spread of this organism in health care facilities is extremely harmful, as it infiltrates the human host's basic defensive line and enters the body through the skin, resulting in nosocomial infections, particularly in hospital intensive care units (ICUs). Due to the availability of various mechanisms of genuine resistance to most antibiotics, the pathogenesis of *P. aeruginosa* is multifactorial, resulting in the creation of a diverse set of cellular structures and extracellular chemicals that play a key role in increasing pathogenicity (1) A rod-shaped GN bacteria *P. aeruginosa* is a common cause for hospital-acquired illnesses. *P. aeruginosa* is responsible for roughly 8-10% of all healthcare-associated infections in the United States (51,000 cases in 2013). Multidrug-resistant strains were detected in roughly 13% of these instances, and a growing number of pan-drug-resistant specimens that could not be treated with any of the anti-

pseudomonal medicines available in the clinic were identified (2).

Burns are one of the most common types of trauma destruction(3). Burns compromise skin integrity and the skin immune system, which protects against pathogenic organisms' activity (4). Nosocomial infection is a major concern for burn patients. Infection is a major source of morbidity and mortality in burn patients in hospitals. Because of their weakened state and the nature of the damage, nosocomial infection is more common in burn patients (5). Wound, by definition, is breaks in skin epithelial integrity and may cause further disruption in skin anatomy, physiology, and its functions. There are two types of wounds known as acute and chronic (6). virulence factors in *P. aeruginosa*. The outer membrane proteins encoded by some gene like OprI and OprL genes of *P. aeruginosa* play important roles in the bacterium's interaction with the environment, as well as its inherent resistance to antibiotics. The presence of these specific outer membrane proteins has been implicated in efflux transport systems that affect cell permeability. Because these proteins are only detected in *P. aeruginosa*, they could be a reliable determinant for identifying P. aeruginosa in clinical samples quickly (7).

Materials and methods

This prospective study was conducted between 1/12/2021 to 1/4/2022, which included 69 clinical samples that collected from patients with burn (n = 53) and wound (n = 16) infections. From Both male and female patients 41 (58.6%),28 (50.9%) Respectively, with age ranged between 1 month to 73 years who attended Al alhussain Teaching Hospital, and Burn Hospital in Medical City, Baghdad Teaching Hospital were incorporated in the study.

Table (1): Frequency of culture positivity according to Source of infection

Positive growth	Source	
	Burn Swab	Wound Swab
P. aeruginosa	53 (60.9)	16 (42.1)
Other bacteria	34 (39.1)	22 (57.9)
Total	87 (69.6)	38 (30.4)
P value	a- 0.078 b- <0.0001* c- 0.109	

*** Represent a significant difference at p<0.05. a; the statistical analysis between positive growth culture (pseudomonas and Other bacteria) and source of infection (Burn and Wound), b; the statistical analysis between culture results (*P. aeruginosa*) and source of infection (Burn and Wound); c: the statistical analysis between culture results (Other bacteria) and source of infection (Burn and Wound).**

Laboratory tests

Isolation of bacteria

The bacterial isolates were diagnosed by observing their ability to grow on the diagnostic media represented by the medium MacConkey agar, blood agar and cetrimide The changes caused by the growing colonies on these media were observed and their phenotypic characteristics were studied in terms of the shape, size and color of the growing colonies, as well as biochemical tests represented by the oxidase and catalase test and IMVc tests.

DNA extraction

This process was made according to the genomic DNA purification Kit complemented by (Trans). Bacterial suspension was prepared when isolate was inoculated in Brain heart broth (BHB) media (incubated at 37°C for 24 h), the turbidity was adjusted for obtaining approximately $1x10^9$ CFU/ml, and then 1ml is transferred of suspension to1.5ml Eppendorf tube and is centrifuged at 14000 xg for 1 min.

Preparation of PCR primers

The primers are prepared depending on the manufacturing instruction by dissolving the lyophilized primers with TE (Tris-EDTA) buffer to make stock solution of concentration of 100 pmole/Ml, On spinning down and stay overnight at 4°C , primers working solution were prepared by diluting the stock solution with TE buffer to get final working solution (10 pmole/Ml) for each primer.

