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# Detection of *exo*A and *opr*D genes expression in clinical isolates of *Pseudomonas aeruginosa*

**A Thesis**

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بسم الله الرَّحْمَنِ الرَّحِيمِ

﴿ وَيَسْـَٔلُونَكَ عَنِ ٱلرُّوحِۖ قُلِ ٱلرُّوحُ مِنْ أَمْرِ
رَبِّي وَمَآ أُوتِيتُم مِّنَ ٱلْعِلْمِ إِلَّا قَلِيلًا ﴾

**صدق الله العظيم**

**آية (٨٥) من سورة الإسراء**

# *Dedication*

## *I dedicate this work to …*

*My martyr brother, may Allah have mercy upon his soul…* ***Hafez***

*The candle of my life, Allah prolongs her life…* ***My Mother***

*My partner and support…My Husband…* ***Ahmed***

*My soul and the secret of my smile…*
*My children...****Haidar, Ali, and Noor***

*My childhood friend .....Rehab*

***Ruqaíya***

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## Summary

*Pseudomonas aeruginosa* has a wide spectrum of antibiotic resistance, the global problem in recent days is the increasing rate of multidrug-resistance, which has resulted in the wide use of medical therapy. It was considered as one of the greatest public pathogens in hospitals, making it to be the main causative agent of nosocomial infections, it widely contributed to severe opportunistic infections, particularly in immune-compromised patients.

The current study includes the diagnosis of 24 isolates of *P. aeruginosa* out of (100) different clinical specimens from wounds, burns, vagina, and urinary tract infections between the ages of (1-69 years), from both genders. The specimens were collected from inpatients and patients who visited Baqubah Teaching Hospital, Al-Batoul Teaching Hospital and Consultative Clinic / Diyala Governorate, during the period from October 2020 to January 2021.

All specimens were diagnosed based on bacteriological and biochemical tests. From (100) specimens, 24(24%) specimens were *P. aeruginosa,* 76(76%) specimens were other bacterial types. All isolates of *P. aeruginosa* were confirmed via tests done by the VITEK2 compact system. These isolates gave positive results for oxidase, catalase, citrate utilization tests. All the isolates were producers of hemolysin. They gave negative results to indole, methyl red, and voges-proskauer tests. The results of the pyocyanin, urease, lipase, protease, and gelatinase tests varied between negative and positive.

Drug susceptibility tests 24 isolates of *P. aeruginosa* were studied by the disk diffusion method against (12) antibiotics and the result showed a different percentage of resistance to each antibiotic as fellow: Imipenem, Meropenem,Ceftazedim, Cefipime, Levofloxacin, Norfloxacin, Ciprofloxacin, Gentamycin, Netilmicin, Azetreonam, Piperacillin/Tazobactam and

Ticarcillin/ Clavulanate (50%, 41.7%, 25%, 75%, 58.3%, 50%, 50%, 58.3%, 50%,75%, 66.7%, and 66.7%) respectively.

As for the molecular study, twelve isolates were DNA extracted by DNA extraction kit. Measurement of the concentration of DNA samples was done by Quantus Fluorometer. The concentrations of all twelve DNA samples were between (14-19)ng/µl. The PCR technique was used for screening the four virulence factors genes (*bla*NDM-1, *pel*A, *opr*D, *exo*A) of DNA of the twelve isolates of *P. aeruginosa*. The result showed that 10(83.3%) isolates were PCR-positive for *exo*A and *opr*D, while 2(16.7%) were PCR-negative. The determination of *pel*A in the twelve DNA of *P. aeruginosa* revealed that PCR was positive in 4(33.3%) isolates and 8(66.7%) were PCR-negative. While the result of the *bla*NDM-1 gene showed that the twelve DNA of *P. aeruginosa* 12(100%) were PCR-negative.

Regarding the study of gene expression, twelve isolates of *P. aeruginosa* were cultured on LB broth and treated by Meropenem. The antibiotic broth for studying the gene expression of *exo*A and *opr*D, and it was compared their gene expression in different body sites. Relative quantification expression ratios of the *exo*A and *opr*D of the cDNA were measured in comparison to the housekeeping gene *trp*E. A marked increase in gene expression was also found after treating the bacterial suspension with the antibiotic Meropenem. The mean value was high for *opr*D in bacterial treated than untreated with no significant difference ($P>0.05$) between bacterial treatment. However, the mean value was high for *exo*A in bacterial treated than untreated with a highly significant difference ($P<0.05$) between bacterial treatment. This indicates an increase in gene expression after treatment with the aforementioned antibiotic, can explain the resistance of bacteria to antibiotics.

