



Molecular Detection and Identification of *oprI* and *lasB* Genes Isolated from *Pseudomonas aeruginosa*

Ciamak Ghazaei ^{1,*}

¹Department of Microbiology, University of Mohaghegh Ardabili, Ardabil, Iran

*Corresponding author: Department of Microbiology, University of Mohaghegh Ardabili, P. O. Box: 179, Ardabil, Iran. Email: ciamakghazaei@yahoo.com

Received 2023 May 17; Revised 2023 December 16; Accepted 2024 January 06.

Abstract

Species *Pseudomonas aeruginosa* is a distinguished opportunistic nosocomial bacterium that is commonly dispersed in nature. A pathogen that causes a large range of infections i.e. chronic as well as acute, with considerable levels of mortality and morbidity. Infections of *P. aeruginosa* are extremely hard to eliminate because of the presence of several virulence determinants as well as the intrinsic resistance against antibiotics. The present study investigated the detection of the virulence genes by polymerase chain reaction (PCR) namely *oprI* and *lasB* in the *P. aeruginosa* that were isolated detection from both human and animal specimens. 120 specimens of *P. aeruginosa* including samples were then collected from hospitals and veterinary laboratories, from which 55 isolates of *P. aeruginosa* were identified and selected. *Pseudomonas aeruginosa* strains were present in 40% of animal sample isolates and 86% of human sample isolates. The PCR results showed that *oprI* genes were detected in 54.16% of isolates obtained from animal samples and 80.76% of isolates obtained from human samples. While, the *lasB* gene was present at 58.33% and 92.30% in the isolates obtained from animal and human samples, respectively. Our results also showed the coexistence of both virulence genes in 32.34% of animal sample isolates and 25.10% of human sample isolations. The detection of gene *oprI* and *lasB* by the PCR technique can be effectively used for the molecular-level identification of *P. aeruginosa*. The identification of such virulence genes in *P. aeruginosa* isolates suggests that these may be linked with varying degrees of the pathogenesis and inherent virulence in this pathogen.

Keywords: *Pseudomonas aeruginosa*, Pathogen, Isolates, Infection, *oprI* and *lasB*

1. Background

Gram-positive *Pseudomonas aeruginosa*, a motile bacterium, is well known as a nosocomial pathogen able to infect almost all tissues. It is an aerobic bacillus that belongs to the Pseudomonadaceae family, which can cause acute as well as chronic infectious causing 18 to 63% of infections and diseases worldwide (1, 2). *Pseudomonas aeruginosa* can defend against diverse physical conditions, and thus can survive in community and hospital backgrounds. It is a cause of various infections i.e. urinary tract infections (UTIs), pneumonia and surgical site infections. It is considered a primary contributing factor to lung infection in cystic fibrosis patients. It can produce biofilm and stick to the lower respiratory tract of human mucin (3). *Pseudomonas aeruginosa* can be eliminated only at the beginning of colonization whereas, a preferable decrease in bacterial density during the colonization to prevent exacerbations (4).

Furthermore, *P. aeruginosa* is responsible for community-acquired infections including ulcerative keratitis, otitis externa and soft tissue infections (5). It is a main health danger, particularly to immune-deficient patients (6). This organism can simply acclimatize to the variations in the environment, hastily build up antibiotic resistance, and generate a range of virulence factors and thus it is connected with significant morbidity. *Pseudomonas aeruginosa* holds a range of virulence genes to assist in disease progression and colonization among a diversity of environments. *Pseudomonas aeruginosa* is a highly resistant pathogen due to its limited susceptibility to certain antimicrobials, which is one of its main pathogenic characteristics. In fact, this is reflected that the majority of resistance genes that are currently known are present in the genome of *P. aeruginosa* (7, 8).

The genes *nan1*, *toxA*, *exoS*, *exoU*, *oprL*, *plcN*, *oprI*, *lasA*, *exoY*, *lasB*, *plcH*, and *oprD* are typically responsible for and contribute to the virulence factors (9). *OprL* as well as *OprI* are outer membrane proteins (OMPs) of *P. aeruginosa*

which play a significant role in the interaction of the bacterium with its environment and also contribute to the innate resistance of *P. aeruginosa* to antibiotics.

Additionally, the occurrence of particular OMPs is concerned in the transport systems i.e. efflux which contributes to cell permeability (10, 11). According to a report, *OprL* has a major impact on the creation of membrane vesicles in *P. aeruginosa* and is a reliable indicator of *P. aeruginosa* infection (12). This second most abundantly present OMP of the *P. aeruginosa* aids in maintaining the bacterial cells' integrity and is also a part of the Tol-OprL multiprotein system where it plays an important role in the transport of carbon sources across cytoplasmic membranes (13-15). Similarly, *P. aeruginosa* has a *lasB* gene that produces Elastase B, a multifunctional protease which is involved in degrading host defence, immunoregulatory proteins as well as epithelial damage and type III secretion system (16). *lasB* gene is considered to be a prospective target for the advancement of a novel chemotherapeutic approach, particularly against multidrug-resistant strains (11).