Table (2): Primers sequences, length, and PCR product

Gene	Primer	Sequences	Expected size (bp)	Refrence
16SrDNA	Forward	5́- TCCTTAGAGTGCCCACCCG-3	956 bp	(Altaai *et al*.,2017)
	Reverse	5 ATGAACAACGTTCTGAAATTCTCTGCT-3´		
Opr I	Forward	5-ATGAACAACGTTCTGAAATTCTCTGCT-3´	249bp	
	Reverse	5-CTTGCGGCTGGCTTTTTCCAG-3		
OprL	Forward	5-ATGGAAATGCTGAAATTCGGC-3´	504bp	
	Reverse	5-CTTCTTCAGCTCGACGCGACG -3´		

Molecular diagnosis of *P. aeruginosa* by 16s rDNA gene using polymerase chain reaction technique

Preparation of PCR mixture from the master mix, size 12.5 µL, 0.5 µL Forward primer, 0.5 µL Reverse primer,3 µL DNA template and Nuclease free water 8.5 µL Where the final volume of the reaction mixture became 25 microliters, and then the contents of the PCR tubes were mixed well and then placed in the PCR apparatus. Then 5 µl of the gene duplication was transferred for electrophoresis onto the prepared 1% agarose gel.

Genetic detection of some virulence factors responsible for multiple resistance to biological factors using polymerase chain reaction (PCR) technique.

The PCR mixture, prepare of the genes encoding the outer membrane proteins oprI, oprL in the same way that the reaction mixture prepares of the 16srDNA gene.

Table (3): PCR Thermal Cycling conditions

Gene	Initial denaturation	No. of cycles	Denaturation	Annealing	Extension	Final extension
16srDNA	94 °C	35	94°C	59°C	72°C	72°C
OprI	94.°C	35	94°C	61.C°	72°C	72°C
OprL	94.°C	35	94°C	65°C	72°C	72°C

Result and discussion

Genomic DNA extraction

The results demonstrated DNA bands from all isolates when carried over to the electrophoresed, in agarose (1%). The gel showed one band in each hole corresponding to the isolates genomics DNA (1).

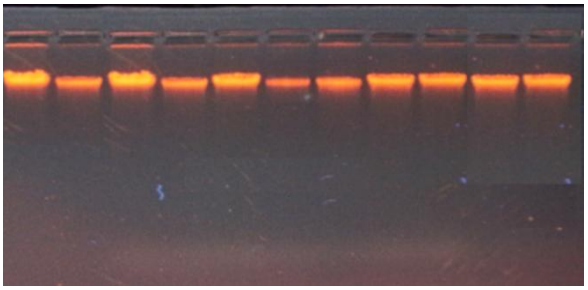

Figure (1): Patterns of 1% of agarose gel electrophoresis reveal DNA bands of Pseudomonas aeruginosa that was extracted.

Amplification of 16S rDNA gene

The extracted DNA was subjected to polymerase chain reaction to amplify 16S rDNA gene for 69 isolates in the present study, after PCR. They were visualized by gel electrophoresis, and were examined by gel electrophoresis. The PCR products gave a sharp band corresponding to a 956bp.The gel electrophoresis compared to the molecular ladder, (1500-100)bp, showing that the band belong to *Pseudomonas aeruginosa* 16S rDNA gene Figure (2).

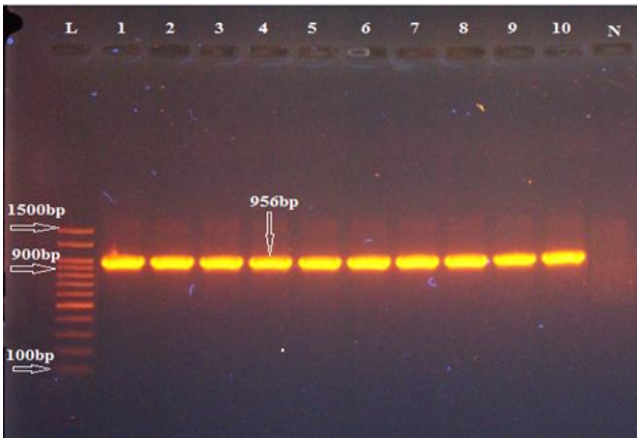

Figure (2): Gel electrophoresis for PCR product of (16SDNA primer) which show 956bp at 59°C, (Agarose 1%, 10min. at 100 voltage and then lowered to 70 volts, 60min.) Visualized under U.V light after staining with ethidium bromide. Lane L: DNA ladder (1500-100)bp, Lanes (1-10) represented positive results,and Lane (N) represented Negative controlDetection of virulace gene in *Pseudomonas aeruginosa*.

