Original Article

Genetic Variations in Multilocus Genes of Clinical *Pseudomonas aeruginosa* in Al-Diwaniyah City, Iraq

Shaimaa Shakir Jawad, Ibtisam Habeeb Al-Azawi

Department of Medical Microbiology, College of Medicine, Al-Qadisiyah University, Al-Diwaniyah, Iraq

Abstract

Background: Because of its availability and comprehensive manner, the multilocus sequence typing (MLST) database was used in this study. As MLST has become more widely used, databases have been created to store and track different sequence types and to enable comparative analysis of allele sequences. **Objective:** This study aimed to detect genetic variations in the multilocus genes of *Pseudomonas aeruginosa* isolates from different clinical sources using sequencing techniques. **Materials and Methods:** A total of 200 samples were collected in this cross-sectional study, which includes burns' swabs (n = 80, 40%), wounds' swabs (n = 66, 33%), and diabetic foot ulcer swabs (n = 54, 27%) from November 2021 to August 2022 from Al-Diwaniyah Teaching Hospital, Feminine and Children Teaching Hospital, and Burns Center admitted patients. The six multilocus gene samples delivered for sequencing were primed Macrogen provided a FASTA-formatted (a DNA and protein sequence alignment software package) DNA sequence chromatogram. **Results:** Of 200 collected samples, only 136 (68%) samples gave positive results for culturing, and of 136 positive culturing samples, only 50 isolates (25.0%) were identified as *P. aeruginosa* isolates. According to the site of infection, 43.1%, 37.5%, and 26.3% of *P. aeruginosa* were isolated from burns, wounds, and diabetic foot ulcers, respectively. Multilocus gene fragments were utilized for direct sequencing to detect genetic alterations in study group samples. Basic Local Alignment Search Tool (BLAST) results showed that the enlarged portions of multilocus genes had genetic alterations. **Conclusions:** Multilocus gene analysis analyzes many genetic loci to evaluate bacterial genetic diversity. Researchers can better comprehend bacterial genetics by examining many genes.

Keywords: Clinical specimens, multilocus genes, *Pseudomonas aeruginosa*, *Pseudomonas aeruginosa* genome sequencing

Introduction

Pseudomonas aeruginosa is a bacterium that can cause infections in different parts of the body. Some common infection sites include skin and soft tissues, the respiratory tract, the urinary tract, and the bloodstream.[1]

When isolates of *P. aeruginosa* are obtained from different sites of infection, they may exhibit different characteristics and patterns of antibiotic resistance.[2] For example, isolates from respiratory tract infections may be more likely to be resistant to antibiotics such as fluoroquinolones and aminoglycosides, whereas isolates from burn and urinary tract infections may be more resistant to beta-lactam antibiotics.[3,4]

Studies have also found that isolates of *P. aeruginosa* from different sites of infection may have different virulence factors, which are molecules that allow the bacterium to cause disease, and isolates from skin and soft-tissue infections may have different types of proteases and exotoxins compared with isolates from respiratory tract infections.[5,6] The quorum sensing system plays an important role in the pathogenicity and biofilm formation of *P. aeruginosa*, and it is essential for bacteria to increase growth and resistance to antibiotics.[7]

Understanding the characteristics of *P. aeruginosa* isolates from different sites of infection can be important for selecting appropriate antibiotic treatments and developing

Access this article online

Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_747_23

Address for correspondence: Dr. Shaimaa Shakir Jawad, Department of Medical Microbiology, College of Medicine, Al-Qadisiyah University, Al-Diwaniyah, Iraq.
E-mail: shaimaa.altofaily@gmail.com

Submission: 13-Jun-2023 **Accepted:** 06-Jul-2023 **Published:** 30-Apr-2026

How to cite this article: Jawad SS, Al-Azawi IH. Genetic variations in multilocus genes of clinical *Pseudomonas aeruginosa* in Al-Diwaniyah city, Iraq. Med J Babylon 2026;23:74-81.

strategies to prevent and treat infections caused by this bacterium.[8]

Multilocus sequence typing (MLST) is a technique used to identify and classify different strains of bacteria based on variations in multiple genetic loci. MLST is commonly used for the detection of multilocus genes in *P. aeruginosa*.[9]

The MLST method involves amplifying and sequencing several housekeeping genes from the bacterial genome, which are usually conserved and evolve slowly. These genes are often selected based on their ability to provide a high discrimination between different strains of the same species.[10] In *P. aeruginosa*, the most commonly used housekeeping genes for MLST are *acsA, aroE, guaA, mutL, nuoD*, and *ppsA.* After sequencing these genes, the resulting data can be analyzed using various software programs to determine the specific sequence types (STs) of each bacterial strain. STs are assigned based on the allelic profiles of the sequenced genes, which can be used to compare and classify different strains of *P. aeruginosa*.[11]

MLST is a powerful tool for epidemiological studies as it allows researchers to track the spread of different strains of *P. aeruginosa* and identify potential outbreaks. It can also be used to investigate the genetic diversity of this pathogen and its evolution over time.[12]

This aimed to detect alteration in the multilocus genes of *P. aeruginosa* using the sequencing technique.