In the veterinary field, antibiotics are administered abundantly for curative as well as prophylaxis purposes (17). Careless use of disinfectants also leads to bacterial adaptations and promotes the proliferation of highly resistant bacteria. Intrinsic resistance of *P. aeruginosa* has led the community as well as nosocomial infections caused by this pathogen, which is a serious concern. In addition to this, *P. aeruginosa* can acquire new resistance mechanisms against a variety of groups of antibiotics like, β -lactam antibiotics, fluoroquinolones and aminoglycosides (18). Hence, it is crucial to enhance our understanding of the resistance patterns and virulence of particular strains of *P. aeruginosa* (19). Thus, to mitigate the spreading risk of the resistant *P. aeruginosa* strain, it is crucial to obtain antimicrobial susceptibility patterns of the *P. aeruginosa* isolates (20). Moreover, the speedy identification of the strains of bacteria responsible for hospital-acquired infections is particularly crucial for making informed treatment decisions for patients. Also, animals and foodstuffs from animals are involved in daily human lives, increasing the risk of transmission of pathogenic strains between humans and animals.

2. Objectives

This study aims to identify presence of two virulence genes, *OprL* and *LasB*, in *P. aeruginosa* strains isolated from the samples obtained from the hospital and veterinary laboratories.

3. Methods

3.1. Isolation of Bacteria and Biochemical Test

One hundred twenty specimens of *P. aeruginosa* were collected from various hospitals and veterinary laboratories in Ardabil, Iran. Out of these 60 samples were from the hospital and the rest of the 60 samples were collected from the veterinary laboratories. Human clinical samples include; exuda of pulmonary, sputum, blood and abscess and veterinary samples were milk samples.

3.2. Sample Processing

The collected samples were cultured on Nutrient and MacConkey agar media and then incubated at 37°C for 24 h to ensure purity and optimal growth. The isolation procedure for *P. aeruginosa* was conducted using the Shukla and Mishra protocol from 2015 (21). Colonies that showed suspicious growth and colony morphology were identified. Additionally, conventional biochemical tests such as catalase test, motility, oxidase test, indole sulfide (SIM), and triple sugar agar (TSI) were performed to identify *P. aeruginosa* (22).

3.3. Bacterial DNA Preparation

Pseudomonas aeruginosa DNA was extracted using a commercially available kit, Cinnapure DNA (Cinnagen, Tehran, Iran), according to the instructions labelled by the manufacturers.

3.4. Detection of Virulence Genes by Polymerase Chain Reaction

The prevalence of virulence genes *oprL*, and *lasB* was determined by polymerase chain reaction (PCR) (23). Specific primers listed in Table 1 are used to amplify virulence genes. As per instructions provided by the supplier, primers were in a lyophilised form dissolved in the nuclease-free water to make up the final concentrations up to 100 pmol/mL. A 25 μ L reaction mixture was prepared to conduct PCR amplification of the *oprL* and *lasB* genes, which included 0.5 μ L of dNTPs (10 mM), 0.5 μ L of each primer (10 pmol), 1.5 μ L of MgCl₂ (25 mM), and 0.2 μ L of Taq DNA polymerase (5 U/ μ L). For PCR amplification of *oprL* and *lasB* genes, a 25 μ L reaction mixture was prepared containing 0.5 μ L of dNTPs (10 mM), 0.5 μ L of each primer (10 pmol), 1.5 μ L of MgCl₂ (25 mM), and 0.2 μ L of Taq DNA polymerase (5 U/ μ L). Both the genes were amplified separately. Strain of *P. aeruginosa* ATCC 27853, was used as a positive control. Agarose gel electrophoresis with a 1% agarose gel stained with ethidium bromide was utilized to visualize the PCR products.

Table 1. Oligonucleotide Primers for Amplification of the Virulence Genes

Primers	PCR Program	Primer Nucleotide Sequence	Amplicon Size (bp)	Reference
<i>oprL</i>	30 cycles: 40 s 94°C, 40 s 57°C, 50 s 72°C	F: 5'-ATGGAAATGCTGAAATTCGGC-3'	504	Xu et al., 2004 (24)
		R: 5'-CTTCTCAGCTCGACGCGACG-3'		
<i>lasB</i>	35 cycles: 30 s 94°C, 40 s 57°C, 50 s 72°C	F: 5'-TTCTACCCGAAGGACTGATAC-3'	153	Zhu et al., 2004 (25)
		R: 5'-AACACCCATGATCGCAAC-3'		

4. Results

4.1. Isolation and Detection of *Pseudomonas aeruginosa*

About 120 specimens *P. aeruginosa* clinical isolates were collected and the identification of these samples was confirmed using conventional bacteriological techniques. Results of the bacteriological test from 76 samples exhibit the green and blue colonies with the sweet grape like scent, indicating the presence of *Pseudomonas* produced on MacConkey agar and it didn't ferment lactose sugar. *Pseudomonas aeruginosa* strains were present in 24 (40%) animal sample isolates and 52 (86%) human sample isolates (Table 2).

