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Research Article

Genetic investigation of virulence factors genes among *Pseudomonas aeruginosa* isolated from clinical sources

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Abstract

Pseudomonas aeruginosa is considered one of the most common causes of infection, especially in immunocompromised patients. Therefore, this study aimed to detect the virulence factor genes in Pseudomonas aeruginosa isolates. The isolates were collected from different sources: 18/100 (18%) burn patients, 13/100 (13%) diabetic foot ulcer patients, and 2/100 (2%) patients suffering from urinary tract infections. The growth rate of Gram-positive bacteria was 79 (26.33%), while the rate of Gram-negative bacteria was 168 (56%). Results of antibiotic susceptibility showed that the highest bacterial resistance was Cefepime 33(100%) while the least resistance was Meropenem reached 16(48.48%). Results of Polymerase chain reaction (PCR) showed that 29 (87.87%), 9 (27.27%), 29(87.87%), 33(100%), 33(100%) and 17(51.51%) of P. aeruginosa isolates had *pilB*, *pilA*, *lasA*, *lasB*, *algD*, and *nan-1* genes, respectively in this work.

1. Introduction

Pseudomonas aeruginosa (P. aeruginosa) is one of the most clinically important Gram-negative bacteria. It is an opportunistic pathogen responsible for about 10 to 20% of nosocomial infections worldwide [1], virulence factors are components that allow an organism to infect a host and are often responsible for patient symptoms [2] *P. aeruginosa* has many virulence factors enzymes and toxins which disrupt host cells and aid bacterial invasion [3] flagella, which are involved in swimming motility within liquid environments [4] pili, which play a role in adhesion to cells and surfaces, biofilm formation, and gene transfer [5]. A distinctive hallmark of *P. aeruginosa* is its propensity for biofilm formation, an intricate process wherein bacterial cells aggregate within a self-produced extracellular matrix. This biofilm formation encompasses a complex architectural arrangement that renders the bacterium impervious to conventional antibiotic regimens and contributes significantly to its clinical impact [6].

2. Materials and Methods

2.1. Ethical approval

Official approvals were obtained to take samples from patients in health institutions, as well as the patients' consent, in addition to following all procedures and instructions recommended by the University of Kufa, College of Science.

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2.2. Patients and Specimens Processing

The current research included data gathered from various clinical sources who were suffering from different disorders. Samples were collected from patients suffering from urinary tract infections, diabetic foot ulcers, and burn injuries in hospitals in Najaf Governorate. The sample collection period extended from September 2024 to January 2025. The samples were placed on blood agar and McCoy medium and incubated at 37°C for 24 hours in a sterile environment [7].

2.3. Collection of the specimens and bacterial identification

All isolates of *P. aeruginosa* were identified by Gram staining some biochemical tests, and oxidase test [7], and finally comfirm by Vitek-2 compact system. Antibiotic susceptibility testing of *P. aeruginosa* was performed according to the Kirby-Bauer method using disk diffusion [8], against different drugs Piperacillin-Tazobactam 100/10 mg, Cefepime 30 mg, Meropenem 10mg, Ofloxacin 5mg , Norfloxacin 10mg, Amikacin (30mg), and Netilmicin (30 mg). The zone diameter was applied base on instructions of the Clinical and Laboratory Standards Institute [9].

2.4. DNA extraction and PCR assay

The DNA extraction micro kit (Favorgen, South Korea) was used to collect all of the nucleic acid for 33 clinical isolates of p.aeruginosa This was done in accordance with the manufacturer's protocol. After ensuring the integrity of the whole DNA sample by storing it in a deep freezer set to -20 degrees Celsius, a PCR analysis was carried out in order to test for the genes listed in Table 1. The equipment for gel documentation was employed for the migration of PCR amplification at 1% agarose, which were dyed with safety stain at a concentration of 0.5 g/ml.