Materials and Methods

In this cross-sectional study, 200 clinical samples were taken from a variety of infection sites, including 80 burns, 66 wounds, and 54 diabetic foot ulcers from patients who were hospitalized in Al-Diwaniyah Teaching Hospital, Feminine and Children Teaching Hospital, and Al-Diwaniyah Burns between November 2021 and August 2022. These patients were males and females of different ages, ranging from 5 months to 62 years. All sample collecting procedures were carried out in a risk-free environment. Sterile transport swabs were used to collect the clinical samples from the patients, and these were then promptly transported to the lab for analysis.

Isolation and identification of *Pseudomonas aeruginosa*

Microbiological standard diagnostic criteria were used to isolate and identify the clinical isolates of *P. aeruginosa*, which included colony morphology, Gram stain, and conventional biochemical tests.[13,14] All isolates were confirmed by Api20E and automated VITEK2 compact system using the GN ID 222 card.

DNA extraction

This method was made according to the genomic DNA purification Kit supplemented by the manufacturing company genomic (Gene aid, USA).

DNA sequencing

In this research, we chose a polymerase chain reaction (PCR) fragment that only partially encompassed the multilocus protein-coding regions. To ascertain the genetic polymorphism of the bacteria samples, immediate sequencing of the amplified fragments was performed. The discriminatory power and phylogenetic distribution of the variations were then assessed by constructing fully complete trees.

PCR amplicon DNA sequencing

The resolved PCR amplicons were then commercially sequenced in both directions as per the sequencing company's recommendations (Macrogen Inc., Geumchen, Seoul, South Korea). To rule out PCR or sequencing artifacts as potential causes of annotation and variance, only clean chromatographs from ABI (Applied Biosystems) sequence files were analyzed. By comparing the retrieved nucleic acid sequences to those from the local samples, the virtual positions and other characteristics of the recoverable PCR fragments were determined.

Sequence data interpretation

The PCR product sequencing data from the selected samples were edited, aligned, and analyzed in comparison with the reference database using the BioEdit Sequence Alignment Editor Software Version 7.1 (DNASTAR, Madison, Wisconsin). All variations found in the sequenced samples were assigned unique numbers, both for the PCR amplicons and the genomic locations where they were found. All identified nucleic acids were tallied using PCR amplicons and the genome. Each bacterial sequencing variant was annotated using Snap Gene Viewer version 4.0.4 (https://www.snapgene.com).

Nucleic acid variations into amino acids

The amino acid sequence for the desired protein was obtained from the Protein Data Bank (http://www.ncbi.nlm.nih.gov).

The Expose website (http://web.expasy.org/translate/) provided a translation of the nucleic acid variants in the coding regions of the genetic loci into a reading frame matching the appropriate amino acid residues in the encoded protein. The "align" script on the BioEdit server matched the cited amino acid sequences to their reported mutant analogs.

Ethical approval

This study was conducted according to the approvals of the College of Medicine, Al-Qadisiyah University, according to the document number 30/3666 in November 9, 2021. The study excluded banned biological materials and genetically modified organisms. *P. aeruginosa* isolates were obtained from hospitalized patients without any additives.

Results

A total of 200 samples were collected in this cross-sectional study, which include burns' swabs (n = 80, 40%), wounds' swabs (n = 66, 33%), and diabetic foot ulcer swabs (n = 54, 27%). These clinical samples were collected from patients admitted to different hospitals represented in Al-Diwaniyah Teaching Hospital, Feminine and Children Teaching Hospital, and Al-Diwaniyah Burns Center.