4.2. Molecular Detection of the Virulence Genes

Polymerase chain reaction analysis done to screen 2 virulence encoding genes, *lasB* and *oprL*. The testing was conducted only for *P. aeruginosa* isolates, and specific primers were utilized for targeting virulence gene i.e. *oprL* and *lasB* that results in the amplicons of 504bp and 153 bp. The *oprL* genes were detected in 54.16% of animal sample isolations and 80.7% of human sample isolates. Results highlighted that 58.33 % of animal sample isolates and 92.30 % of human sample isolates showed the presence of *lasB* genes. However, PCR results showed the coexistence of both the virulence genes in 32.34% of animal sample isolates and 25.10% of human sample isolates (Figure 1).

The expression of *oprL* and *lasB* were higher in the exoda of pulmonary compared to all other samples and the least expression was found in abscess and blood. Isolates from sputum showed the prevalence of the genes *oprL* and *lasB*, by more than 50% (Figure 2).

4.3. Statistical Analysis

We have used a descriptive statistics for the analysis, to know the prevalence of virulence genes; *lasB* and *oprL*, in all of *P. aeruginosa* isolates obtained from both human and animal clinical samples collected from the hospital and the veterinary laboratories. With the help of comparative analysis, the prevalence of these genes in the different isolates obtained from different clinical samples, were also investigated.

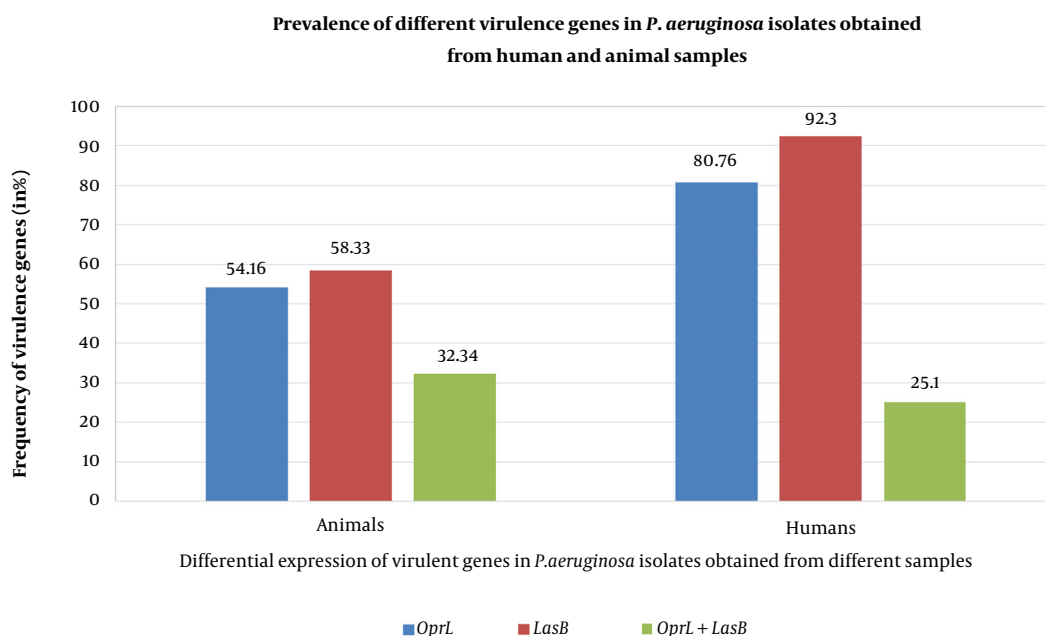
5. Discussion

The ubiquitous and dangerous nature of *P. aeruginosa* can be attributed to genetic adaptability, its ability to acquire antimicrobial resistance AMR, and its capacity to express various virulence factors. Additionally, its capacity to form biofilms adds to its notoriety as a pathogen (26). Numerous studies reported the disease outbreaks caused by *P. aeruginosa* that accredited both the environmental source as well as hospital-acquired infections. *Pseudomonas aeruginosa* is found in environmental sources and is linked to severe infections in immunocompromised hosts, patients with severe burns, and those with surgical injuries (27, 28). This situation is due to the prospective colonization factors such as pili and the alginates and extensive drug resistance strains of *P. aeruginosa* in the hospitals (29). Moreover, this organism is one of the most vital sources of disease in patients suffering from cystic fibrosis. Estimates reveal that more than 80% of cystic fibrosis patients are infected with *P. Aeruginosa* (30). *Pseudomonas aeruginosa* can consume a wide range of organic materials as energy sources and hence it can exist in the environment over an extended period (31). So the exact quick detection of *P. aeruginosa* and the information about its susceptibility profile is of great importance.

