folia medica

Folia Medica 66(3):361-369
DOI: 10.3897/folmed.66.e124561

Original Article

Molecular Investigation of *LasA*, *LasB*, and *PIV* Genes in Clinical Isolates of *Pseudomonas aeruginosa* in Mazandaran Province, North Iran

Sahar Mohammadi Baladezaee[1], Mehrdad Gholami[2], Elham Amiri[3], Hamid Reza Goli[2,4]

[1] *Sana Institute of Higher Education, Sari, Iran*
[2] *Department of Medical Microbiology and Virology, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*
[3] *Student Research Committee, Mazandaran University of Medical Sciences, Sari, Iran*
[4] *Molecular and Cell Biology Research Center, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*

Corresponding author: Hamid Reza Goli, Department of Medical Microbiology and Virology, Faculty of Medicine, Mazandaran University of Medical Sciences, Farah Abad Blvd., Khazar square, Sari, Mazandaran, Iran; Email: goli59@gmail.com; Tel.: +981133543081

Received: 2 April 2024 ♦ **Accepted:** 4 June 2024 ♦ **Published:** 30 June 2024

Citation: Baladezaee AM, Gholami M, Amiri E, Goli HR. Molecular investigation of *LasA*, *LasB*, and *PIV* genes in clinical isolates of *Pseudomonas aeruginosa* in Mazandaran Province, North Iran. Folia Med (Plovdiv) 2024;66(3):361-369. doi: 10.3897/folmed.66.e124561.

Abstract

Aims: *Pseudomonas aeruginosa* plays an important role in hospital infections caused by several virulence factors, such as elastase and proteases. This study aimed to evaluate the prevalence of *LasA, LasB,* and *PIV* genes, encoding these enzymes, in clinical isolates of *P. aeruginosa*.

Materials and methods: One hundred clinical isolates were collected from patients admitted to educational and therapeutic hospitals of Mazandaran Province, North Iran. The isolates were identified by the standard microbiological and biochemical tests. The bacterial DNA was extracted by the alkaline lysis method, and the presence of relevant genes was detected using the PCR method. The data were analyzed using SPSS v. 23 and the chi-square test. A p-value <0.05 was considered statistically significant.

Results: In 100 clinical isolates of *P. aeruginosa*, the *LasA*, *LasB,* and *PIV* genes were presented with a frequency of 97%, 96%, and 97%, respectively. Of the total number of samples, 39 patients were female and 61 were male. Also, the majority of the patients were between 61 and 70 years old.

Conclusion: *LasA*, *LasB*, and *PIV* are highly prevalent in clinical isolates of *P. aeruginosa*, indicating the importance of these genes as key virulence factors in *P. aeruginosa* pathogenicity in this region.

Keywords

LasA, *LasB*, *PIV, Pseudomonas aeruginosa*

INTRODUCTION

Pseudomonas aeruginosa is a motile opportunistic gram-negative nosocomial bacillus presented in different environments and mammalian tissues.[1,2] This organism is responsible for a considerable rate of hospital infections, including bacteremia, pneumonia, burns, and urinary tract infections, especially in immunocompromised patients.[3] Due to the increasing antibiotic resistance in *P. aeruginosa*, this organism is grouped as a “critical priority” bacteria by the World Health Organization (WHO).[4] This organism can adapt to harsh environments by secreting the different

Copyright by authors. This is an open access article distributed under the terms of the Creative Commons Attribution License (CC-BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

PENSOFT.

pathogenic factors.[5] Most *P. aeruginosa* strains produce one or more extracellular pigments, such as pyoverdine, pyocyanin, pyorubin, and pyomelanin.[6] Some intracellular or extracellular pathogenic factors are essential for the establishment or maintenance of infection through highly complex regulatory circuits and signaling systems. These virulence factors include extracellular proteases, toxins, type III secretion system, cell envelope components, protease IV (*PIV*), elastase A (staphylolysin, *LasA*), elastase B (pseudolysin, *LasB*), alkaline protease (*AprA*), lipase C, phospholipase C, exotoxin A, exoenzyme S, aminopeptidase, and esterase A.[4,7,8]