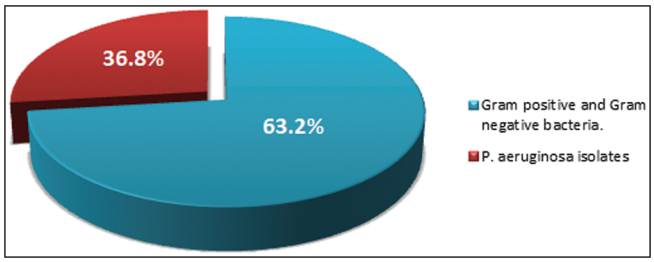

Figure 1: Isolation rate of *Pseudomonas aeruginosa* isolated from clinical samples

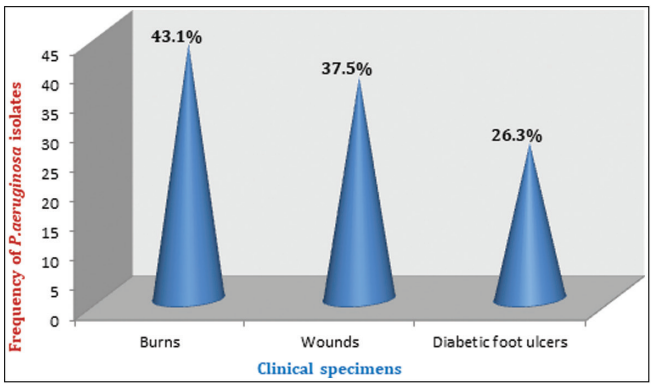

Figure 2: Distribution of *Pseudomonas aeruginosa* in clinical samples

Of 200 collected samples, only 136 (63.2%) samples gave positive results for culturing, and of 136 positive culturing samples, only 50 isolates (36.8%) were identified to be as *P. aeruginosa* isolates depending on culture characteristics and biochemical tests [Figure 1]. Other than *P. aeruginosa*, different Gram-positive and Gram-negative bacteria were detected in the other positive cultures.

The 50 *P. aeruginosa* isolates distributed according to the source of clinical samples were as follows: 43.1%, 37.5%, and 26.3% in burns, wounds, and diabetic foot ulcers, respectively [Figure 2].

Using the PCR results, which reflect amplified fragments of multilocus genes, direct sequencing was utilized to detect any potential genetic alterations in samples from the study group. Six samples including several genes were sequenced in this study, and primers are listed in Table 1. The DNA sequence chromatogram in FASTA format (a DNA and protein sequence alignment software package) was among the data collected from the Macrogen Corporation. The amplified regions of multilocus genes have experienced genetic alterations, according to Basic Local Alignment Search Tool (BLAST) results for comparing DNA from research samples (Query) with the sequence regarded as the reference that is preserved in gene bank at NCBI [Table 2].

Three genes including *trpE*, *ppsA*, and *nuoD* of *P. aeruginosa* isolated from burns were recorded in the gene bank with accession number [Table 3].

Table 1: Housekeeping genes of *Pseudomonas aeruginosa* for multilocus sequence typing

Gene		Primer sequence (5′_3′)	Amplicon size (bp)	Reference
nuoD	F	ACCGCCACCCGTATCTG	1042	pubmlst.org
	R	TCTCGCCCATCTTGACCA		
ppsA	F	GGGTAGCAAGGCGATCAAGATG	1034	pubmlst.org
	R	GGTTCTCTTCTTCCGGCTCGTAG		
trpE	F	GCCGATCCCTCCGAGGAAAATG	919	pubmlst.org
	R	CCCGGCGCTTGTTGATGGTT		

Table 2: Number of the isolates of *Pseudomonas aeruginosa* (*trpE*, *ppsA*, *and nuoD*) genes, and source of isolation with date of collection and country

Sequence Id	Organism	Isolation source	Collection date
Seq1	*Pseudomonas aeruginosa* (trpE, ppsA, and nuoD)	Burn	January 9, 2022
Seq2	*Pseudomonas aeruginosa* (trpE, ppsA, and nuoD)	Burn	January 10, 2022
Seq3	*Pseudomonas aeruginosa* (trpE, ppsA, and nuoD)	Wound	February 22, 2022
Seq4	*Pseudomonas aeruginosa* (trpE, ppsA, and nuoD)	Wound	March 27, 2022
Seq5	*Pseudomonas aeruginosa* (trpE, ppsA, and nuoD)	DFU	April 12, 2022
Seq6	*Pseudomonas aeruginosa* (trpE, ppsA, and nuoD)	DFU	May 10, 2022

DFU: diabetic foot ulcer

Table 3: Gene bank accession number to three genes for two isolates of *Pseudomonas aeruginosa* (*trpE*, *ppsA*, and *nuoD*) burn source

Isolate no.	trpE accession no.	ppsA accession no.	nuoD accession no.
1	OP832217	OP832218	OP832219
2	OP832224	OP832225	OP832226

Table 4: MLST analysis to identify MLST sequence types (STs), nearest ST, and allelic profiles of six *Pseudomonas aeruginosa* isolates from the different specimen sources with three housekeeping genes (*nuoD*, *ppsA*, and *trpE*)