Pseudomonas aeruginosa draws interest as a dreadful pathogen for consumer health of a variety of diseases in humans as well as from the food obtained from animals and is a source of multi-drug resistant characters that are interchangeable in pathogens of humans and animals. One of the major struggles tackling the world is resistance to antibiotics. Hence it is vital to identify *P. aeruginosa* specifically and rapidly and also recognize the susceptibility patterns of *P. aeruginosa* strains. This may evade unnecessary treatments with less effective antibiotic and prevents the development of pathogens that are resistant to antibiotics (32). Pathogens use the fabrication of virulence factors during early colonization and acute infection as a survival strategy to evade the host's immune system. This is an important mechanism that allows the pathogen to persist in the host. A large number of the virulence traits and the cell-associated

Table 2. The Distribution of *Pseudomonas aeruginosa* Isolates Among Diverse Specimens

Sl. No.	Specimens	Number of <i>P. aeruginosa</i> Isolates; No. (%)
1	Animal samples (60)	24 (40)
2	Human samples (60)	52 (86)

**Figure 1.** Frequency of the virulence genes (*oprL*, *lasB* and *oprL + lasB*) among *Pseudomonas aeruginosa* isolates obtained from different samples

or secreted compounds of both low and high molecular weights are identified as crucial in initiating infections caused by *P. aeruginosa* (33, 34). While such virulence traits and cell-associated or secreted compounds play a significant role in promoting growth and persistence, they can also cause severe damage to host tissues and weaken immune responses (35). Earlier studies used as a basis to screen various virulence genes in the *P. aeruginosa* isolates obtained from the different samples. Billard-Pomare and coworkers' study showed the real-time PCR of the *oprL* gene has a higher specificity and is a more suitable one than the culture for demonstrating bacterial colonization (36). Similarly, another study compared the efficacy of PCR and traditional culture for colonization determination and concluded that PCR has elevated specificity as well as sensitivity in comparison to the culture (37). In 2014, another study proved that some sputum samples tested positive by the PCR polymerase chain reaction analysis, while their cultures were found negative. Polymerase chain reaction samples become positive prior in comparison with the culture examinations in most

of the cases as the samples transformed to positive at some stage in the period of hospitalization. Thus, they suggested that the PCR method has adequate sensitivity as well as specificity to distinguish colonized patients (38).

Of the virulence genes studied in this study, the *oprL* gene along with maintenance of bacterial cell integrity recently has been found to play a significant role in protecting bacterial cells from oxidative stress and also play a crucial role in conferring resistance against different antiseptics and antibiotics (13-15, 24). Also, molecular identification of the *oprL* gene with PCR provides a rapid and reliable method to identify *P. aeruginosa* strains (39). Another virulence gene studied in *Pseudomonas aeruginosa*; *lasB* encodes for the most important protease an extracellular collagenase (an elastase enzyme). It contributes to the bacterial virulence mechanism by cleaving the components like elastin or collagen of the host tissues along with disruption of the cellular junctions to cause host tissue damage aiding bacterial invasion. It can break down immunoglobulins G and A and is also involved in the destruction of fibronectin,

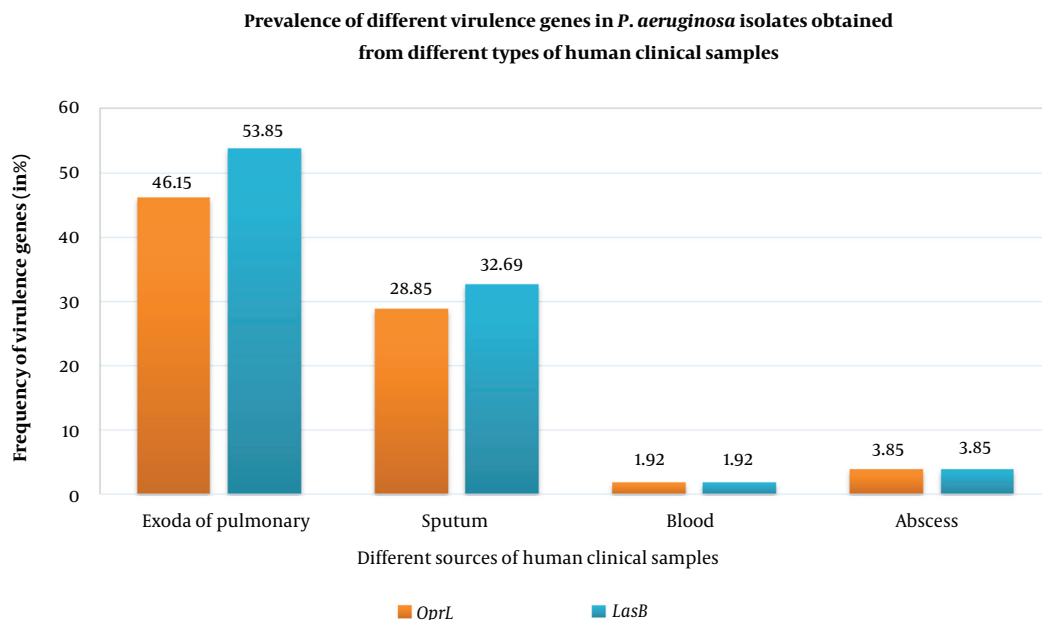


Figure 2. Presence of virulence genes in *Pseudomonas aeruginosa* isolates obtained from different human clinical samples

which can expose ligands for bacterial attachment (40, 41). Also, some studies showed its role in immune modulation along with another protease via inhibition of flagellin-mediated immune system recognition and detection. The presence of the *lasB* gene in *P. aeruginosa* isolates isolated from different environments and clinical samples, indicates its significance in the survival of the bacteria in diverse settings. *lasB* mutation reduces the invasion of *P. aeruginosa* and so molecular detection of this gene also helps to identify *P. aeruginosa* (24, 40, 42, 43).