The highest mean value of the *exo*A was in wound specimens, while the least mean value of it was in urine specimens. Based on the *opr*D, the highest mean value was in burn specimens, while the least mean value was in wound specimens.

# *Table of Contents*


| *Title* | | *Page No.* |
|---|---|---|
| *Summary* | | *I-III* |
| *Table of contents* | | *IV* |
| *List of tables* | | *VIII* |
| *List of figures* | | *IX* |
| *List of abbreviations* | | *X* |
| ***Chapter One : Introduction*** | | ***1-3*** |
| ***Chapter Two : Literature Review*** | | ***4*** |
| 2.1 | *Pseudomonas aerugenosa* | 4 |
| 2.2 | Classification of *Pseudomonas aeruginosa* | 5 |
| 2.3 | Diagnosis of *Pseudomonas aeruginosa* | 6 |
| 2.3.1 | Molecular methods | 6 |
| 2.4 | Epidemiology of *Pseudomonas aeruginosa* | 9 |
| 2.5 | Antibiotic resistance in *Pseudomonas aeruginosa* | 10 |
| 2.6 | Pathogenicity of *Pseudomonas aeruginosa* | 13 |
| 2.7 | Virulence factors of *pseudomonas aeruginosa* | 17 |
| 2.8 | The cell-associated virulence factors | 18 |
| 2.9 | Secretion virulence factors (extracellular factors) | 19 |
| 2.10 | Genomic of *Pseudomonas aeruginosa* | 21 |
| 2.11 | Virulence genes in *Pseudomonas aeruginosa* | 23 |
| ***Chapter Three: Materials & Methods*** | | ***26*** |
| 3.1 | Materials | 26 |
| 3.1.1 | Apparatus and equipment | 26 |
| 3.1.2 | The chemicals and biological materials | 28 |
| 3.1.3 | Culture media | 29 |
| 3.1.4 | Antibiotics | 30 |

| | | |
|---|---|---|
| 3.1.5 | Antibiotic powder | 30 |
| 3.1.6 | Kits | 31 |
| 3.2 | Methods | 32 |
| 3.2.1 | Media preparation and other technique sterilization | 32 |
| 3.2.2 | Laboratory prepared culture media | 32 |
| 3.2.2.1 | Pseudomonas agar base | 32 |
| 3.2.2.2 | Blood agar medium | 32 |
| 3.2.2.3 | MacConkey agar | 33 |
| 3.2.2.4 | Nutrient agar | 33 |
| 3.2.2.5 | Nutrient broth +15% glycerol | 33 |
| 3.2.2.6 | Brain heart infusion broth | 33 |
| 3.2.2.7 | Egg-yolk agar | 33 |
| 3.2.2.8 | Skim milk agar | 34 |
| 3.2.2.9 | Gelatin liquefaction medium | 34 |
| 3.2.2.10 | Urea agar medium | 34 |
| 3.2.2.11 | Muller Hinton agar | 34 |
| 3.2.2.12 | LB broth | 35 |
| 3.2.3 | Preparation of solutions | 35 |
| 3.2.3.1 | McFarland turbidity standard | 35 |
| 3.2.3.2 | Normal saline solution | 35 |
| 3.2.4 | Collection of patient's samples | 35 |
| 3.2.5 | Isolation of *Pseudomonas aeruginosa* | 36 |
| 3.2.6 | Identification of clinical *P. aeruginosa isolates* | 36 |
| 3.2.6.1 | Biochemical examination | 36 |
| 3.2.6.1.1 | Catalase test (slide test) | 36 |
| 3.2.6.1.2 | Oxidase test | 37 |
| 3.2.6.1.3 | IMViC tests | 37 |
| 3.2.6.1.4 | Triple sugar-iron (TSI) test | 38 |