Isolates	Source	Allelic profile				
		nuoD	ppsA	trpE	ST	Nearest (ST)
1	Burn	4	13	7	264[a]	-
2	Burn	4	13	7	264[a]	-
3	Wound	1	2	4	Not hited	235
4	Wound	1	2	4	Not hited	235
5	DFU	14	4	7	Not hited	244
6	DFU	14	4	7	Not hited	244

DFU: diabetic foot ulcer
[a]Star noval allel. Not hited mean (not matched)

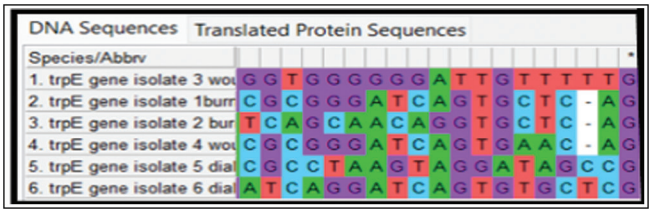

Figure 3: Alignment of *trpE* gene of *Pseudomonas aeruginosa* with the referenced genome from NCBI (gene bank)

Table 5: Genetic Variations of *trpE* gene of *Pseudomonas aeruginosa*

No. of isolate	Identity%	Genetic variation	Type of mutation
1	100%	C….T	Transition
		G….T	Transversion
2	100%	A….G	Transition
		T….G	Transversion
		C….G	Transition
		C….T	Transition
3	100%	C….T	Transition
		G….C	Transversion
		C….A	Transversion
		G….A	Transition
		A….G	
		With deletion	
4	100%	G….C	Transversion
5	100%	G….T	Transversion
		C….T	Transition
		T….C	Transition
6	100%	C….T	Transition
		G….T	Transition
		A….T	Transversion

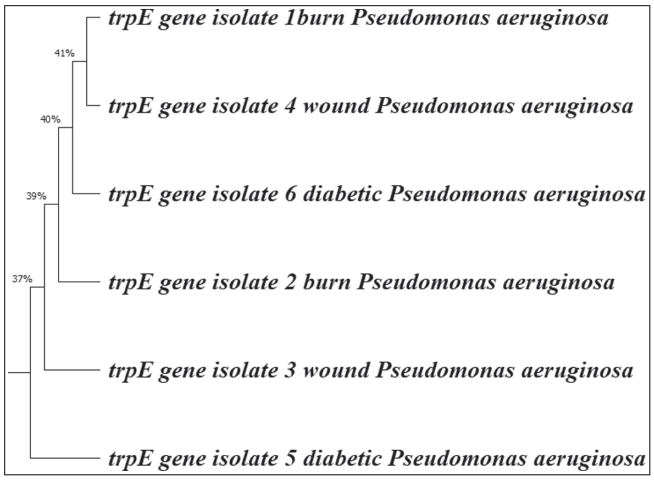

Figure 4: Phylogenetic tree for *trpE* gene for *Pseudomonas aeruginosa* constructed by UPGMA (MLST) analysis

ST was detected for two isolates from burn source, whereas others do not match when compared with the ST of *P. aeruginosa* using the Omics tool. So, not matched once were compared to the nearest ST as mentioned in Table 4.

The significant mutation rate found when the *trpE* gene was aligned suggested that there were changes in the gene between the isolates [Figure 3 and Table 5].

Figure 4 shows the phylogenetic tree of the *trpE* gene, one of the three chosen housekeeping genes of *P. aeruginosa*. According to the findings, it had a diverse impact on bacterial genes, resulting in a narrow range of similarity with a large number of mutations.

Alignment of *ppsA* gene shows genetic variation of this gene between the isolates [Figure 5 and Table 6]. These differences could result in an altered gene expression in the isolates.

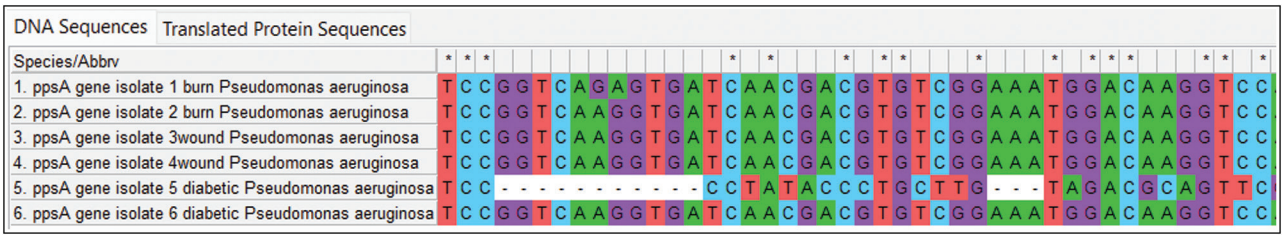

Figure 5: Alignment of *ppsA* gene of *Pseudomonas aeruginosa* with the referenced genome from NCBI (gene bank)