Among them, elastase A, elastase B, and protease IV produced by *P. aeruginosa* cause the destruction of host tissue and immune components.[4] The elastase A and elastase B enzymes encoded by the *LasA* and *LasB* genes have proteolytic activity damaging the lung and skin tissues.[4] Staphylolysin is a serine protease in the M23 metallopeptidase family and is produced as an inactive precursor, containing signal peptide, propeptide, and mature catalytic domains.[9] Also, it is a limited elastinolytic enzyme and increases the activity of elastase B, resulting in the destruction of elastin in connective tissues, blood vessels, and lung tissue.[4,9] Recently, it has been shown that the expression of elastase A is related to antibiotic resistance in clinical isolates of *P. aeruginosa*.[10] In addition, elastase B is considered a neutral extracellular metalloprotease belonging to the thermolysin M4 family and is controlled by the quorum sensing (QS) system.[11] This enzyme is the most secreted protein by type II secretion system in *P. aeruginosa*.[7] Elastase B hydrolyzes a wide range of substrates, including elastin, collagen, surfactants, mucin, immunoglobulins, cytokines, laminin, fibronectin, vitronectin, and antimicrobial peptides.[4] It is thought that this enzyme can play a significant role in the early stages of infection caused by *P. aeruginosa* by the destruction of the host tissue and the components of the innate immune system.[12] In addition, protease IV (*PIV*), encoded by the *PIV* gene, is a lysyl endopeptidase that cleaves the carboxyl end of lysine-containing peptides.[8] This enzyme destroys many proteins, including immunoglobulin, supplements, fibrinogen, plasminogen, and surfactant proteins, resulting in the host immune depression.[4] *PIV* is recognized as an important pathogenic agent of *P. aeruginosa* in human corneal and pulmonary infections, and a mutation in the *PIV* gene leads to a decrease of bacterial virulence in the cornea.[8]

AIM

Considering the importance of pathogenic and invasive strains of *P. aeruginosa*, and the role of elastase A, elastase B, and protease IV, this study aimed to investigate the prevalence of *LasA*, *LasB,* and *PIV* genes in clinical isolates of *P. aeruginosa*.

MATERIALS AND METHODS

Ethical approval statements and consent to participation

This study was conducted according to the Declarations of Helsinki. All participants signed a written informed consent form. The classifying information of patients was kept secret. The inclusion criteria for determining patients' enrollment included observing a hospital infection after 48 hours, non-repetition of the clinical sample, and positive microbial culture results for *P. aeruginosa*. Also, patients who did not fill out the informed consent form or decided to withdraw from the study, patients infected with other bacteria, and patients who entered from other hospitals to our centers were excluded from the study. In addition, the Iran National Committee for Ethics in Biomedical Research approved this study with IR.MAZUMS.REC.1397.368 National Ethical Code.

Sample collection and bacterial identification

For this descriptive-analytical study, 100 non-duplicated clinical isolates of *P. aeruginosa* were estimated according to the following formula, where **n** is the sample size, **z** – the value of standard normal distribution (Z-statistic) at 95% confidence level (z=1.96), **p** is the prevalence of virulence genes in *P. aeruginosa* (p=89%)[13], and **d** is the maximum error rate=0.061.

$$n = \frac{z_{\alpha/2}^2 \times p(1-P)}{d^2}$$

The *P. aeruginosa* isolates were collected from different clinical samples, including blood, catheters, eye secretion, wound, respiratory, stool, and urine. The samples were collected from patients admitted to educational and therapeutic hospitals affiliated with the Mazandaran University of Medical Sciences, North Iran, from January to December 2022. The isolates were identified by the standard microbiological and biochemical tests, such as gram staining, the colony odor, size, shape and pigment, cultivation on Triple Sugar Iron agar, motility, catalase and oxidase test, OF (Oxidation-Fermentation) test, growth at 42°C and on cetrimide agar.[14]

Genomic DNA extraction and PCR

DNA extraction of the isolates was done using an alkaline lysis buffer as previously described.[15] Briefly, a lysing buffer was prepared by suspending 0.5 g of sodium dodecyl sulfate (SDS) and 0.4 g of NaOH in 200 mL of sterile deionized water. Then, several colonies of bacteria were dissolved in 20 µL of this buffer and heated at 95°C for 10 minutes. Next, the suspension was centrifuged at 13,000 rpm for one minute, and the supernatant was discarded. Finally, 180 µL

of the sterile deionized water was added to the microtube. The quality of the extracted genomic DNAs was checked by the measurement of optical density (OD) using a NanoDrop (ND 1000, USA) and electrophoresis on a 1.5% agarose gel (Sigma, Germany).

As shown in **Table 1**, the specific primers for the detection of the studied genes were chosen from the previously conducted research. The PCR reactions were done at a final volume of 15 μL. The amounts of the PCR materials and the amplification conditions and temperatures are shown in **Table 2**. The PCR products were electrophoresed on a 1% agarose gel (Sigma) containing the safe stain (SinaClon, Iran), and were visualized using a Gel Documentation system (UVITEC Cambridge, UK).

Statistical analysis

The data collected from the results of this study were imported to SPSS v. 23. Then, the chi-square test was used for statistical analysis of the data. A *p*-value of less than 0.05 was considered statistically significant.

RESULTS

One hundred *P. aeruginosa* clinical isolates in this study were collected from patients hospitalized in BuAli Sina (n=12), Fatemeh Al-Zahra (n=8), Imam Khomeini (n=35), Razi (n=31), and Zare (n=14) educational and therapeutic hospitals which are the pediatric, heart, general, infectious, and burn centers, respectively, in North Iran. In addition, 42 isolates were collected from women and 58 from men. The age range of patients was from 1 to 90 years old, while the most isolates were obtained from 61-70 (25%) and 51-60 (17%) year-old patients.