Table 1: Primer Sequence and condition of thermocycles

Target	Name gene	The sequence (5' to 3')	Anneali ng (°C)	Size of product bp	Reference
lasA	*lasA-F* *lasA-R*	GCAGCACAAAAGATCCC GAAATGCAGGTGCGGTC	59 C°	1075 bp	Fazeli and Momtaz [10]
lasB	*lasB -F* *lasB -R*	GGAATGAACGAAGCGTTCTC GGTCCAGTAGTAGCGGTTGG	55 C°	284 bp	Fazeli and Momtaz [10]
pilA	*pilA -F* *pilA -R*	ACAGCATCCAACTGAGCG TTGACTTCCTCCAGGCTG	59 C°	1675 bp	Fazeli and Momtaz [10]
pilB	*pilB -F* *pilB -R*	ATGAACGACAGCATCCAACT GGGTGTTGACGCGAAAGTCGAT	57 C°	826 bp	Heidary et al., [11]
nan-1	*nan-1-F* *nan-1-R*	ATGAATACTTATTTTGATAT CTAAATCCATGCTCTGACCC	50 C°	1316 bp	Lanotte et al., [12]
algD	*algD-F* *algD -R*	CGTCTGCCGCGAGATCGGCT GACCTCGACGGTCTTGCGGA	63 C°	313 bp	Fazeli and Momtaz [10]

3. Result

3.1. Patients and bacterial growth

Samples for this study were collected from three different sources: diabetic foot ulcer patients, urinary tract infection patients, and burn injury patients. The results of bacterial culture showed that 66% of the samples were Gram-negative and 25% Gram-positive bacteria, while 6% and 3% had mixed growth and no growth, respectively, for burn injury patients. The results of the culture for diabetic foot ulcer patients were 59% Gram-negative bacteria growth and 31% Gram-positive bacteria growth, while 4% and 6% had mixed growth and no growth, respectively. As for the growth results of the UTI culture, they were as follows: 43% Gram-negative bacteria growth and 23% Gram-positive bacteria growth, while no growth was seen in 34% (table 2). We notice a high rate of non-growth in samples isolated from urinary tract infections. This may be due to fungal or viral infections, or perhaps physiological problems. Through the results of the Vitek-2 system, it was found that 11% of the isolates were *Pseudomonas aeruginosa*.

Table 2: Results of culture media of 300 specimens obtained from burn patients, diabetic foot ulcers, and UTIs.

		Burns Patient	Diabetic foot ulcer	Utis
Growth	Gram-negative	66 (66%)	59 (59%)	43 (43%)
	Gram-Positive	25 (25%)	31 (31%)	23 (23%)
	Mix growth	6(6%)	4(4%)	0 (0)
No growth		3(3%)	6(6%)	34 (34%)
Total		100(100%)	100(100%)	100(100%)

3.2. Antimicrobial susceptibility of *P. aeruginosa* isolates

The results of the current study showed high resistance by *P. aeruginosa* isolates to an important group of drugs, where the highest resistance was recorded to the antibiotic Cefepime, and Netilmicin, which reached 100%, followed by resistance to antibiotics Netilmicin and which reached 96.9%, for each. Whereas the bacteria recorded the lowest resistance rate to the antibiotic Meropenem, which reached 48.4%, while the other drugs showed diverse resistance, as shown in Table 3.

Table 3: Antibacterial agent susceptibility of *P. aeruginosa* isolates.

Antibacterial agent	Resistance (%)	Intermediate (%)	Sensitive (%)
Piperacillin-Tazobactam	21 (63.63)	8 (24.24)	4 (12.12)
Cefepime	33 (100)	0 (0)	0 (0)
Netilmicin	32(96.96)	0(0)	1(3.03)
Amikacin	29 (87.87)	3 (9.09)	1 (3.03)
Meropenem	16 (48.48)	4 (12.12)	13(39.39)
Norfloxacin	30 (90.90)	1 (3.03)	2 (6.06)
Ofloxacin	31(93.93)	1 (3.03)	1 (3.03)

3.3. Molecular detection of virulence factor genes in *P. aeruginosa* isolates.

Results of PCR about resistance genes showed that 33(100%), 33(100%), 29(87.87%), 29(87.87%),17(51.51%) and 9(27.27%) of P. aeruginosa isolates were harbored *lasB, algD, lasA, pilB, nan-1* and *pilA* genes, respectively (figure 1,2,3,4,5 and 6).