The presence of a number of virulence genes was investigated in isolates of *P. aeruginosa* bacteria

Opr I and Opr L within the genotype of *P. aeruginosa*, the presence of which is a gain for this bacterium, which enhances the increase in its resistance to antibiotics. PCR was performed for all *P. aeruginosa* isolates from burns and wounds, using specialized primers targeting the specific sequence of these genes,

including the bacterial genotype. For the purpose of detecting bacterial isolates that possess these genes opr L, opr I and after preparing the reaction mixture for each gene with a volume of 25 microliters, The reaction was carried out. After that, the multiplication product was left on the agarose gel at a concentration of 1% for a period of 90 minutes. When examining the gel under ultraviolet rays, one band was observed on the gel at the same level as for the isolates that possess these genes. This indicates the association of the primer with its complementary sequence in DNA strand. The estimation of the molecular weights of the resulting bundles was done using standard markers with a size of 100-1500 base pairs, which are shown on the (L-path) on the gel. The obtained results showed a similarity in the molecular weight of the resulting bundles when compared with the volume guide of 100 base pairs.

Detection of opr L.

Table (4): Genotyping of Opr L genes in *P. aeruginosa*.

Genes	OPR L Opr L	
	Positive	Negative
Burn	47 (88.7)	6 (11.3)
Wound	12 (75)	4 (25)
Total	59 (85.5)	10 (14.5)
P Value	0.173	

Table (4) shows result of conventional PCR revealed from fifty-nine of *Pseudomonas aeruginosa* (85.5%) were positive for opr L gene from total isolated. This gene did not appear in 6 isolates from burns and four from wounds, because the primer of this gene was not able to find its complementary sequence within the genetic structure of these isolates. It may be due to mutations or to rearranging the sequences of this gene within the genetic structure of those isolates. Thus it did not succeed in forming the replication product of the target gene, although these isolates gave a positive result when molecular diagnosed by the 16SrDNA gene .Besides the results of the traditional tests that indicated the bacteria p.aeruginosa. The results were obtained after the end of the PCR program for Gene Opr L and carry-over 5 µl product with standard markers on the gel to detect the presence of this gene, and to identify the molecular weight of the resulting bundles, and after the migration process is completed and examining Gel under UV source. The majority of the isolates showed bands specific for this gene the Molecular weight 504 base pairs when compared to standard DNA markers known molecular weight as shown in figure (3) These result full compitable with study of baghdad university, (8) Which score (85%) of isolated harbored of this gene (opr L), (9) show a result a little less

than our study for opr L gen score 77.77% while the study by (10), of baghdad university ,(11) study from wasit city and (7) in Egypt, The gene appeared in 100% of their isolates.

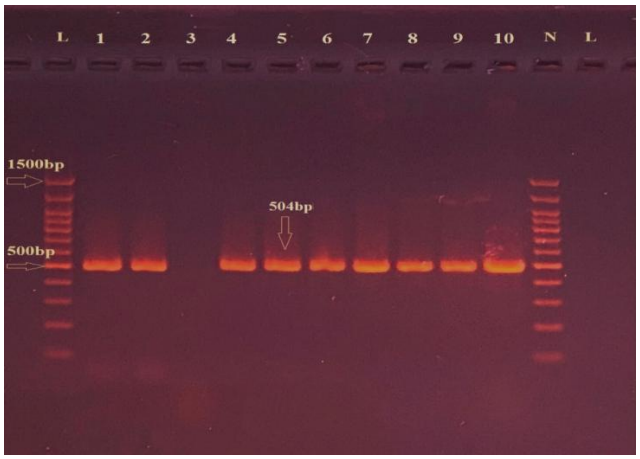

Figure (3): Gel electrophoresis for PCR product of (Opr L primer).
which show 504bp at 65°C , (Agarose 1%, 10min. at 100 voltage and then lowered to 70 volts, 60min.) Visualized under U.V light after staining with ethidium bromide.
Lane L: DNA ladder (1500-100)bp,
Lanes (1-2 and 4-10) represented positive results,
Lane (3) represented Negative result and Lane (N) represented Negative control.