| | | |
|---|---|---|
| 3.2.6.1.5 | Grown at 42°C test | 38 |
| 3.2.6.2 | VITEK2 apparatus system | 38 |
| 3.2.7 | Preservation of bacterial isolates | 39 |
| 3.2.7.1 | Short-period preservation | 39 |
| 3.2.7.2 | Long-period preservation | 39 |
| 3.2.8 | Antibiotics susceptibility (Kirby-Bauer method) | 39 |
| 3.2.8.1 | Antimicrobial agents used | 40 |
| 3.2.9 | Molecular Study of *Pseudomonas aeruginosa* | 40 |
| 3.2.9.1 | DNA extraction by extraction kit | 40 |
| 3.2.9.2 | Quantitation and purity of DNA | 42 |
| 3.2.9.3 | Primer preparation | 42 |
| 3.2.9.4 | Reaction setup and PCR program | 43 |
| 3.2.9.5 | Agarose gel electrophoresis | 44 |
| 3.2.9.5.1 | Preparation of agarose | 44 |
| 3.2.9.5.2 | The casting of the horizontal agarose gel | 45 |
| 3.2.9.5.3 | DNA loading | 45 |
| 3.2.10 | Gene expression | 45 |
| 3.2.10.1 | Preparation control broth | 45 |
| 3.2.10.2 | Preparation antibiotic broth | 45 |
| 3.2.10.3 | RNA purification of the (control, antibiotic)broth | 46 |
| 3.2.10.4 | Determine RNA yield | 47 |
| 3.2.10.5 | Primer preparation | 48 |
| 3.2.10.6 | Reaction setup and thermal cycling protocol | 48 |
| 3.2.11 | Statistical analysis | 49 |
| ***Chapter Four: Results*** | | ***50*** |
| 4.1 | Specimens collection | 50 |
| 4.2 | Isolation and Identification of *P. aeruginosa* | 50 |
| 4.3 | Biochemical tests and virulence factors | 51 |

| | | |
|---|---|---|
| 4.4 | Confirmation of *P. aeruginosa* identified | 52 |
| 4.5 | Antibiotics susceptibility test of *P. aeruginosa* isolates | 54 |
| 4.6 | Molecular study of *P. aeruginosa* | 56 |
| 4.6.1 | Concentration and purity of DNA | 56 |
| 4.6.2 | Detection of some virulence genes by PCR | 56 |
| 4.6.2.1 | Detection of *bla*NDM-1 | 57 |
| 4.6.2.2 | Detection of *pel*A | 57 |
| 4.6.2.3 | Detection of *exo*A | 58 |
| 4.6.2.4 | Detection of *opr*D | 58 |
| 4.7 | Gene expression study | 59 |
| 4.7.1 | Gene expression of the *opr*D and *exo*A | 60 |
| ***Chapter five: Discussion*** | | ***63*** |
| 5.1 | Specimens collection | 63 |
| 5.2 | Antibiotics susceptibility test of *P. aeruginosa* isolates | 64 |
| 5.3 | Molecular study of *Pseudomonas aeruginosa* | 69 |
| 5.3.1 | Molecular detection of different genes | 69 |
| 5.3.2 | Gene expression study | 74 |
| ***Chapter Six: Conclusions & Recommendations*** | | ***79*** |
| 6.1 | Conclusions | 79 |
| 6.2 | Recommendations | 80 |
| ***Appendices*** | | ***81*** |
| ***References*** | | ***88*** |

# *List of Tables*


| *Table No.* | *Table Title* | *Page No.* |
|---|---|---|
| (3-1) | The apparatus and equipment that was used in the experimental study. | 26 |
| (3-2) | The chemical and biological materials were used in the experimental study. | 28 |
| (3-3) | The culture media were used in the experimental study. | 29 |
| (3-4) | The antibiotic disks were used in the experimental study. | 30 |
| (3-5) | The antibiotic powder was used in the experimental study**.** | 30 |
| (3-6) | The kits that were used in the experimental study. | 31 |
| (3-7) | Volume and concentration of primer. | 42 |
| (3-8) | The sequence of primers of virulence genes used in PCR reaction | 43 |
| (3-9) | PCR reaction components and volumes. | 43 |
| (3-10) | PCR program used in the current study. | 44 |
| (3-11) | RNA Concentration (ng/µl) | 47 |
| (3-12) | Material of RT-qPCR technique. | 49 |
| (3-13) | Amplification steps of the gene expression of RT-qPCR technique. | 49 |
| (4-1) | Biochemical tests and virulence factors of of *P. aeruginosa*. | 51 |
| (4-2) | Biochemical tests results of *P. aeruginosa* by VITEK2 compact system | 52 |
| (4-3) | Comparative bacterial types with infection sites, diseases, and anthropometric characters of patients are calculated by $X^2$ test. | 53 |
| (4-4) | Frequency and percentage of sensitivity and resistance of antibiotics are calculated by $X^2$ test. | 55 |
| (4-5) | Frequency and percentage of genes are measured by conventional PCR for 12 isolates are calculated by $X^2$ test. | 56 |