Fig. 1 shows the results of electrophoresis of the studied genes' products in the PCR method. The prevalence of *LasA*, *LasB*, and *PIV* genes in 100 clinical isolates of *P. aeruginosa* were 97%, 96%, and 95%, respectively. The prevalence of *LasA*, *LasB*, and *PIV* genes in the five hospitals in this study is shown in **Fig. 2**.

The assessment of the *LasA*, *LasB*, and *PIV* virulence genes of *P. aeruginosa* clinical isolates based on hospital wards and the type of clinical samples are shown in **Tables 3, 4**. There was a significant relation between the presence of the *LasB* gene and the sample type (*p*=0.00). However, we did not find any correlation between the presence of virulence genes and other studied variables.

Also, 2, 2, 94, and 2 isolates in this study produced brown, blue-green, green, and yellow pigments, respectively. Among them, all isolates with brown and blue-green pigment were carrying the *LasA*, *LasB*, and *PIV* genes. However, among 94 isolates with the green pigment, 92 (97.87%), 90 (95.74%), and 90 (95.74%) isolates contained the *LasA*, *LasB*, and *PIV* genes, respectively. In addition, among 2 isolates with the yellow pigment, 1, 2, and 1 isolates contained the *LasA*, *LasB*, and *PIV* genes, respectively.

In addition, **Table 5** shows the relationship between the age range of the patients and the presence of virulence genes, while there was no statistical significance.

On the other hand, among 61 males included in this study, 59 (96.72%), 58 (95.08%), and 59 (96.72%) patients had the *P. aeruginosa* isolates containing the *LasA*, *LasB*, and *PIV* genes, respectively. Also, 38 (97.43%), 38 (97.43%), and 36 (92.30%) *P. aeruginosa* carrying the *LasA*, *LasB*, and *PIV* genes were isolated from 39 females, respectively.

Table 1. The sequences of the desired primers in this study

Genes	5′ to 3′ sequence of the primers	Product Size (bp)	References
LasA	F: GCAGCACAAAAGATCCC R: GAAATGCAGGTGCGGTC	1075	[16]
LasB	F:ACACAATACATATCAACTTCGC R: AGTGTGTTTAGAATGGTGATC	284	[17]
PIV	F: GCCGGCTACCGCGACGGCTTC R: TCAGGGCGCGAAGTAGCGGGAG	756	[18]

Table 2. The amounts of the materials and the amplification conditions in this study

Genes	The amounts of materials (μL)				The amplification conditions					
	M.M	Each primers	DNA	D.W	Initial denaturation	Denaturation	Annealing	Extension	Final extension	Cycles
LasA	7.5	0.5	1	5.5	95°C (5 min)	95°C (40 sec)	60°C (35 sec)	72°C (1 min)	72°C (10 min)	34
LasB	7.5	1.5	3	1.5	95°C (10 min)	95°C (30 sec)	64°C (30 sec)	72°C (30 sec)	72°C (10 min)	35
PIV	7.5	0.25	0.5	6.5	95°C (5 min)	95°C (30 sec)	68°C (35 sec)	72°C (1 min)	72°C (10 min)	34

M.M: Master Mix; D.W: Distilled Water

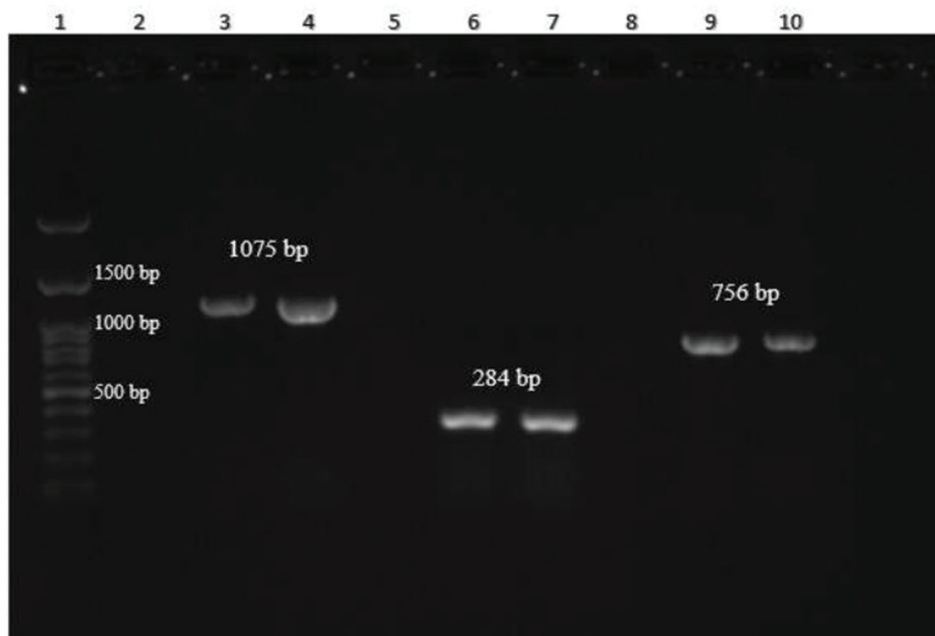

Figure 1. Electrophoresis result of PCR product of three genes *LasA*, *LasB* and *PIV*. **Line 1**, DNA Ladder 100 bp plus; **Line 2**, negative control (Master Mix without DNA) for the *LasA* gene; **Line 3**, positive control of the *LasA* gene; **Line 4**, positive sample carrying the *LasA* gene (1075 bp); **Line 5**, negative control for the *LasB* gene; **Line 6**, positive control of the *LasB* gene (284 bp); **Line 7**, positive sample carrying the *LasB* gene; **Line 8**, negative control for the *PIV* gene; **Line 9**, positive control of the *PIV* gene; **Line 10**, positive sample carrying the *PIV* gene (756 bp).