So in the current study, overall 76 isolates of *P. aeruginosa* were cultured for the occurrence of the specific virulence genes, *oprL* and *lasB*. Among 24 animal sample isolates, the *oprL* genes were detected in 54.16% and *lasB* genes in 58.33%. The coexistence of both virulence genes was observed in 32.34% of animal sample isolates. On the other hand, 80.76% of human sample isolates expressed *oprL* genes and 92.30% showed the presence of *lasB* genes. 25.10% of human sample isolates showed the coexistence of both *oprL* and *lasB* genes. Pathogenicity associated with *P. aeruginosa* is obviously multifactorial. According to one study, *oprL* was promised in AMR antibiotic resistance along with bio-film formation directs for numerous troubles regarding the presence of *P. aeruginosa* infections can complicate the management and thus make them difficult to treat (44). In the current study, several isolates detected showed the presence of *lasB* gene and results of this study are in accordance with

the earlier studies (45). Our study results suggested that all strains isolated from either human or animal samples harboured at least one of the virulence genes being tested.

The frequency of *P. aeruginosa* and expression of its virulence genes depend upon different factors like; the nature of its environment and the extent to which it contaminates as well as the immune response of the patients and also the strain virulence (46). Some strains exhibit improved adaptation to certain conditions originating in the infectious sites which may come back to the vast geographical and environmental sources due to differences in distribution of virulence genes. The absence of virulence factor genes in the strain is attributed to the fact that *P. aeruginosa* infection can result in genome reduction, which increases its ability to adhere host (47). Differences in distribution of virulence factor genes within the populations suggest that certain *P. aeruginosa* strains may be better adapted to specific circumstances present in certain sites of infection (48).

To sum up, the combination of *oprL* and *lasB* genes in PCR provides a reliable method for the identification of *P. aeruginosa*. The presence or absence of different virulence genes among the *P. aeruginosa* isolates suggests that they are associated with varying levels of inherent virulence and pathogenicity, which may have different impacts on the disease progression and outcome. Moreover, *P. aeruginosa* strains; isolated from veterinary samples are studied occasionally. Since animals and animal-related

food products are part of daily human lives, it potentially increases the risk of transfer of pathogenic *P. aeruginosa* strains between them. Hence, a significant advantage of our study was the collection of strains obtained from both human and veterinary samples sequentially, enabling us to determine the prevalence of virulence factor genes, *oprL* and *lasB*. Thus, data from our study can be useful for the health care workers as well as for the people working in veterinary areas to develop the proper infection control measurements and to set up a surveillance system to minimise the spread of pathogenic *P. aeruginosa* isolates.

Footnotes

Authors' Contribution: Study concept and design: Ciamak Ghazaei; analysis and interpretation of data: Ciamak Ghazaei; drafting of the manuscript: Ciamak Ghazaei.

Conflict of Interests: There is no conflict of interest.

Data Availability: The data presented in this study are openly available for readers.

Funding/Support: There is no funding for this article.