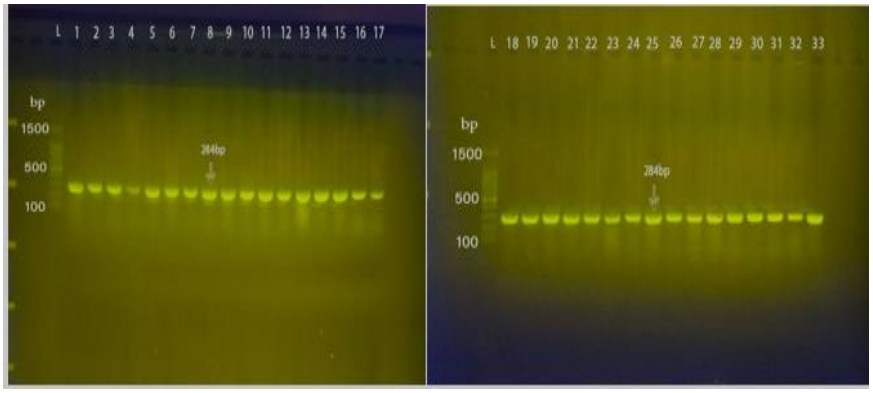

Figure 1: PCR products for *lasB* gene of *P. aeruginosa* isolates

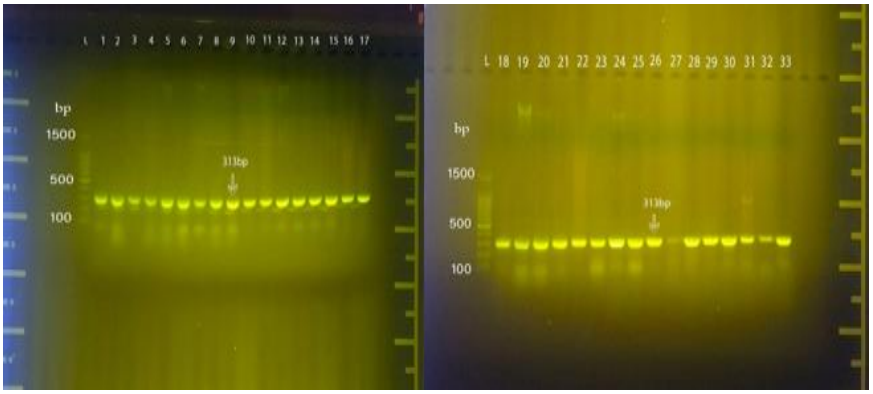

Figure 2: PCR products for *algD* gene of *P. aeruginosa* isolates

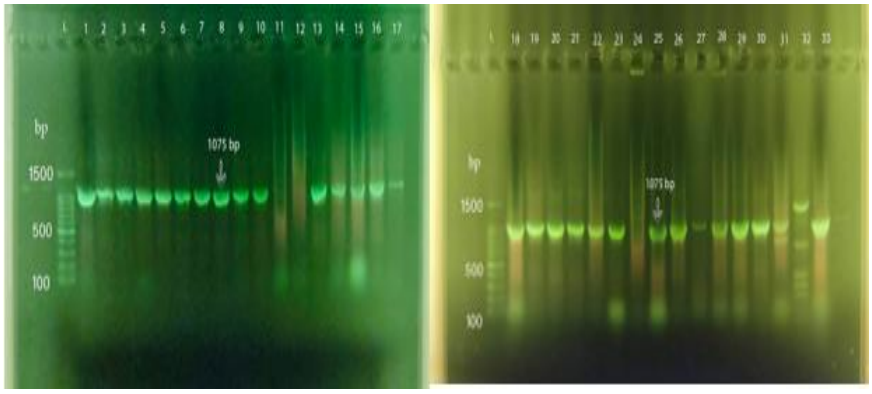

Figure 3: PCR products for *lasA* gene of *P. aeruginosa* isolates

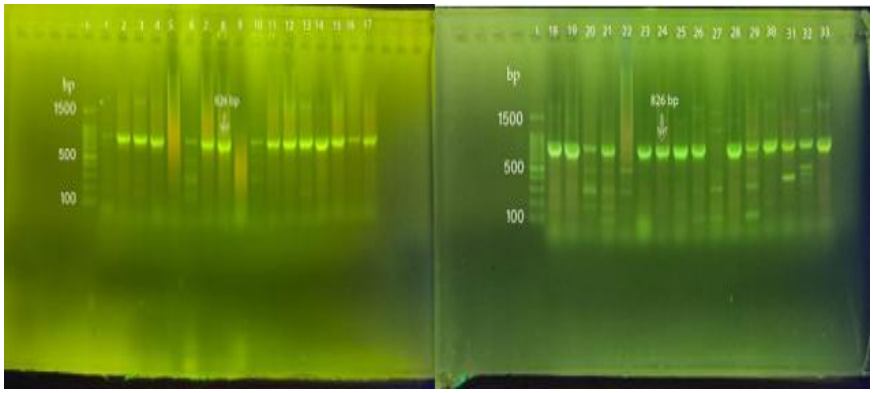

Figure 4: PCR products for *pilB* gene of *P. aeruginosa* isolates

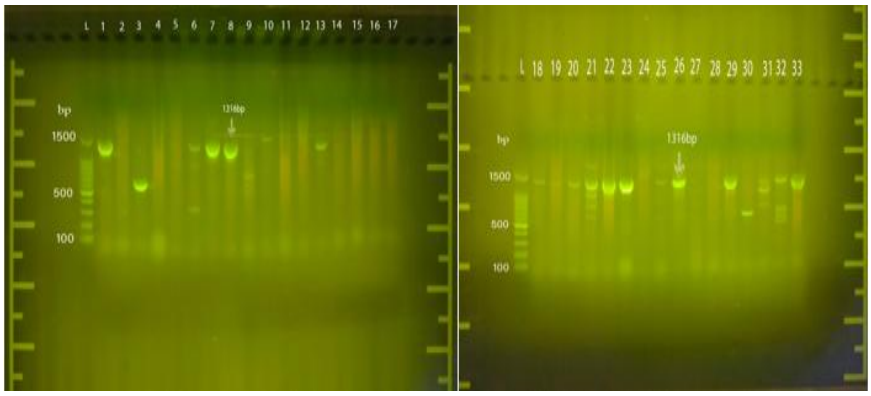

Figure 5: PCR products for *nan-1* gene of *P. aeruginosa* isolates

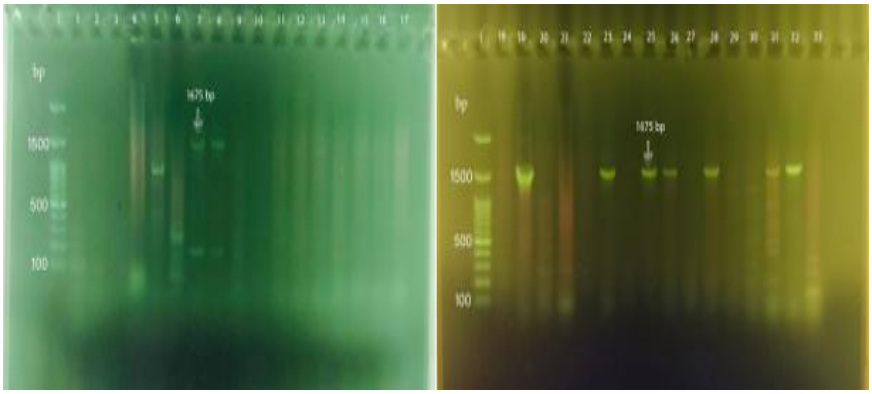

Figure 6: PCR products for *pilA* gene of *P. aeruginosa* isolates

4. Discussion

Pseudomonas aeruginosa causes hospital infections, especially in immunocompromised individuals. This work has shown that *P. aeruginosa* is highly resistant to most drugs, which is consistent with many studies.

The results of the current study showed a high prevalence of virulence factor genes among Pseudomonas aeruginosa isolates isolated from different clinical sources. This indicates a worrying indicator, especially the possibility of these genes being transferred to other bacterial strains. The results of molecular analysis support and reinforce the widespread prevalence and pathogenicity of *P. aeruginosa* in health institutions in the city of Najaf. In a local study conducted in hospitals in Babylon Governorate via [13], it was found that *P. aeruginosa* isolates were (71.75%) resistant to Norfloxacin, (64.12%) to Piperacillin-tazobactam, and (79.39%) to Amikacin,(93.90%) to Cefepime (93.90%) while their resistance to Meropenem was (81.68%).W%).

In a local study conducted by [14] in Anbar, Iraq, to detect the virulence factor genes of P. aeruginosa, samples were collected from infected patients from the National Center for Educational Laboratories, the Educational Burns 84 Hospital, and the Ramadi Educational Hospital. It showed that the presence of the *algD* gene was 100%, and *pilA* was 92%, and *pilB* was 4%.

While globally also agreed with our results In a study conducted via [15] in Brazil, clinical samples were taken from different sources and it was found that the presence of the *pilA* gene was 14.1%, the nan-1 gene was 14.1%, the *LasB* gene was 89.9%, the *pilB* gene was 27.3%, and the *algD* gene was 75.7%.