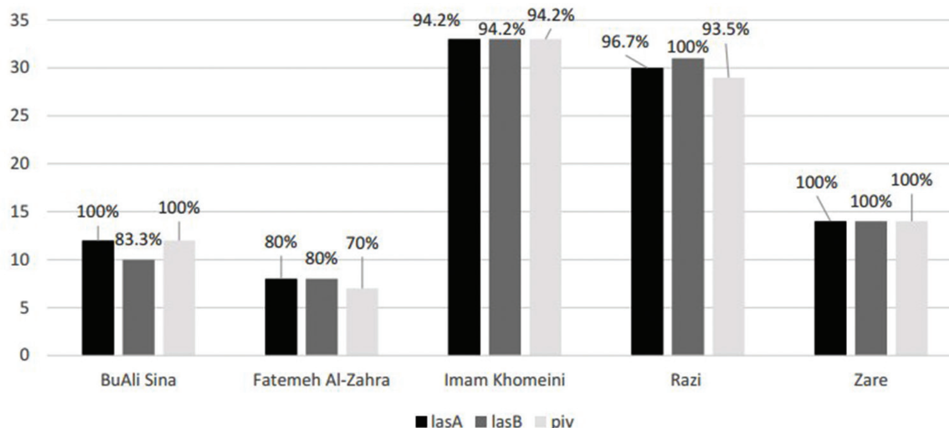

Figure 2. The prevalence of the *LasA*, *LasB*, and *PIV* genes in different hospitals.

DISCUSSION

Pseudomonas aeruginosa is a ubiquitous Gram-negative opportunistic pathogen with ecological and health significance.[19] This organism is one of the main causes of nosocomial infections, especially in immunocompromised patients, such as cystic fibrosis.[20] Some infections included bacteremia, pneumonia, burns, lung, and urinary tract infections.[3] This pathogen can adapt to environmental changes, is quickly resistant to antibiotics, and produces various pathogenic factors.[2] Recurrent drug resistance and continued colonization of bacteria have led to the difficulty of the treatment and eradication of *P. aeruginosa*.[1] This organism has a wide range of virulence factors, including LPS, flagella, and pili (for adhesion/colonization), secretion systems (for secretion of effectors), enzymes and toxins (for damaging), quorum sensing system (for bacterial communication), and biofilm (for drug resistance).[1] In particular, three extracellular proteases in *P. aeruginosa*, including elastase A (*LasA*), elastase B (*LasB*), and protease IV (*PIV*), are the significant virulence factors, destroying host tissue and immune components.[20] The *LasA* and *LasB* genes encode elastases with proteolytic activity, causing damage to lung tissue and skin. Therefore, the expression of these genes is related to the pathogenicity of species, especially in patients with burned skin.[21] The elastinolytic activity of *LasA* in-

Table 3. Number (%) of the *LasA*, *LasB*, and *PIV* virulence genes in clinical isolates of *P. aeruginosa* based on hospital departments

Hospital wards (number)	*LasA*		*LasB*		*PIV*	
	Yes	No	Yes	No	Yes	No
ICU (n=49)	47 (95.91)	2 (4.08)	48 (97.95)	1 (2.04)	46 (93.87)	3 (6.12)
Burn (n=6)	6 (100)	-	6 (100)	-	6 (100)	-
BICU (n=3)	2 (66.66)	1 (33.33)	3 (100)	-	3 (100)	-
NICU (n=1)	1 (100)	-	1 (100)	-	1 (100)	-
CCU (n=5)	5 (100)	-	5 (100)	-	5 (100)	-
Emergency (13)	13 (100)	-	13 (100)	-	12 (93.30)	1 (7.69)
Internal (n=9)	9 (100)	-	8 (88.88)	1 (11.11)	9 (100)	-
Neurology (n=2)	2 (100)	-	2 (100)	-	2 (100)	-
Oncology (n=1)	1 (100)	-	1 (100)	-	1 (100)	-
Surgery (n=6)	6 (100)	-	5 (83.33)	1 (16.66)	5 (83.33)	1 (16.66)
Pediatric (n=5)	5 (100)	-	4 (80)	1 (20)	5 (100)	-
N=100	97	3	96	4	95	5
p-value	0.59		0.12		0.88	

ICU: Intensive care unit; BICU: Burn intensive care unit; NICU: Neonate intensive care unit; CCU: Cardiac care unit.