# *List of Figures*


| ***Fig. No.*** | ***Figure Title*** | ***Page No.*** |
|---|---|---|
| (2-1) | The basic theory of real-time PCR using the SYBR® Green technique. | 8 |
| (2-2) | Antibiotic target sites. | 13 |
| (2-3) | *P. aeruginosa* Pathogenesis and major virulence factors. | 18 |
| (2-4) | The five developmental stages in biofilm formation. | 21 |
| (4-1a) | Gel electrophoresis of *bla*NDM-1 in *P. aeruginosa* in TAE buffer, stained with ethidium bromide using 100V for approximately 75min. M: 100bp ladder marker; lanes all isolates negative results of *bla*NDM-1 PCR product. | 57 |
| (4-1b) | Gel electrophoresis of *pel*A in *P. aeruginosa* in TAE buffer, stained with ethidium bromide using 100V for approximately 75min. M: 100bp ladder marker; lanes 1,4,7,8,9,10,11,12 negative results; lanes 2,3,5,6: 116bp of *pel*A PCR product. | 57 |
| (4-1c) | Gel electrophoresis of *exo*A in *P. aeruginosa* in TAE buffer, stained with ethidium bromide using 100V for approximately 75min. M: 100bp ladder marker; lanes 1,9 negative results; lanes 2,3,4,5,6,7,8,10,11,12 :397bp of *exo*A PCR product**.** | 58 |
| (4-1d) | Gel electrophoresis of *opr*D in *P. aeruginosa* in TAE buffer, stained with ethidium bromide using 100V for approximately 75min. M: 100bp ladder marker; lanes 1,9 negative results; lanes 2,3,4,5,6,7,8,10,11,12 : 193bp of *opr*D PCR product. | 59 |
| (4-2) | Expression of *exo*A and *opr*D in *Pseudomonas aeruginosa* that isolated from different infection sites are calculated by *F test* for 12 isolates. | 61 |
| (4-3) | Comparative *exo*A and *opr*D genes with antibiotic treating. | 62 |

# *List of Abbreviations*

| *Symbols* | *The meaning* |
|---|---|
| CDC | Central for Disease Control |
| CF | Cystic fibrosis |
| ISS | International space station |
| MDR | Multi Drug Resistance |
| XDR | Extensively Drug Resistance |
| PDR | Pan Drug Resistance |
| PCR | Polymerase Chain Reaction |
| RT-qPCR | Quantitative real-time Polymerase Chain Reaction |
| DNA | Deoxyribonucleic acid |
| RNA | Ribonucleic acid |
| ITUs | Intensive treatment unit |
| CCUs | Critical care unit |
| ICUs | Intensive care unit |
| WHO | World Health Organization |
| QS | Quarium Sensing |
| NI | Nosocomial Infection |
| UTIs | Urinary Tract Infection |
| PID | Pelvic Infection Diseases |
| IUD | Intrauterine device |
| LPS | Lipopolysaccharide |
| EPS | Exopolysaccharide |
| CLSI | Clinical and Laboratory Standards Institute |
| IS | Insertion sequence |
| ETA | ExotoxinA |
| ADP | Adenosine diphosphate |
| NDM | New Delhi Metallo |
| PFGE | Pulsed-field gel electrophoresis |
| bp | Base pair |
| µg | Microgram |
| µL | Microliter |
| UV | Ultraviolate |

# *Chapter One*

## 1.1 Introduction

*Pseudomonas aeruginosa* is a pervasive environmental bacteria. It has appeared as an opportunistic pathogen of main clinical relevance (Bassetti *et al*., 2018). It was considered as one of the greatest public pathogens in hospitals, and it widely contributed to severe opportunistic infections, particularly in immune-compromised patients (Pachori *et al*., 2019). According to the Centre for Disease Control, more than 51,000 clinical *P. aeruginosa* infections were reported each year in the USA with 400 deaths per year (CDC, 2018).