Table 6: Genetic variations of *ppsA* gene of *Pseudomonas aeruginosa*

No. of isolate	Identity%	Genetic variation	Type of mutation
1	100	No	
2	100	No	
3	100	No	
4	100	No	Transversion
5	100	Deletion	
		T….C	Transition
		A….C	Transversion
		A….G	Transition
		C….T	Transition
6	100	No	

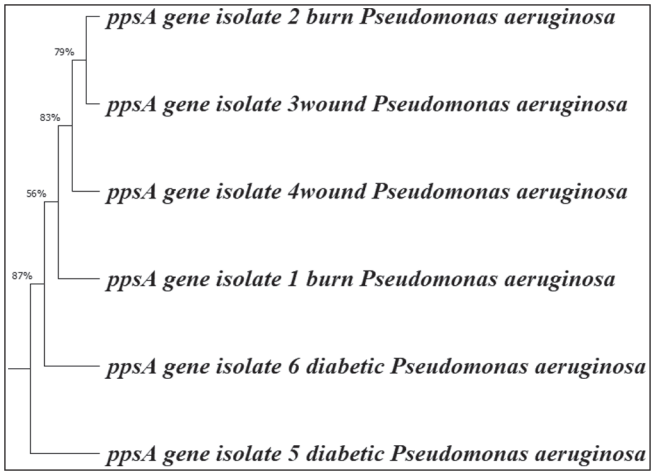

Figure 6: Phylogenetic tree for *ppsA* gene for *Pseudomonas aeruginosa* constructed by UPGMA (MLST) analysis

Phylogenetic tree for *ppsA* gene [Figure 6] for six isolates from different sources. Surprisingly, the result for the isolates that have the same source was separated from each other by a number of mutations.

Alignment of *nuoD* gene [Figure 7] reveals single nucleotide polymorphism (SNP) for the isolates.[1-5] Different SNPs were mentioned in Table 7.

Phylogenetic difference range between 45% and 91%, where the diabetic isolates were close together even though they share 45% of similarity. Other isolates from other sources were separated by high number of mutations [Figure 8].

Discussion

P. aeruginosa is a terrifying infectious agent in high-risk populations with burns, diabetic foot, and wound infections because of the severity of the patient's condition and the prevalence of antibiotic resistance with a high mortality rate.[15,16] Worldwide, burn injuries are a major public health issue. Both foreign and endogenous bacteria can colonize burn sites if they find an ideal environment. The burn team has a significant challenge in the form of infection, which is a leading cause of high morbidity and mortality in burn patients.[17] In Iraq, the fatality rate from burn injuries was significantly elevated.[18] Because of compromised immunity, infections are more likely to develop, which can result in sepsis.[19] Between 50% and 75% of burn patients who survive early treatment die from infection-related complications.[20] During the second

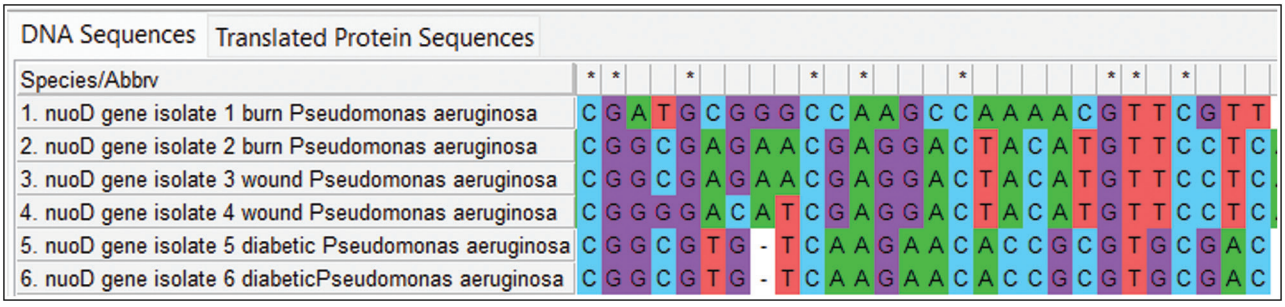

Figure 7: Alignment of *nuoD* gene of *Pseudomonas aeruginosa* with the referenced genome from NCBI (gene bank)

Table 7: Genetic variations of *nuoD* gene of *Pseudomonas aeruginosa*

No. of isolate	Identity%	Genetic variation	Type of mutation
1	100%	G….A	Transition
		C….T	Transition
		A….C	Transversion
		T….C	Transition
		A….C	Transversion
2	100%	No	
3	100%	G….C	Transversion
4	100%	Deletion	
		T….A	Transversion
		A….C	Transversion
		T….C	Transition
5	100%	T….C	Transition
		T….A	Transversion
		Deletion	
6	100%	Deletion	