Table 4. Number (%) of the *LasA*, *LasB*, and *PIV* virulence genes in clinical isolates of *P. aeruginosa* based on sample types

Specimens (number)	*LasA*		*LasB*		*PIV*	
	Yes	No	Yes	No	Yes	No
Blood (n=13)	13 (100)	-	12 (92.3)	1 (7.69)	12 (92.3)	1 (7.69)
Eye secretion (n=2)	2 (100)	-	1 (50)	1 (50)	2 (100)	-
Respiratory tract (n=37)	36 (97.29)	1 (2.70)	36 (97.29)	1 (2.70)	35 (94.59)	2 (5.4)
Stool (n=2)	2 (100)	-	2 (100)	-	1 (50)	1 (50)
Urine (n=26)	25 (96.15)	1 (3.84)	26 (100)	-	25 (96.15)	1 (3.84)
Burn wound (n=20)	19 (95)	1 (5)	19 (95)	1 (5)	20 (100)	-
N=100	97	3	96	4	95	5
p-value	0.99		0.00		0.13	

Table 5. Relationship between the age range of the patients and the presence of virulence genes

Age range in years (number)	*LasA*		*LasB*		*PIV*	
	Yes	No	Yes	No	Yes	No
1-10 (n=11)	11 (100)	-	10 (90.90)	1 (9.09)	11 (100)	-
11-20 (n=6)	6 (100)	-	6 (100)	-	5 (83.33)	1 (16.66)
21-30 (n=7)	7 (100)	-	6 (85.71)	1 (14.28)	7 (100)	-
31-40 (n=13)	12 (92.30)	1 (7.69)	13 (100)	-	13 (100)	-
41-50 (n=12)	12 (100)	-	12 (100)	-	12 (100)	-
51-60 (17)	16 (94.11)	1 (5.88)	15 (88.23)	2 (11.76)	17 (100)	-
61-70 (n=25)	25 (100)	-	25 (100)	-	22 (88)	3 (12)
71-80 (n=7)	6 (85.71)	1 (14.28)	7 (100)	-	6 (85.71)	1 (14.28)
81-90 (n=2)	2 (100)	-	2 (100)	-	2 (100)	-
N=100	97	3	96	4	95	5
p-value	0.59		0.42		0.35	

creases this activity in other proteases, including *LasB*, destroying connective tissue, blood vessels, and lung tissue by *P. aeruginosa*.[4] During the colonization of the organism in cystic fibrosis lung disease, the staphylolytic function of *LasA* has probably been considered an important advantage in *P. aeruginosa* over *Staphylococcus aureus*.[9] Also, the overexpressed *LasA* could be related to antibiotic resistance in *P. aeruginosa*.[10] The *LasB* gene, encoding the *LasB* enzyme, is highly conserved among environmental and clinical strains of *P. aeruginosa* and is present in more than 98% of the strains.[7] Apart from the elastinolytic activity, *LasB* causes the destruction of many proteins in the human immune system and the loss of tight epithelial connections.[4] Also, *LasB* is involved in the activation of proteins derived from bacteria, including flagellin, exotoxins, and other proteases necessary for the pathogenicity of *P. aeruginosa*.[22] According to the study in mouse models of infections, it was concluded that the secretion of *LasB* by clinical isolates of *P. aeruginosa* causes diffuse hemorrhagic alveolar damage.[7] Another study showed that high levels of *LasB* activity in respiratory isolates obtained from intensive care unit patients were associated with increased 30-day mortality.[23] Protease IV also destroys most of the host's immune system proteins and causes corneal and lung infections in humans and invertebrate hosts by *P. aeruginosa*.[8]

We detected that 97%, 96%, and 95% of *P. aeruginosa* clinical isolates in the present study contained the *LasA*, *LasB*, and *PIV* genes, respectively. However, all isolates collected from the burn center were positive for all studied genes, indicating the significance of pathogenesis. Also, the considerable prevalence of these genes was detected in the clinical isolates collected from the pediatric and infectious centers in this region.

De Sousa et al. investigated the frequency of *LasA* and *LasB* virulence genes in *P. aeruginosa* isolated from urinary tract infections (UTIs), while all isolates contained the *LasB* gene and none of them had the *LasA* gene.[24] The frequency of the *LasB* gene was consistent with the results of our study, but in the case of the *LasA* gene, the results were contradictory. Like elastase B, elastase A destroys elastin and affects the epithelial cells.[4] Another research by Gervasoni et al. collected 23 *P. aeruginosa* isolates from the water samples, from which 95.6% were carrying the *LasA* and *LasB* genes.[25] This organism can be a significant threat to human and animal health, as water supply systems can serve as a dissemination source for *P. aeruginosa*.[25] However, despite the different sample types, the prevalence of *LasA* and *LasB* genes in our study was in close agreement with the aforementioned study, indicating the significance of the environmental sources. On the other hand, Camargo et al. investigated the frequency of some virulence and antibiotic resistance genes in 119 *P. aeruginosa* clinical isolates, while all isolates contained the *LasB* gene and only one isolate lacked the *LasA* gene.[26] The slight difference between the abovementioned research and our results can be due to the different sample types; however, both studies showed the significance of these genes in pathogenesis. In addition, Mokhtari et al. conducted a study on 60 human and 60 animal isolates of *P. aeruginosa*, and their results showed a 93.3% and 30% prevalence of the *LasB* gene in human and animal isolates, respectively.[27] The slight discrepancy with our research may be due to the unequal number of samples or the difference in geographical area.