It was reported that *P. aeruginosa* has a wide spectrum of antibiotic resistance, making it to be the main causative agent of nosocomial infections (Moradali *et al.*,2017). This bacterium is a facultative anaerobic Gram-negative that colonizes a various collection of habitats, including surgical equipment and catheters (Rasamiravaka *et al*., 2015). It is one of the most common pathogens in intensive care units (ICUs) causing severe respiratory, urinary tract infections, bacteremia, wound sepsis. As well, it is the main cause of life-threatening infections in burn patients (Dou *et al*., 2017).

It is mainly dangerous for patients with severe wounds, cystic fibrosis (CF), and cancer. The infection strategy of *P. aeruginosa* depends on the production of numerous cell-associated and secreted molecules, including various proteases and toxins (Moradali *et al*., 2017). *P. aeruginosa* can cause life-threatening infections in patients with compromised immune system. Hence, it is a leading cause of clinical infections all over the world, especially in patients admitted to critical care units recovering from post-operative surgical wounds, burns, traumas, and pre-existing lung diseases such as cystic fibrosis (Baker *et al*., 2016).

The pathogenicity of *P. aeruginosa* was caused by some virulence factors, which are modulated by the expression of different genes including, *opr*I, *opr*D, and *tox*A. It was shown that there is a correlation between particular virulence factors and the distinct manifestations of *P. aeruginosa* infections (Nikbin *et al.,* 2012 ). The global problem in recent days is the increasing rate of multidrug-resistance (MDR) of *P. aeruginosa* strains which has resulted in the wide use of medical therapy (Dogonchi *et al*.,2018). *P. aeruginosa* infections can become established at various host sites and progress into life threatening acute or chronic infections (Lorenz *et al*., 2019; Moradali *et al*., 2017).

The flexibility and complication of the huge genome of *P. aeruginosa* (6.3Mbp) determine the evolutionary adaptations of this species (Cristina and Eusébio, 2013). It consists of a conserved essential genome with strain-specific districts that allow strains to gain or shed genomic fragments in an extension of environments to boost survival traits (Wendt and Joon-Heo, 2016). It was demonstrated that *P. aeruginosa* can catch and integrate clusters of genes that impart resistance to antibiotics and increase virulence. It was shown that this resistance is a result of the acquisition of mobile elements such as class 1 integrons, which are the antibiotic cassettes associated with them and lead to increased multi-drug-resistant *P. aeruginosa* (Ebrahimpour *et al*., 2018). Gene expression has been defined as the "production of a visible phenotype of a gene which is usually determined by protein synthesis" (Alberts *et al,* 2002).

The term 'gene expression' is often used with mRNA measurements synonymously. In addition, most studies often assume that single genes were associated with individual gene products for simplicity, while in fact, individual genes may ultimately lead to a variety of transcripts and proteoforms (Aebersold *et al*., 2018; Salovska *et al*., 2020). Furthermore,

RNA–RNA interactions of partly overlapping mRNA transcripts may lead to post-transcriptional regulatory events by modulating the interaction of RNA-binding proteins and impact gene expression levels (Gilet *et al*., 2020; Toledo-Arana and Lasa, 2020).

It was shown that there are variances between clinical isolates of *P. aeruginosa* that were isolated from different infection sites of human body in the gene expression of different genes which play an important role in the pathogenicity of this bacterium (Willcox *et al*., 2008). *opr*D is gene encoded for a Carbapenem-specific porin whose reduction or absence causes primary Carbapenem resistance. Porins are three-partite protein channels in *P. aeruginosa's* outer membrane that selectively transfer hydrophilic molecules based on their size and charge (Courvalin *et al.,* 2010). ExotoxinA is a toxin that inhibits protein synthesis by causing ADP ribosylation of eukaryotic elongation factor, which is similar to the mechanism of feat of diphtheria poison (Hossein *at el*., 2015).

Therefore, it is important to study the gene expression of different genes that play a role in virulence in *P. aeruginosa* isolated from different clinical isolates.The aim of the current study, determine the gene expression of different genes including *bla*NDM-1, *pel*A, *exo*A, and *opr*D in *Psudomonas aeruginosa* that were isolated from burn, wound, urine, and vagina infections. The steps to verify the aim are as follows:

**1.** The isolation and identification of *P. aeruginosa* from different clinical specimens.

**2.** Determine the antibiotic susceptibility of *P. aeruginosa* isolates.

**3.** Detection of some virulence factors genes.

**4.** Determine the gene expression of the genes in *P. aeruginosa* that were isolated from different infection sites.