In a study conducted via [16] in Mazandaran Province, northern Iran, to detect the prevalence of virulence factor genes of *P. aeruginosa* isolated from patients admitted to educational and therapeutic hospitals in Iran, it was indicated that the percentage of *lasB* genes was (96%), while the prevalence of the *lasA* gene was (97%).

References

[1] T. Ikeno, K. Fukuda, M. Ogawa, M. Honda, T. Tanabe, and H. Taniguchi. Small and rough colony pseudomonas aeruginosa with elevated biofilm formation ability isolated in hospitalized patients. *Microbiology and Immunology*, 51(10):929–938, 2007.

[2] C. Liao, X. Huang, Q. Wang, D. Yao, and W. Lu. Virulence factors of pseudomonas aeruginosa and antivirulence strategies to combat its drug resistance. *Frontiers in Cellular and Infection Microbiology*, 12:926758, 2022.

[3] V. V. Veetilvalappil, A. Manuel, J. M. Aranjani, R. Tawale, and A. Koteshwara. Pathogenic arsenal of pseudomonas aeruginosa: an update on virulence factors. *Future Microbiology*, 17(6):465–481, 2022.

[4] S. L. Gellatly and R. E. Hancock. Pseudomonas aeruginosa: new insights into pathogenesis and host defenses. *Pathogens and Disease*, 67(3):159–173, 2013.

[5] I. Jurado-Martín, M. Sainz-Mejías, and S. McClean. Pseudomonas aeruginosa: an audacious pathogen with an adaptable arsenal of virulence factors. *International Journal of Molecular Sciences*, 22(6):3128, 2021.

[6] S. Sharma, J. Mohler, S. D. Mahajan, S. A. Schwartz, L. Bruggemann, and R. Aalinkeel. Microbial biofilm: a review on formation, infection, antibiotic resistance, control measures, and innovative treatment. *Microorganisms*, 11(6):1614, 2023.

[7] J. G. Collee, A. G. Fraser, B. P. Marmion, and A. Simmons. *Practical Medical Microbiology*. Churchill Livingstone, USA, 14th edition, 1996.

[8] A. W. Bauer, W. M. Kirby, J. C. Sherris, and M. Turck. Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, 45(4):493–496, 1966.

[9] CLSI. *Performance Standards for Antimicrobial Susceptibility Testing. 31st ed. CLSI supplement M100*. Clinical and Laboratory Standards Institute, 2021.

[10] N. Fazeli and H. Momtaz. Virulence gene profiles of multidrug-resistant pseudomonas aeruginosa isolated from iranian hospital infections. *Iranian Red Crescent Medical Journal*, 16(10):e15722, 2014.

[11] Z. Heidary, E. Bandani, M. Eftekhary, and A. A. Jafari. Virulence genes profile of multidrug resistant pseudomonas aeruginosa isolated from iranian children with utis. *Acta Medica Iranica*, pages 201–210, 2016.

[12] P. Lanotte, S. Watt, L. Mereghetti, N. Dartiguelongue, A. Rastegar-Lari, A. Goudeau, and R. Quentin. Genetic features of pseudomonas aeruginosa isolates from cystic fibrosis patients compared with those of isolates from other origins. *Journal of Medical Microbiology*, 53(1):73–81, 2004.

[13] A. A. Almuttairi and A. A. Abdulla. Occurrence of class 1, 2, and 3 integrons among multidrug-resistant pseudomonas aeruginosa in babylon province, iraq. *Medical Journal of Babylon*, 20(1):181–187, 2023.

[14] T. A. Al-Mohammed and H. M. Mahmood. Carbapenem resistance related with biofilm formation and pilin genes in clinical pseudomonas aeruginosa isolates. *Iraqi Journal of Pharmaceutical Sciences (P-ISSN*, 33(1):1683–3597, 2024. E-ISSN 2521-3512),), 72–78.

[15] F. Nitz, B. O. de Melo, L. C. N. da Silva, A. de Souza Monteiro, S. G. Marques, V. Monteiro-Neto, others, and M. R. Q. Bomfim. Molecular detection of drug-resistance genes of blaoxa-23, blaoxa-51, and mcr-1 in clinical isolates of pseudomonas aeruginosa. *Microorganisms*, 9(4):786, 2021.

[16] S. M. Baladezaee, M. Gholami, E. Amiri, and H. R. Goli. Molecular investigation of lasa, lasb, and piv genes in clinical isolates of pseudomonas aeruginosa in mazandaran province, north iran. *Folia Medica*, 66(3):361–369, 2024.