Another study conducted in Egypt on 30 *P. aeruginosa* isolated from the burn, blood, and pulmonary samples showed a 100% frequency of the *LasB* gene, indicating the importance of elastase B for the survival of *P. aeruginosa* in different environments.[28] The discrepancy may be due to the smaller number of samples compared to our study **(Table 4)**. Also, Nikbin et al. conducted a study on 268 *P. aeruginosa* clinical isolates collected from burn, wound, and pulmonary tract infections, and showed a 100% prevalence of the *LasB* gene. It is clearly established that the virulence of *P. aeruginosa* is multifactorial. Elastase B is one of the most important proteases of this Gram-negative bacillus.[29] This enzyme has a high presence in clinical samples related to burn, wound, and lung infections, resulting in elastin and collagen destruction in tight epithelial connections.[4] Engel et al. showed that a mutant *P. aeruginosa* without the *PIV* gene can lose the ability to cause keratitis. Based on the results of the mentioned study, doses of 50 ng to 200 ng of pure PIV could lead to eye epithelial defects within 3 hours. Also, 20 ng of active protease IV or 200 ng of heat-inactivated protease IV had no effect on eye tissue.[30] However, the *PIV* gene was detected in both isolates collected from the eye secretions in our study. Also, Li et al. demonstrated that the three main proteases of *P. aeruginosa*, *LasA*, *LasB*, and *PIV*, are under the control of the quorum sensing (QS) system, and these enzymes activate each other in a cascade manner.[31] This indicates the significant role of *LasB*, as a leader in this group, in the protease-related pathogenesis of *P. aeruginosa*. However, there was nearly no difference in the *LasA*, *LasB*, and *PIV* gene prevalence in the present study.

CONCLUSIONS

Pseudomonas aeruginosa has multiple pathogenic factors and complex signaling systems providing the conditions for pathogenesis. This pathogen can colonize different surfaces and produce several virulence factors. *LasA*, *LasB*, and *PIV* are grouped as significant virulence factors, controlled by QS, sensing *P. aeruginosa*, which affects different proteins. Due to the high frequency of *LasA*, *LasB,* and *PIV* genes in our study, we can conclude that proteases are suitable targets to control the pathogenesis of this organism in patients. Despite the fact that *P. aeruginosa*'s pathogenicity is multifactorial, it is advised that future research look into the expression levels of these proteins in *P. aeruginosa* isolates because of the high prevalence of these enzymes. It is also suggested that research be done on discovering potent substances to block the synthesis or function of these proteins in order to lessen the severity of illness in hospitalized patients.

Limitations

The limitation of this study is the lack of investigation of other important and effective proteases in aggravating the pathogenicity of *Pseudomonas aeruginosa*. Also, we can investigate the expression levels of these enzymes in our isolates in future research.

Ethics

Ethical approval statements and consent for participation

This study was conducted according to the Declarations of Helsinki. A written informed consent form was signed by all participants. The classifying information of patients was kept secret. Moreover, the Iran National Committee for Ethics in Biomedical Research approved this study with IR.MAZUMS.REC.1397.368 national ethical code.

Consent for publication

The authors have all approved the submission of this article. Also, this manuscript has not been published and is not under review. This article is reviewed and approved by all co-authors.

Author contributions

Conceptualization: H.R.G.; Data curation: H.R.G., S.M., E.A., and M.G.; Formal analysis: S.M. and M.G.; Investigation: H.R.G., S.M., E.A., and M.G.; Methodology: H.R.G., E.A., and S.M.; Project administration: H.R.G.; Software: H.R.G. and M.G.; Supervision: H.R.G.; Validation: H.R.G.; Visualization: H.R.G., S.M. and M.G.; Writing - original draft: S.M.; Writing - review & editing: H.R.G., S.M., E.A., and MG.

Conflict of Interests

The authors declare no conflict of interest.

Funding

This study reports a database of a master's thesis recorded and completed at Sana Institute of Higher Education, Sari, Iran, but was not sponsored by any organization.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Acknowledgments

We thank the Zare, Razi, Bu-Ali Sina, Fatemeh Zahra, and Imam Khomeini hospitals' staff for giving patients' information and the collection of the clinical isolates.