This gene (opr L) is one of the most common genes in *p. aeruginosa*, which explains the reason for its large presence in most isolates, this is because it encodes lipoproteins, which is a component of the outer membranes of P. aeruginosa, and in achieving the pathogenicity of many Bacterial infections. It is one of the factors of virulence, as well as being a part of the efflux pump systems for antibiotics and toxins that affect bacterial cells as it directly affects the permeability of bacteria Cell membranes, thus preventing antibiotics and toxins from affecting to bacterial cells. This, in turn leads, to an increase in resistance (12). This has been evident in the multiple resistance of all isolates under the current study to more than one antibiotic. All isolates possess this ability in resistance may be due to the success of this gene in expression and thus be one of the reasons for the increase in resistance. Moreover, this gene is used in the direct diagnosis of P. aeruginosa isolated from different pathological, because its presence is limited to this bacteria among the species belonging to pseudomonadaceae (13).

Detection of opr I

Table (5): Genotyping of Opr I in *P. aeruginosa*.

Genes	OprL O Opr I OO	
	Positive	Negative
Burn	53(100)	0(0)
Wound	16 (100)	0 (0)
Total	69 (100)	0(0)
P value	0.027*	

*** Represent a significant difference at p<0.05.**

The results shown in the Table (5) showed that all isolates of *Psedomonas aeruginosa* are isolated from burns and wounds had the OprI gene in proportion 100%. The result was obtained after the completion of the PCR program for the gene opr I and the migration of the product on the agarose gel for the purpose of detecting the presence of the duplicated bundles of this gene estimate the molecular weight of those bundles by comparing with standard marker 100 base pair size. The results showed that the doubled bundle sizes of the opr I gene were identical with the expected size of 249 base pairs. as show in the figure(4).

These results are compatible with these recorded by (10) in Baghdad Iraq, (7) in Egypt. While the study by (8) score 87.5% were positive for this gene.

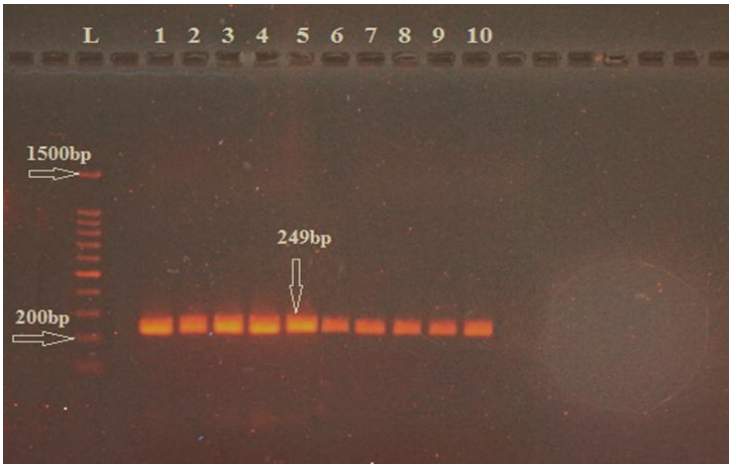

Figure (4): Gel electrophoresis for PCR product of (OprI primer) which show 249 bp at 61°C , (Agarose 1%, 10min. at 100 voltage and then lowered to 70 volts, 60min.) Visualized under U.V light after staining with ethidium bromide.
Lane L: DNA ladder (1500-100)bp , Lanes ((1-10) represented positive results and Lane (N) represented Negative control.

