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Genetic and Molecular Mapping of Bacteria in Chronic Ulcerative Infections among Diabetics Patients

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Abstract

Research investigated *Pseudomonas aeruginosa* genetic profiles that infect diabetic foot ulcers through DNA sequencing examination of samples obtained in Wasit province, Iraq. Standard PCR procedures were used to test ulcers of 90 diabetic patients for *P. aeruginosa* through swab collection. DNA sequencing followed to generate results for six positive samples that received accession numbers for submission to NCBI GenBank. The local strains analyzed through MEGA-11 software demonstrated genetic similarities to a reference strain from India based on NCBI-BLAST database results with identity ranges from 99.15 % to 99.68 % and mutation ranges from 0.0003 % to 0.001 %. Genetic sequencing proved to be highly effective for bacterial pathogen identification in persistent infections according to these research findings. The studied gene marker shows potential value as both a tool for evolutionary research and diagnostic improvement for clinical *P. aeruginosa* monitoring.

Keywords: Molecular detection, Diabetic foot ulcers, *Pseudomonas aeruginosa*.

1. Introduction

Building process and activation mode of the bacterial 30S ribosomal subunit required for protein generation directed by 16S rRNA gene directs [1, 2]. This genetic marker combines both stable and mutable sections to serve as a commonly adopted tool for bacterial classification and evolutionary pattern investigation [3, 4].

The opportunistic pathogen *Pseudomonas aeruginosa* exists as a Gram-negative bacterium which typically produces infections during healthcare-related settings. This pathogen poses serious threats particularly among those whose immune systems are weak [5]. *P. aeruginosa* becomes a particularly difficult pathogen because its arsenal includes virulence factors that include adhesins plus

toxins together with advanced secretion systems which allow it to attach to surfaces while also invading tissues and evading immune responses [6, 7]. Because *P. aeruginosa* demonstrates strong resistance to many antibiotics it becomes challenging to both treat and control infections [8]. People with diabetes who develop diabetic foot ulcers (DFUs) commonly face infection from *Pseudomonas aeruginosa* which acts as a frequent and dangerous bacterium. Diabetic foot ulcers emerge from multiple medical reasons such as impaired wound repair alongside nerve damage and inadequate blood circulation and anatomy abnormalities [9, 10]. The infection of these ulcers produces prolonged hospitalization and reduced patient quality of life together with antibiotic escalation which can ultimately cause necessary amputation according to Navarro-Flores and Cauli [11]. The exact determination of bacterial infection agents at an early stage remains vital for achieving successful treatment and strengthening both infection control procedures and treatment results [12, 13].

The 16S rRNA gene serves as a valuable analytical tool for determining bacterial diversity together with its evolutionary lineages. Diagnostic techniques have improved extensively along with our comprehension of bacterial disease mechanisms because of this gene [14]. Iraqi research on *P. aeruginosa* resistance and virulence genes exists [15-18]. There is minimal work with 16S rRNA gene for phylogenetic analysis. The research assesses genetic similarity between *P. aeruginosa* strains which infect diabetic foot ulcers to understand their diagnostic significance as well as their evolutionary relationships.

2. Materials and Methods

2.1. Sample Collection

A total of 90 diabetic patients with foot ulcers were enrolled from several private clinics throughout Wasit province, Iraq. Using sterile techniques, swab samples were carefully taken directly from the ulcer sites and placed into labeled, sterile tubes. The samples were then transported on ice to the laboratory and stored at –20 °C until they were ready for further analysis.

2.1.1. DNA Extraction and PCR Amplification

The Presto™ Mini gDNA Bacteria Kit from Geneaid in Taiwan enabled the extraction of genomic DNA from swab samples following the producer guidelines. DNA purity and concentration evaluation occurred through analysis on a Nanodrop spectrophotometer produced by Thermo Scientific based in UK. The AccuPower® PCR PreMix Kit (Bioneer, Korea) performed conventional PCR detecting the

P. aeruginosa 16S rRNA gene (F: 5'-TGC CTG GTA GTG GGG GAT AA-3', R: 5'-CCC CTT GCG GTT AGA CTA GC-3') with a 1325 bp product size. In a Thermo Scientific thermal cycler the reactions received an initial denaturation stage at 95 °C for 5 minutes followed by 35 cycles with denaturation at 95 °C for 30 seconds and annealing at 56 °C for 30 seconds and extension at 72 °C for 30 seconds and a final extension stage at 72 °C for 5 minutes.