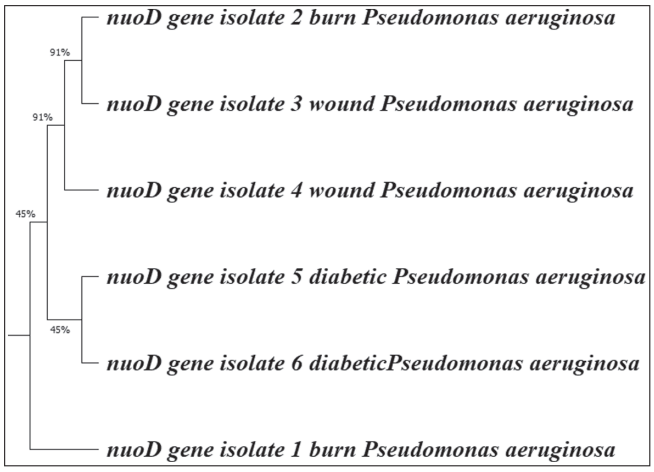

Figure 8: Phylogenetic tree for *nuoD* gene for *Pseudomonas aeruginosa*

week after a burn, *P. aeruginosa* may colonize and infect the wound.[21]

In the current study, the rates of *P. aeruginosa* isolated from burns and wound swabs were 43.1% and 37.5%, respectively [Figure 2]. A recent study in Babylon recorded a higher isolation rate from burns than this study obtained, which is 78.63%, whereas the percentage of isolation from wounds was less, which is 5.34%.[22] Previous local study had documented that *P. aeruginosa* rates isolated from burn and wound swabs were 58.5% and 66.7%, respectively,[23] whereas *P. aeruginosa* isolation rates from burns were recorded as 18.7% in Najaf Hospitals and 40.84% in Basrah.[24,25] Also, another study isolated *P. aeruginosa* from burns and wounds by 57.6% and 44.0%, respectively.[26] A 28.5% of *P. aeruginosa* isolates were recovered from Burn Center in Al-Najaf.[27]

When comparing the STs of the burn source isolates with the ST of *P. aeruginosa*, only two of the isolates matched. Therefore, pairs that did not match once were compared to the nearest ST. The results were in agreement with a study of three housekeeping genes' traits. All three genes' dN/dS ratios were calculated, and for *nuoD* and *trpE*, the values were 1.6741 and 2.3272, respectively.

Alignment of the *trpE* gene [Figure 3] revealed a high mutation rate, indicating that the gene has changed significantly between the isolates. Resistance or sensitivity to the mutation depends on whether the gene locus and overall function are preserved. As this function is crucial for the bacterium, it can be expressed in many ways. It was clear on the MLST effect that they shared the same locus of the gene but used it differently because of the amount of mutations present, which affected the coding protein. The findings corroborated those of researchers who reported on the draft genome sequencing of a unique ST3351 (identified by MLST) *P. aeruginosa* strain isolated from a Mexico patient and found to have high levels of resistance and mutation.[28] These results have the potential to improve our understanding of acquired resistance in human-infecting *P. aeruginosa* lineages.

A phylogenetic tree for the *trpE* gene [Figure 4] had a wide variety of effects on the genes of bacteria, which has led to a constricted range of genetic similarity with a significant number of mutations. Researchers confirmed this by showing that isolates from the same patient collected at different times and places were not genetically identical when linked to the infection site, demonstrating the high discriminatory power of MLST profiling. Disparities in gene expression between isolates can be traced back to these alignment discrepancies.

Interestingly, the *ppsA* gene phylogenetic tree for six isolates from different sources revealed that the isolates from the same source were separated from each other by a number of mutations [Figure 6], which is due to the genetic differences that have different effects on the bacteria as it required for infection. The genetic variations suggest that gene function in each isolate will be expressed differently from the vital role that the housekeeping genes play for the bacteria; therefore, any defect will affect bacterial infection or infection severity.