P.aeruginosa isolates possess Opr I gene (100%).The reason for the high frequency of this gene in all *P. aeruginosa* isolates from burn and wound infection, is that the sequence of this gene is highly conserved with in the

genetic makeup of *P.aeruginosa*, and most species of the genus (Pseudomonas fluorescent) to which this bacteria belongs Therefore, it was used as an important indicator in the molecular classification and phylogeny of *Pseudomonas* rRNA group I (14) . It is also the smallest genes for encoding outer membrane proteins in *P. aeruginosa* (15). Moreover, the Opr I protein, which encodes by the Opr I gene, plays an important role in the interaction of bacteria with the environment by binding the outer membrane of bacteria to the peptidoglycan layer (16) Thus ,this maintains the bacterial cell, in addition to providing latent resistance for P aeruginosa towards different types of antibiotics because it is part of the Efflux pump system this affects on the cell membrane permeability and ,Thus, is an effective barrier to entry of these antibiotics and a main component in the ejection of antibiotics that enter outside the bacterial cell (7).

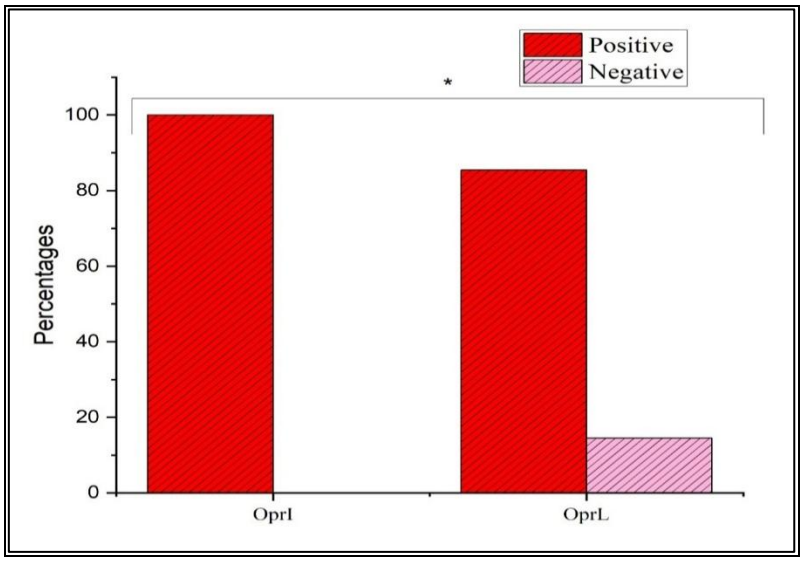

Figure (5): Genotyping of OprL and OPRL genes in *P. aeruginosa* in addition to the roles of both OprL and OprI genes encoding outer membrane proteins in maintaining bacterial cell shape and membrane integrity (Lin et al. 2010, et al.) Thus, the possession of this gene by bacteria and its successful expression increases bacterial resistance, and there by, the potential to cause *P. aeruginosa* severe infection in chronically infected patients is inevitable due to the weak resistance mechanisms and the inability to penetrate the outer membrane barrier of this bacteria.

Conclusions

The present study showed that Pseudomonas aeruginosa were isolated from burn' patients high than wound patient at a rate (60.9) while the wound (42.1), The results of the detection of virulence genes showed that P. aeruginosa had both opr I 100% and opr L 85.5 for all isolated.