References

- Kang CI, Kim SH, Kim HB, Park SW, Choe YJ, Oh MD, et al. Pseudomonas aeruginosa bacteremia: risk factors for mortality and influence of delayed receipt of effective antimicrobial therapy on clinical outcome. *Clin Infect Dis*. 2003;37(6):745-51. [PubMed ID: 12955633]. <https://doi.org/10.1086/377200>.
- Odumosu BT, Adeniyi BA, Chandra R. Analysis of integrons and associated gene cassettes in clinical isolates of multidrug resistant Pseudomonas aeruginosa from Southwest Nigeria. *Ann Clin Microbiol Antimicrob*. 2013;12:29. [PubMed ID: 24143920]. [PubMed Central ID: PMC3842740]. <https://doi.org/10.1186/1476-0711-12-29>.
- Whiteley M, Bangera MG, Bumgarner RE, Parsek MR, Teitzel GM, Lory S, et al. Gene expression in Pseudomonas aeruginosa biofilms. *Nature*. 2001;413(6858):860-4. [PubMed ID: 11677611]. <https://doi.org/10.1038/35101627>.
- Canton R, Cobos N, de Gracia J, Baquero F, Honorato J, Gartner S, et al. Antimicrobial therapy for pulmonary pathogenic colonisation and infection by Pseudomonas aeruginosa in cystic fibrosis patients. *Clin Microbiol Infect*. 2005;11(9):690-703. [PubMed ID: 16104983]. <https://doi.org/10.1111/j.1469-0691.2005.01217.x>.
- Hoge R, Pelzer A, Rosenau F, Wilhelm S. "Weapons of a pathogen: Proteases and their role in virulence of Pseudomonas aeruginosa". *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology*. Microbial biotechnology; 2010. p. 383-95.
- Nanvazadeh F, Khosravi AD, Zolfaghari MR, Parhizgari N. Genotyping of Pseudomonas aeruginosa strains isolated from burn patients by RAPD-PCR. *Burns*. 2013;39(7):1409-13. [PubMed ID: 23773789]. <https://doi.org/10.1016/j.burns.2013.03.008>.
- Balasubramanian D, Schnepfer L, Kumari H, Mathee K. A dynamic and intricate regulatory network determines Pseudomonas aeruginosa virulence. *Nucleic Acids Res*. 2013;41(1):1-20. [PubMed ID: 23143271]. [PubMed Central ID: PMC3592444]. <https://doi.org/10.1093/nar/gks1039>.
- Khatab MA, Nour MS, Elsheshtawy NM. Genetic Identification of Pseudomonas aeruginosa Virulence Genes among Different Isolates. *J Microb Biochem Technol*. 2015;7:274-7.
- Haghi F, Zeighami H, Monazami A, Toutouchi F, Nazaralian S, Naderi G. Diversity of virulence genes in multidrug resistant Pseudomonas aeruginosa isolated from burn wound infections. *Microb Pathog*. 2018;115:251-6. [PubMed ID: 29273509]. <https://doi.org/10.1016/j.micpath.2017.12.052>.
- Fernandez L, Breidenstein EB, Hancock RE. Creeping baselines and adaptive resistance to antibiotics. *Drug Resist Updat*. 2011;14(1):1-21. [PubMed ID: 21288762]. <https://doi.org/10.1016/j.drugp.2011.01.001>.
- Wong A, Rodrigue N, Kassen R. Genomics of adaptation during experimental evolution of the opportunistic pathogen Pseudomonas aeruginosa. *PLoS Genet*. 2012;8(9): e1002928. [PubMed ID: 23028345]. [PubMed Central ID: PMC3441735]. <https://doi.org/10.1371/journal.pgen.1002928>.
- Wessel AK, Liew J, Kwon T, Marcotte EM, Whiteley M. Role of Pseudomonas aeruginosa peptidoglycan-associated outer membrane proteins in vesicle formation. *J Bacteriol*. 2013;195(2):213-9. [PubMed ID: 23123904]. [PubMed Central ID: PMC3553829]. <https://doi.org/10.1128/JB.01253-12>.
- Rodriguez-Herva JJ, Ramos-Gonzalez MI, Ramos JL. The Pseudomonas putida peptidoglycan-associated outer membrane lipoprotein is involved in maintenance of the integrity of the cell envelope. *J Bacteriol*. 1996;178(6):1699-706. [PubMed ID: 8626299]. [PubMed Central ID: PMC177856]. <https://doi.org/10.1128/jb.178.6.1699-1706.1996>.
- Llamas MA, Rodriguez-Herva JJ, Hancock RE, Bitter W, Tommassen J, Ramos JL. Role of Pseudomonas putida tol-*oprL* gene products in uptake of solutes through the cytoplasmic membrane. *J Bacteriol*. 2003;185(16):4707-16. [PubMed ID: 12896989]. [PubMed Central ID: PMC166457]. <https://doi.org/10.1128/JB.185.16.4707-4716.2003>.
- Panmanee W, Gomez F, Witte D, Pancholi V, Britigan BE, Hassett DJ. The peptidoglycan-associated lipoprotein OprL helps protect a Pseudomonas aeruginosa mutant devoid of the transactivator OxyR from hydrogen peroxide-mediated killing during planktonic and biofilm culture. *J Bacteriol*. 2008;190(10):3658-69. [PubMed ID: 18310335]. [PubMed Central ID: PMC2395008]. <https://doi.org/10.1128/JB.00022-08>.
- Dulon S, Leduc D, Cottrell GS, D'Alayer J, Hansen KK, Bunnett NW, et al. Pseudomonas aeruginosa elastase disables proteinase-activated receptor 2 in respiratory epithelial cells. *Am J Respir Cell Mol Biol*. 2005;32(5):411-9. [PubMed ID: 15705968]. <https://doi.org/10.1165/rcmb.2004-0274OC>.
- Dandachi I, Sokhn ES, Dahdouh EA, Azar E, El-Bazzal B, Rolain JM, et al. Prevalence and characterization of multi-drug-resistant gram-negative bacilli isolated from Lebanese poultry: A nationwide study. *Front Microbiol*. 2018;9:550. [PubMed ID: 29628921]. [PubMed Central ID: PMC5876231]. <https://doi.org/10.3389/fmicb.2018.00550>.
- Filiatrault MJ, Picardo KF, Ngai H, Passador L, Iglewski BH. Identification of Pseudomonas aeruginosa genes involved in virulence and anaerobic growth. *Infect Immun*. 2006;74(7):4237-45. [PubMed ID: 16790798]. [PubMed Central ID: PMC1489737]. <https://doi.org/10.1128/IAI.02014-05>.
- Matar GM. Editorial: Pseudomonas and acinetobacter: From drug resistance to pathogenesis. *Front Cell Infect Microbiol*. 2018;8:68. [PubMed ID: 29594065]. [PubMed Central ID: PMC5859085]. <https://doi.org/10.3389/fcimb.2018.00068>.
- Sawa T, Shimizu M, Moriyama K, Wiener-Kronish JP. Association between Pseudomonas aeruginosa type III secretion, antibiotic resistance, and clinical outcome: a review. *Crit Care*. 2014;18(6):668. [PubMed ID: 25672496]. [PubMed Central ID: PMC4331484]. <https://doi.org/10.1186/s13054-014-0668-9>.
- Shukla S, Mishra P. Pseudomonas aeruginosa infection in broiler chicks in Jabalpur. *Int J Extensive Res*. 2015;6:37-9.