REFERENCES

1. Liao S, Zhang Y, Pan X, et al. Antibacterial activity and mechanism of silver nanoparticles against multidrug-resistant Pseudomonas aeruginosa. Int J Nanomedicine 2019; 114:1469–87.
2. Tuon FF, Dantas LR, Suss PH, et al. Pathogenesis of the Pseudomonas aeruginosa biofilm: A review. Pathogens 2022; 11(3):300.
3. Adesoji AT, Onuh JP, Palang IP, et al. Prevalence of multi-drug resistant Pseudomonas aeruginosa isolated from selected residential sewages in Dutsin-Ma, Katsina State, Nigeria. J Public Health Afr 2023; 14(2).
4. Jurado-Martín I, Sainz-Mejías M, McClean S. Pseudomonas aeruginosa: an audacious pathogen with an adaptable arsenal of virulence factors. Int J Molec Sci 2021; 22(6):3128.
5. Qin S, Xiao W, Zhou C, et al. Pseudomonas aeruginosa: Pathogenesis, virulence factors, antibiotic resistance, interaction with host, technology advances and emerging therapeutics. Signal Transduct Target Ther 2022; 7(1):199.
6. Kothari A, Kumar SK, Singh V, et al. Association of multidrug resistance behavior of clinical Pseudomonas aeruginosa to pigment coloration. Eur J Med Res 2022; 27(1):1–13.
7. Everett MJ, Davies DT, Leiris S, et al. Chemical optimization of selective Pseudomonas aeruginosa LasB elastase inhibitors and their impact on LasB-mediated activation of IL-1β in cellular and animal infection models. ACS Infectious Diseases 2023; 9(2):270–82.
8. Kim T-H, Li X-H, Lee J-H. Alleviation of Pseudomonas aeruginosa infection by propeptide-mediated inhibition of protease IV. Microbiology Spectrum 2021; 9(2):e00782–21.
9. Grande KK, Gustin JK, Kessler E, et al. Identification of critical residues in the propeptide of LasA protease of Pseudomonas aeruginosa involved in the formation of a stable mature protease. J Bacteriol 2007; 189(11):3960–8.
10. Krishnan G, Sethumadhavan A, Muthusamy S, et al. Antibiotic resistant clinical isolates of Pseudomonas aeruginosa harbor LasA gene. Internet J Microbiol 2019; 16:125.
11. Santajit S, Kong-Ngoen T, Chongsa-Nguan M, et al. Human single-chain antibodies that neutralize elastolytic activity of Pseudomonas aeruginosa LasB. Pathogens 2021; 10(6):765.
12. Bastaert F, Kheir S, Saint-Criq V, et al. Pseudomonas aeruginosa LasB subverts alveolar macrophage activity by interfering with bacterial killing through downregulation of innate immune defense, reactive oxygen species generation, and complement activation. Frontiers Immunol 2018; 9:1675.
13. Ghanem SM, Abd El-Baky RM, Abourehab MA, et al. Prevalence of quorum sensing and virulence factor genes among Pseudomonas aeruginosa isolated from patients suffering from different infections and their association with antimicrobial resistance. Infect Drug Resist 2023; 2371–5.
14. 1Derakhshan S, Hosseinzadeh A, Hedayati MA, et al. Prevalence rate of resistance to ciprofloxacin among clinical Pseudomonas aeruginosa

isolates in Kurdistan province, west of Iran. SJKU 2020; 24(6):56-67.
15. Šipošová NŠ, Liptáková V, Kvasnová S, et al. Genetic diversity of Acinetobacter spp. adapted to heavy metal polluted environments. Nova Biotechnologica et Chimica 2017; 16(1):42–7.
16. Fazeli N, Momtaz H. Virulence gene profiles of multidrug-resistant Pseudomonas aeruginosa isolated from Iranian hospital infections. Iran Red Crescent Med J 2014; 16(10).
17. Poursina S, Ahmadi M, Fazeli F, et al. Assessment of virulence factors and antimicrobial resistance among the Pseudomonas aeruginosa strains isolated from animal meat and carcass samples. Vet Med Sci 2023; 9(1):315–25.
18. Caballero A, Thibodeaux B, Marquart M, et al. Pseudomonas keratitis: protease IV gene conservation, distribution, and production relative to virulence and other Pseudomonas proteases. Invest Ophthalmol Vis Sci 2004; 45(2):522–30.
19. Stead D. Grouping of plant-pathogenic and some other Pseudomonas spp. by using cellular fatty acid profiles. Int J Syst Evol Microbiol 1992; 42(2):281–95.
20. Li X-H, Lee J-H. Quorum sensing-dependent post-secretional activation of extracellular proteases in Pseudomonas aeruginosa. J Biolog Chem 2019; 294(51):19635–44.
21. Kruczek C, Kottapalli KR, Dissanaike S, et al. Major transcriptome changes accompany the growth of Pseudomonas aeruginosa in blood from patients with severe thermal injuries. PloS One 2016; 11(3):e0149229.
22. Camberlein V, Jézéquel G, Haupenthal J, et al. The structures and binding modes of small-molecule inhibitors of Pseudomonas aeruginosa elastase LasB. Antibiotics 2022; 11(8):1060.
23. Zupetic J, Peñaloza HF, Bain W, et al. Elastase activity from Pseudomonas aeruginosa respiratory isolates and ICU mortality. Chest 2021; 160(5):1624–33.
24. De Sousa T, Hébraud M, Alves O, et al. Study of antimicrobial resistance, biofilm formation, and motility of Pseudomonas aeruginosa derived from urine samples. Microorganisms 2023; 11(5):1345.
25. Gervasoni LF, Peixoto IC, Imperador AC, et al. Relationship between antibiotic resistance, biofilm formation, virulence factors and source of origin of Pseudomonas aeruginosa environmental isolates with regard to the presence of metallo-β-lactamase-encoding genes. Microbial Pathogenesis 2023; 182:106223.
26. Camargo CH, Yamada AY, Souza ARd, et al. Genomics and antimicrobial susceptibility of clinical Pseudomonas aeruginosa isolates from hospitals in Brazil. Pathogens 2023; 12(7):918.
27. Mokhtari K, Amini K. Identification of virulence genes in Pseudomonas aeruginosa isolated from human and animal samples by multiplex-PCR and their antibiotic resistance pattern. Iran J Med Microbiol 2019; 13(4):294–301.
28. Khattab M, Nour M, ElSheshtawy N. Genetic identification of Pseudomonas aeruginosa virulence genes among different isolates. J Microb Biochem Technol 2015; 7(5):274–7.
29. Nikbin V, Aslani MM, Sharafi Z, et al. Molecular identification and detection of virulence genes among Pseudomonas aeruginosa isolated from different infectious origins. Iran J Microbiol 2012; 4(3):118.
30. Engel LS, Hill JM, Moreau JM, et al. Pseudomonas aeruginosa protease IV produces corneal damage and contributes to bacterial virulence. Invest Ophth Vis Sci 1998; 39(3):662–5.
31. Li L-L, Malone JE, Iglewski BH. Regulation of the Pseudomonas aeruginosa quorum-sensing regulator VqsR. J Bacteriol 2007; 189(12):4367–74.