2.2. Gel Electrophoresis

The results of PCR products became visible through agarose gel electrophoresis using ethidium bromide-stained 1.5 % agarose. The gels operated under 100 volts for a duration of 90 minutes before UV transilluminator visualization (Clinx Science, China). A digital Nikon camera served for image acquisition.

2.3. DNA Sequencing and Phylogenetic Analysis

The DNA sequences of six PCR-positive isolates were sent to Macrogen Inc. (Korea) for their sequencing analysis. Sequence data from the obtained DNA samples were sent to NCBI GenBank to acquire accession numbers. Sequence alignment and phylogenetic analysis occurred through MEGA Version 11 while identity comparisons happened with the NCBI-BLAST database and MSA Viewer. The study evaluated sequence similarity relationships and evolutionary evolution through homology testing and tree-building methods.

2.4. Ethical Approval

This study was conducted following the approval of the Scientific Committee at the College of Medicine, University of Wasit.

2.5. Statistical Analysis

GraphPad Prism software performed the analysis of collected data. We analyzed data using the t-test at a p-value < 0.05 for determining statistical significance.

3. Results and Discussion

The conventional PCR analysis of 16S rRNA gene aimed at *Pseudomonas aeruginosa* detection found this bacterium in 18.89 % (17 out of 90) of samples from diabetic foot ulcer patients as shown in table 1.

Table 1: Prevalence of *Pseudomonas aeruginosa* Among DFU Patients

Total Number of Samples	Positive Samples	Negative Samples	Prevalence (%)
90	17	73	18.89%

Six *P. aeruginosa* samples confirmed by PCR underwent sequencing where their DNA sequences were submitted to GenBank and assigned accession numbers PQ650121.1, PQ650122.1, PQ650123.1, PQ650124.1, PQ650125.1, and PQ650126.1.

The phylogenetic trees with multiple sequence alignment and homology evaluation were performed through MEGA-11 software and NCBI tools that included MSA viewer and BLAST.

High levels of sequence similarity were observed between *P. aeruginosa* samples collected from the local area and reference Indian *P. aeruginosa* strain MW073526.1. Based on their percentage identity ranging from 99.15 % to 99.68 % and their mutation percentage ranging from 0.0003 % to 0.001 % as demonstrated in tables 4, 5 and 6. Moreover, a summary of genetic sequences for *Pseudomonas aeruginosa* strains are shown in table 2, and table 3.

Table 2: Local IQ.HASS Isolates and of *Pseudomonas aeruginosa* with Corresponding GenBank Accession Numbers

Sequence ID	Strain Name	Accession Number
Seq1	Pseudomonas aeruginosa IQ.HASS isolate No.1	PQ650121.1
Seq2	Pseudomonas aeruginosa IQ.HASS isolate No.2	PQ650122.1
Seq3	Pseudomonas aeruginosa IQ.HASS isolate No.3	PQ650123.1
Seq4	Pseudomonas aeruginosa IQ.HASS isolate No.4	PQ650124.1
Seq5	Pseudomonas aeruginosa IQ.HASS isolate No.5	PQ650125.1
Seq6	Pseudomonas aeruginosa IQ.HASS isolate No.6	PQ650126.1

Table 3: Table: International Reference Strains of *Pseudomonas aeruginosa* with Corresponding GenBank Accession Numbers