22. Lennette EH, Balows A, Hausler WJ, Shadomy HJ, American Society for M. *Manual of clinical microbiology*. 4th ed ed. Washington, D.C: American Society for Microbiology Washington, D.C; 1985. p. XVI-1149 p. : il. 29 cm.
23. Nikbin VS, Aslani MM, Sharafi Z, Hashemipour M, Shahcheraghi F, Ebrahimipour GH. Molecular identification and detection of virulence genes among *Pseudomonas aeruginosa* isolated from different infectious origins. *Iran J Microbiol*. 2012;**4**(3):118-23. [PubMed ID: 23066485]. [PubMed Central ID: PMC3465536].
24. Xu J, Moore JE, Murphy PG, Millar BC, Elborn JS. Early detection of *Pseudomonas aeruginosa*-comparison of conventional versus molecular (PCR) detection directly from adult patients with cystic fibrosis (CF). *Ann Clin Microbiol Antimicrob*. 2004;**3**:21. [PubMed ID: 15496232]. [PubMed Central ID: PMC529303]. <https://doi.org/10.1186/1476-0711-3-21>.
25. Zhu H, Bandara R, Conibear TC, Thuruthyl SJ, Rice SA, Kjelleberg S, et al. *Pseudomonas aeruginosa* with lasI quorum-sensing deficiency during corneal infection. *Invest Ophthalmol Vis Sci*. 2004;**45**(6):1897-903. [PubMed ID: 15161855]. <https://doi.org/10.1167/iov.03-0980>.
26. Silva LV, Galdino AC, Nunes AP, dos Santos KR, Moreira BM, Cacci LC, et al. Virulence attributes in Brazilian clinical isolates of *Pseudomonas aeruginosa*. *Int J Med Microbiol*. 2014;**304**(8):990-1000. [PubMed ID: 25127423]. <https://doi.org/10.1016/j.ijmm.2014.07.001>.
27. Gomes MZ, Oliveira RV, Machado CR, Conceição Mde S, Souza CV, Lourenço MC, et al. Factors associated with epidemic multiresistant *Pseudomonas aeruginosa* infections in a hospital with AIDS-predominant admissions. *Braz J Infect Dis*. 2012;**16**(3):219-25. [PubMed ID: 22729187]. <https://doi.org/10.1590/s1413-86702012000300001>.
28. Hickey C, Schaible B, Nguyen S, Hurley D, Sri Kumar S, Fanning S, et al. Increased virulence of bloodstream over peripheral isolates of *P. Aeruginosa* identified through post-transcriptional regulation of virulence factors. *Front Cell Infect Microbiol*. 2018;**8**:357. [PubMed ID: 30416988]. [PubMed Central ID: PMC6212473]. <https://doi.org/10.3389/fcimb.2018.00357>.
29. Feizabadi MM, Majnooni A, Nomanpour B, Fatolahzadeh B, Raji N, Delfani S, et al. Direct detection of *Pseudomonas aeruginosa* from patients with healthcare associated pneumonia by real time PCR. *Infect Genet Evol*. 2010;**10**(8):1247-51. [PubMed ID: 20728580]. <https://doi.org/10.1016/j.meegid.2010.08.008>.
30. Saiman L, Siegel J. Infection control in cystic fibrosis. *Clin Microbiol Rev*. 2004;**17**(1):57-71. [PubMed ID: 14726455]. [PubMed Central ID: PMC321464]. <https://doi.org/10.1128/CMR.17.1.57-71.2004>.
31. Tumeo E, Gbaguidi-Haore H, Patry I, Bertrand X, Thouverez M, Talon D. Are antibiotic-resistant *Pseudomonas aeruginosa* isolated from hospitalised patients recovered in the hospital effluents? *Int J Hyg Environ Health*. 2008;**211**(1-2):200-4. [PubMed ID: 17459769]. <https://doi.org/10.1016/j.ijheh.2007.02.010>.
32. Hamisi Z, Tuntufye HN, Shahada F. Antimicrobial resistance Phenotypes of *Escherichia coli* isolated from tropical free range chickens. *Int J Sci Res*. 2014;**3**(9):34-7.
33. Strateva T. Contribution of an arsenal of virulence factors to pathogenesis of *Pseudomonas aeruginosa* infections. *Ann Microbiol*. 2011;**61**. <https://doi.org/10.1007/s13213-011-0273-y>.
34. Foulkes DM, McLean K, Haneef AS, Fernig DG, Winstanley C, Berry N, et al. *Pseudomonas aeruginosa* toxin exoA as a therapeutic target in the treatment of bacterial infections. *Microorganisms*. 2019;**7**(12). [PubMed ID: 31888268]. [PubMed Central ID: PMC6955817]. <https://doi.org/10.3390/microorganisms7120707>.
35. Azam MW, Khan AU. Updates on the pathogenicity status of *Pseudomonas aeruginosa*. *Drug Discov Today*. 2019;**24**(1):350-9. [PubMed ID: 30036575]. <https://doi.org/10.1016/j.drudis.2018.07.003>.