Молекулярное исследование генов *LasA*, *LasB* и *PIV* в клинических изолятах *Pseudomonas aeruginosa* в провинции Мазендеран, Северный Иран

Сахар Мохамади Баладезаее[1], Мехрад Голами[2], Елхам Амири[3], Хамид Реза Голи[2,4]

[1] *Институт высшего образования „Сана", Сари, Иран*

[2] *Кафедра медицинской микробиологии и вирусологии, Факультет медицины, Университет медицинских наук Мазандарана, Сари, Иран*

[3] *Студенческий научно-исследовательский комитет, Университет медицинских наук Мазандарана, Сари, Иран*

[4] *Исследовательский центр молекулярной и клеточной биологии, Факультет медицины, Университет медицинских наук Мазандарана, Сари, Иран*

Адрес для корреспондецнии: Хамид Реза Голи, Кафедра медицинской микробиологии и вирусологии, Факультет медицины, Университет медицинских наук Мазандарана, бул. „Фарах Абад", площадь „Казар", Сари, Иран; Email: goli59@gmail.com; тел.: +981133543081

Дата получения: 2 апреля 2024 ♦ **Дата приемки:** 4 июня 2024 ♦ **Дата публикации:** 30 июня 2024

Образец цитирования: Baladezaee AM, Gholami M, Amiri E, Goli HR. Molecular investigation of *LasA*, *LasB*, and *PIV* genes in clinical isolates of *Pseudomonas aeruginosa* in Mazandaran Province, North Iran. Folia Med (Plovdiv) 2024;66(3):361-369. doi: 10.3897/folmed.66.e124561.

Резюме

Цели: *Pseudomonas aeruginosa* играет важную роль в госпитальных инфекциях, вызванных несколькими факторами вирулентности, такими как эластаза и протеазы. Целью данного исследования была оценка распространённости генов *LasA, LasB* и *PIV,* кодирующих эти ферменты, в клинических изолятах *P. aeruginosa*.

Материалы и методы: Сто клинических изолятов были собраны у пациентов, поступивших в образовательные и терапевтические больницы провинции Мазандаран, Северный Иран. Изоляты были идентифицированы с помощью стандартных микробиологических и биохимических тестов. Бактериальная ДНК была извлечена методом щёлочного лизиса, а наличие соответствующих генов было обнаружено с помощью метода ПЦР. Данные были проанализированы с помощью SPSS v. 23 и критерия хи-квадрат. Значение $p<0.05$ считалось статистически значимым.

Результаты: В 100 клинических изолятах *P. aeruginosa* гены *LasA, LasB* и *PIV* были представлены с частотой 97%, 96% и 97% соответственно. Из общего числа образцов 39 пациентов были женщинами и 61 мужчинами. Также большинство пациентов были в возрасте от 61 до 70 лет.

Заключение: *LasA, LasB* и *PIV* широко распространены в клинических изолятах *P. aeruginosa*, что указывает на важность этих генов как ключевых факторов вирулентности в патогенности *P. aeruginosa* в этом регионе.

Ключевые слова

LasA, *LasB*, *PIV, Pseudomonas aeruginosa*