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References

1. Abd Al-Mayali, M. and Salman, E. D. (2020). Bacteriological and Molecular Study of Fluor quinolones Resistance in Pseudomonas aeruginosa isolated from Different Clinical Sources. Iraqi Journal of Science, 2204-2214. dissertation, M. Sc. Thesis Submitted to the Council of the Institute of Genetic Engineering and Biotechnology for Post Graduate Studies, University of Baghdad. 60-63.
2. Wagner, S.; Sommer, R.; Hinsberger, S.; Lu, C.; Hartmann, R. W.; Empting, M., *et al*. (2016). Novel strategies for the treatment of Pseudomonas aeruginosa infections. Journal of medicinal chemistry, 59(13): 5929-5969
3. Atilla, A.; Tomak, L.; Katrancı, A. O.; Ceylan, A. and Kılıç, S. S. (2015). Mortality risk factors in burn care units considering the clinical significance of acinetobacter infections. Ulus Travma Acil Cerrahi Derg, 21(1): 34-38.
4. Aleksiewicz, R.; Kostro, K.; Kostrzewski, M.; Lisiecka, B.; Bojarski, M. and Mucha, P. A. (2015). Percentage of CD4+, CD8+, and CD25+ T lymphocytes in peripheral blood of pigs in the course of experimental burns and necrectomy. Journal of Veterinary Research, 59(3): 401-410.
5. Risan, F. A.; Salih, M. K. and Salih, T. A. (2020). Estimation of IL-17A and IFN-y in the Burn of Patients that Afflicted by Different Bacterial Types. International Journal of Psychosocial Rehabilitation, 24(05).
6. Che Soh, N. A.; Rapi, H. S.; Mohd Azam, N. S.; Santhanam, R. K.; Assaw, S.; Haron, M. N., *et al*. (2020). Acute Wound Healing Potential of Marine Worm, Diopatra claparedii Grube, 1878 Aqueous Extract on Sprague Dawley Rats. Evidence-Based Complementary and Alternative Medicine, 2020.
7. Khattab, M. A.; Nour, M. S. and ElSheshtawy, N. M. (2015). Genetic identification of Pseudomonas aeruginosa virulence genes among different isolates. Journal of Microbial and Biochemical Technology, 7(5): 274-7.
8. Ahmed, R.A. (2017). Molecular study and evaluation of DNA modification by DNA of pseudomonas aeruginosa isolates isolated from sources. M.SC. Thesis, College of Science, University of Baghdad.
9. Gholami, A.; Majidpour, A.; Talebi-Taher, M.; Boustanshenas, M. and Adabi, M. (2016). PCR-based assay for the rapid and precise distinction of Pseudomonas aeruginosa from other Pseudomonas species recovered from burns patients. Journal of preventive medicine and hygiene, 57(2): E81
10. Al-Mayyahi, A. W. (2018). Detection of (exoT, exoY, exo S and exoU) Genes In Pseudomonas aeruginosa Isolate From Different Clinical Sources (Doctoral dissertation, M. Sc. Thesis Submitted to the Council of the Institute of Genetic Engineering and Biotechnology For Post Graduate Studies, University of Baghdad. 60-63.
11. Al-Saeedi, R. H. A. and Raheema, R. H. (2019). Molecular Diagnosis of some Virulence Genes in Pseudomonas aeruginosa Clinical Isolates in Wasit Province. Indian Journal of Public Health, 10(04).
12. Firoved, A. M.; Ornatowski, W. and Deretic, V. (2004). Microarray analysis reveals induction of lipoprotein genes in mucoid Pseudomonas aeruginosa: implications for inflammation in cystic fibrosis. Infect Immun 72: 5012–5018.
13. Hassan, K. I.; Rafik, S. A. and Mussum, K. (2012). Molecular identification of *Pseudomonas aeruginosa* isolated from Hospitals in Kurdistan region. Journal of Advanced Medical Research. 2(3): 90 -98.
14. De Vos, D.; Bouton, C.; Sarniguet, A.; De Vos, P.; Vauterin, M. and Cornelis, P. (1998). Sequence diversity of the oprI gene, coding for major outer membrane lipoprotein I, among rRNA group I pseudomonads. Journal of bacteriology, 180(24): 6551–6556.
15. Remans, K.; Vercammen, K,; Bodilis, J. and Cornelis, P. (2010). Genome-wide analysis and literature-based survey of lipoproteins in *Pseudomonas aeruginosa*. Microbiology (Reading, England), 156(9): 2597–2607.
16. Mutharia, L. M. and Hancock, R. E. W. (1985). Monoclonal antibody for an outer membrane lipoprotein of the Pseudomonas fluorescens group of the family Pseudomonadaceae. International Journal

of Systematic and Evolutionary Microbiology, 35: 381–390.

17. Sana'a, N. H. (2013). Detection of Exo A and OPR I genes in *Pseudomonas aeruginosa* using polymerase chain reaction. Iraqi Journal of Biotechnology, 12(1), 44-50.