The phylogenetic difference in the *nuoD* gene [Figure 8] ranges between 45% and 91% of the diabetics isolates that were close together even though they share 45% of similarity. Other isolates were separated by a high number of the mutations, and it is important to know that this mutation will affect the function of the house keeping gene. The results were quite similar to the research[28] depending on the MLST data, and some strains were

novel. *P. aeruginosa* strains exhibit clusters that are different from those of other *P. aeruginosa* strains, according to trees built for individual and concatenated genes. These groups essentially corresponded to where the isolates were found. These findings confirm what we already knew, which is that there are *P. aeruginosa* strains in the water. Based on MLST and phylogenetic analyses of seven housekeeping genes, it can be said that the ocean *P. aeruginosa* strains have diverged from other isolates and formed a unique cluster.[29]

Several studies have used multilocus gene analysis to investigate the genetic diversity of *P. aeruginosa* populations. These studies have identified multiple clonal complexes or groups of genetically related strains, within the species.[30] One study found that *P. aeruginosa* isolates from environmental sources tended to be more diverse genetically than isolates from clinical sources, which were more likely to belong to specific clonal complexes.[31] Another study found that certain clonal complexes were more commonly associated with infections in cystic fibrosis patients, whereas others were more commonly found in environmental samples.[32]

Overall, multilocus gene analysis has provided valuable insights into the genetic diversity and population structure of *P. aeruginosa*. These findings can help researchers better understand the epidemiology and virulence of this important pathogen.[33]

Conclusion

Multilocus gene analysis is a technique used to study the genetic diversity of bacterial populations by analyzing multiple genetic loci simultaneously. By analyzing multiple genes, researchers can obtain a more comprehensive understanding of the genetic structure of a bacterial population.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Bobrov AG, Getnet D, Swierczewski B, Jacobs A, Medina-Rojas M, Tyner S, *et al*. Evaluation of *Pseudomonas aeruginosa* pathogenesis and therapeutics in military-relevant animal infection models. APMIS 2022;130:436-57.
2. Eladawy M, El-Mowafy M, El-Sokkary MM, Barwa R. Antimicrobial resistance and virulence characteristics in ERIC-PCR typed biofilm forming isolates of *P. aeruginosa*. Microb Pathog 2021;158:105042.
3. Al-Wahid AA, Al-Azawi IH. Occurrence of plasmid-mediated carbapenem resistance genes among *Pseudomonas aeruginosa* isolated from clinical and hospital environmental samples in Al-Nasseryia city, Iraq. Int J Pharm Res 2020;12:4542.
4. Daji M, Ade TI, Cletus HS, Bello AM, Joseph P. Antimicrobial resistance pattern of clinical isolates of *Pseudomonas aeruginosa* from urinary tract infections in Wukari, Taraba state, Nigeria. J Biochem Microbiol Biotechnol 2022;10:25-8.
5. Căpățînă D, Feier B, Hosu O, Tertiș M, Cristea C. Analytical methods for the characterization and diagnosis of infection with *Pseudomonas aeruginosa*: A critical review. Anal Chim Acta 2022;1204:339696.
6. Qin S, Xiao W, Zhou C, Pu Q, Deng X, Lan L, *et al*. *Pseudomonas aeruginosa*: Pathogenesis, virulence factors, antibiotic resistance, interaction with host, technology advances and emerging therapeutics. Signal Transduct Target Ther 2022;7:199.
7. Al-Azawi IH, Al-Shabbani HA. Molecular study of quorum sensing and biofilm formation genes in *Pseudomonas aeruginosa* isolated from UTIs patients. Int J Health Sci 2022;6:12121-34.
8. Al-Ishaq RK, Skariah S, Büsselberg D. Bacteriophage treatment: Critical evaluation of its application on World Health Organization priority pathogens. Viruses 2020;13:51.
9. Chuan J, Xu H, Hammill DL, Hale L, Chen W, Li X. Clasnip: A web-based intraspecies classifier and multi-locus sequence typing for pathogenic microorganisms using fragmented sequences. PeerJ 2023;11:e14490.
10. Yates MC, Derry AM, Cristescu ME. Environmental RNA: A revolution in ecological resolution? Trends Ecol Evol 2021;36:601-9.
11. Cabrera R, Fernández-Barat L, Vazquez N, Alcaraz V, López-Aladid R, Bueno L, *et al*. Antimicrobial resistance mechanisms, molecular epidemiology and virulence factors in *Pseudomonas aeruginosa* strains associated to patients with bronchiectasis. In: B56. WHAT'S UP IN THE PNEUMONIA WORLD? American Thoracic Society; 2022. p. A3111-A3111.
12. Simar SR, Hanson BM, Arias C. A techniques in bacterial strain typing: Past, present, and future. Curr Opin Infect Dis 2021;34:339.
13. Goodfellow M, Peter KÃ, Busse H-J, Trujillo ME, Ludwig W, Suzuki KI, *et al*. Bergey's Manual of Systematic Bacteriology: Volume 5: The Actinobacteria. New York, NY: Springer New York; 2012.
14. Tille P. Bailey & Scott's Diagnostic Microbiology-E-Book. Elsevier Health Sciences; 2015.
15. Jneid J, Lavigne JP, La Scola B, Cassir N. The diabetic foot microbiota: A review. Hum Microbiome J 2017;5-6:1-6.
16. Huang Y, Mu L, Zhao X, Han Y, Guo B. Bacterial growth-induced tobramycin smart release self-healing hydrogel for *Pseudomonas aeruginosa*-infected burn wound healing. ACS Nano 2022;16:13022-36.
17. Weber J, McManus A; Nursing Committee of the International Society for Burn Injuries. Infection control in burn patients. Burns 2004;30:A16-24.
18. Obaid EM, Baiee HA. Epidemiological and clinical characteristics of burn injuries among hospitalized patients in Babylon Province, Med J Babylon 2022;19:9
19. Farina JA, Rosique MJ, Rosique R. G Curbing inflammation in burn patients. Int J Inflamm 2013;2013:715645.
20. Chaudhary NA, Munawar MD, Khan MT, Rehan K, Sadiq A, Bhatti HW, *et al*. Epidemiology, bacteriological profile, and antibiotic sensitivity pattern of burn wounds in the burn unit of a tertiary care hospital. Cureus 2019;11:e4794.
21. Naqvi SZA, Naqvi SA, Usman M, Naqvi SBS. Burn wound infection: Significance of rule of nine in microbial surveillance. Professional Med J 2014;21:869-73.
22. Almuttairi AA, Abdulla AA. Occurrence of class 1, 2, and 3 integrons among multidrug-resistant *Pseudomonas aeruginosa* in Babylon Province, Iraq. Med J Babylon 2023;182.
23. Abdulzahra AN. Study Multidrug Resistance Factors of *Pseudomonas aeruginosa* Isolated from Burns and Wounds by Phenotypic and Molecular Methods. Unpublished Master's Thesis. College of Education for Pure Science/Ibin–Al-Haitham. University of Baghdad; 2020.