36. Billard-Pomares T, Herwegh S, Wizla-Derambure N, Turck D, Courcol R, Husson MO. Application of quantitative PCR to the diagnosis and monitoring of *Pseudomonas aeruginosa* colonization in 5-18-year-old cystic fibrosis patients. *J Med Microbiol*. 2011;**60**(Pt 2):157-61. [PubMed ID: 20947668]. <https://doi.org/10.1099/jmm.0.023838-0>.
37. Logan C, Habington A, Lennon G, Cronin F, O'Sullivan N. Evaluation of the efficacy of real-time polymerase chain reaction for the routine early detection of *Pseudomonas aeruginosa* in cystic fibrosis sputum and throat swab specimens. *Diagn Microbiol Infect Dis*. 2010;**68**(4):358-65. [PubMed ID: 20884156]. <https://doi.org/10.1016/j.diagmicrobio.2010.07.012>.
38. Rashno Tae S, Khansarinejad B, Abtahi H, Najafimosleh M, Ghaznavi-Rad E. Detection of algD, oprL and exoA genes by new specific primers as an efficient, rapid and accurate procedure for direct diagnosis of *Pseudomonas aeruginosa* strains in clinical samples. *Jundishapur J Microbiol*. 2014;**7**(10). e13583. [PubMed ID: 25632330]. [PubMed Central ID: PMC4295320]. <https://doi.org/10.5812/jjm.13583>.
39. Algammal AM, Mabrok M, Sivaramasamy E, Youssef FM, Atwa MH, El-Kholy AW, et al. Emerging MDR-*Pseudomonas aeruginosa* in fish commonly harbor oprL and toxA virulence genes and bla(TEM), bla(CTX-M), and tetA antibiotic-resistance genes. *Sci Rep*. 2020;**10**(1):15961. [PubMed ID: 32994450]. [PubMed Central ID: PMC7524749]. <https://doi.org/10.1038/s41598-020-72264-4>.
40. Lomholt JA, Poulsen K, Kilian M. Epidemic population structure of *Pseudomonas aeruginosa*: evidence for a clone that is pathogenic to the eye and that has a distinct combination of virulence factors. *Infect Immun*. 2001;**69**(10):6284-95. [PubMed ID: 11553572]. [PubMed Central ID: PMC98763]. <https://doi.org/10.1128/IAI.69.10.6284-6295.2001>.
41. Tumbarello M, De Pascale G, Trecarichi EM, Spanu T, Antonicelli F, Maviglia R, et al. Clinical outcomes of *Pseudomonas aeruginosa* pneumonia in intensive care unit patients. *Intensive Care Med*. 2013;**39**(4):682-92. [PubMed ID: 23370828]. <https://doi.org/10.1007/s00134-013-2828-9>.
42. Cowell BA, Twining SS, Hobden JA, Kwong MSF, Fleiszig SMJ. Mutation of lasA and lasB reduces *Pseudomonas aeruginosa* invasion of epithelial cells. *Microbiology (Reading)*. 2003;**149**(Pt 8):2291-9. [PubMed ID: 12904569]. <https://doi.org/10.1099/mic.0.26280-0>.
43. Casilag F, Lorenz A, Krueger J, Klawonn F, Weiss S, Haussler S. The lasB elastase of *Pseudomonas aeruginosa* acts in concert with alkaline protease aprA to prevent flagellin-mediated immune recognition. *Infect Immun*. 2016;**84**(1):162-71. [PubMed ID: 26502908]. [PubMed Central ID: PMC4693985]. <https://doi.org/10.1128/IAI.00939-15>.
44. Moghaddam MM, Khodi S, Mirhosseini AF. Quorum sensing in bacteria and a glance on *Pseudomonas aeruginosa*. *Clin Microbiol*. 2014;**3**(4).
45. Nicas TI, Iglewski BH. Production of elastase and other exoproducts by environmental isolates of *Pseudomonas aeruginosa*. *J Clin Microbiol*. 1986;**23**(5):967-9. [PubMed ID: 3086372]. [PubMed Central ID: PMC268764]. <https://doi.org/10.1128/jcm.23.5.967-969.1986>.
46. Aljebory I. PCR detection of some virulence genes of *Pseudomonas aeruginosa* in Kirkuk city, Iraq. *J Pharm Sci Res*. 2018;**10**:1068-71.
47. Lee DG, Urbach JM, Wu G, Liberati NT, Feinbaum RL, Miyata S, et al. Genomic analysis reveals that *Pseudomonas aeruginosa* virulence is combinatorial. *Genome Biol*. 2006;**7**(10):R90. [PubMed ID: 17038190]. [PubMed Central ID: PMC1794565]. <https://doi.org/10.1186/gb-2006-7-10-r90>.
48. Lanotte P, Watt S, Mereghetti L, Dartiguelongue N, Rastegar-Lari A, Goudeau A, et al. Genetic features of *Pseudomonas aeruginosa* isolates from cystic fibrosis patients compared with those of isolates from other origins. *J Med Microbiol*. 2004;**53**(Pt 1):73-81. [PubMed ID: 14663109]. <https://doi.org/10.1099/jmm.0.05324-0>.