24. Rasool AA, Almohana AM. Dissemination multi-drug and carbapenems resistant *Pseudomonas aeruginosa* among clinical isolates in Najaf Hospitals. Al-Kufa Univ J Biol 2017;9.
25. Al- Simary, M, B J. Isolation and Characterization of Bacteriophages Against MDR *Pseudomonas aeruginosa* Isolated from Burn Patients in Basrah Governorate-Iraq. PhD thesis. Department of Biology, Faculty of Science, University of Kuf; 2018.
26. Al-kazrage HAD. Inhibition of Virulence Factors in *Pseudomonas aeruginosa* Isolated from Clinical Samples Using Galardin Loaded AgPEG Nanocomposite. Unpublished PhD Thesis. College of Biotechnology, Al-Nahrain University; 2021.
27. Al-Janahi HCL, Khalil, SA, Almohana AM, Al-sherees HA. A dissemination of new delhi metallo-β-lactamase (blandm) gene in *Pseudomonas aeruginosa* isolates from burn center in Najaf, Iraq. Int J Inf Res Rev 2020;7:7071-6.
28. Rosales-Reyes R, Esposito F, Fuga B, Cerdeira L, Gayosso-Vázquez C, Lezana-Fernández JL, *et al*. Draft genome sequence of a *Pseudomonas aeruginosa* sequence type 3351 strain exhibiting high-level resistance to polymyxins in a pediatric patient with cystic fibrosis in Mexico. Microbiol Resour Announc 2020;9:e01261-19.
29. Khan NH, M A, Yoshizawa S, Hosoya S, Yokota A, Kogure K. Multilocus sequence typing and phylogenetic analyses of *Pseudomonas aeruginosa* isolates from the ocean. Appl Environ Microbiol 2008;74:6194-205.
30. Tönnies H, Prior K, Harmsen D, Mellmann A. Establishment and evaluation of a core genome multilocus sequence typing scheme for whole-genome sequence-based typing of *Pseudomonas aeruginosa*. J Clin Microbiol 2021;59:e01987-20.
31. Ozer EA, Nnah E, Didelot X, Whitaker RJ, Hauser AR. The population structure of *Pseudomonas aeruginosa* is characterized by genetic isolation of exoU+ and exoS+ lineages. Genome Biol Evol 2019;11:1780-96.
32. Rossi E, La Rosa R, Bartell JA, Marvig RL, Haagensen JA, Sommer LM, *et al*. *Pseudomonas aeruginosa* adaptation and evolution in patients with cystic fibrosis. Nat Rev Microbiol 2021;19:331-42.
33. Muthukumarasamy U, Preusse M, Kordes A, Koska M, Schniederjans M, Khaledi A, *et al*. Single-nucleotide polymorphism-based genetic diversity analysis of clinical *Pseudomonas aeruginosa* isolates. Genome Biol Evol 2020;12:396-406.