Strain Name	Accession Number
Pseudomonas aeruginosa strain KDWS03/India	MW073526.1
Pseudomonas aeruginosa strain RA5/India	MH160762.1
Pseudomonas aeruginosa strain PSA01/India	MH128359.1
Pseudomonas aeruginosa strain TEN01/India	KP282446.1
Pseudomonas aeruginosa strain MK13/India	KF530282.1
Pseudomonas aeruginosa strain lytW/China	PQ632476.1
Pseudomonas aeruginosa strain S2/India	PQ579840.1
Pseudomonas aeruginosa strain S-M-01/Indonesia	PQ573347.1
Pseudomonas aeruginosa strain FB3/Pakistan	HQ658764.1
Pseudomonas aeruginosa strain SOU01/India	MT860290.1
Pseudomonas aeruginosa strain VETSOU/India	MT889999.1
Pseudomonas aeruginosa strain EN18/Iran	MH142640.1
Pseudomonas aeruginosa strain CIFRIB-10/India	OP554288.1
Pseudomonas aeruginosa strain ARASP187T/Iran	MH489017.1
Pseudomonas aeruginosa strain RB4/Nigeria	MK271756.1
Pseudomonas aeruginosa strain SN2/Thailand	LC485221.1
Pseudomonas aeruginosa strain DM23/China	QR574359.1
Pseudomonas aeruginosa strain IHR-BDM6/India	KP403282.1
Pseudomonas aeruginosa strain RPB21/India	MT772196.1

Seq1 *Pseudomonas aeruginosa* IQ.HASS isolate No.1 (PQ650121.1), Seq2 *Pseudomonas aeruginosa* IQ.HASS isolate No.2 (PQ650122.1), Seq3 *Pseudomonas aeruginosa* IQ.HASS isolate No.3 (PQ650123.1), Seq4 *Pseudomonas aeruginosa* IQ.HASS isolate No.4 (PQ650124.1), Seq5 *Pseudomonas aeruginosa* IQ.HASS isolate No.5 (PQ650125.1), and Seq6 *Pseudomonas aeruginosa* IQ.HASS isolate No.6 (PQ650126.1).

Additionally, the following international strains were included: *Pseudomonas aeruginosa* strain KDWS03/India (MW073526.1), RA5/India (MH160762.1), PSA01/India (MH128359.1), TEN01/India (KP282446.1), MK13/India (KF530282.1), lytW/China (PQ632476.1), S2/India (PQ579840.1), S-M-01/Indonesia (PQ573347.1), FB3/Pakistan (HQ658764.1), SOU01/India (MT860290.1), VETSOU/India (MT889999.1), EN18/Iran (MH142640.1), CIFRIB-10/India (OP554288.1), ARASP187T/Iran (MH489017.1), RB4/Nigeria (MK271756.1), SN2/Thailand (LC485221.1), DM23/China (QR574359.1), IHR-BDM6/India (KP403282.1), and RPB21/India (MT772196.1).

Each strain analysis contains data about strain identification with public database accession numbers followed by genetic region sequence information. The study of genetic variations and

relationships and pathogen-specific characteristics depends fundamentally on this type of data for comparing different strains of opportunistic pathogens.

Table 4: GenBank Accession Numbers for Sequenced *P. aeruginosa* Isolates from DFU Patients

Isolate Code	GenBank Accession Number	Sequence Type	Source
IQ.HASS-1	PQ650121.1	16S rRNA gene (partial)	Diabetic Foot Ulcer
IQ.HASS-2	PQ650122.1	16S rRNA gene (partial)	Diabetic Foot Ulcer
IQ.HASS-3	PQ650123.1	16S rRNA gene (partial)	Diabetic Foot Ulcer
IQ.HASS-4	PQ650124.1	16S rRNA gene (partial)	Diabetic Foot Ulcer
IQ.HASS-5	PQ650125.1	16S rRNA gene (partial)	Diabetic Foot Ulcer
IQ.HASS-6	PQ650126.1	16S rRNA gene (partial)	Diabetic Foot Ulcer

Table 5: Homology Sequence identity (%) for local and NCBI-BLAST *P. aeruginosa* isolates

Local isolate		NCBI isolate			
Name	Access No.	Species	Country	Access No.	%
IQ.HASS-1	PQ650121.1	*P. aeruginosa*	India	MW073526.1	99.23
IQ.HASS-2	PQ650122.1	*P. aeruginosa*	India	MW073526.1	99.27
IQ.HASS-3	PQ650123.1	*P. aeruginosa*	India	MW073526.1	99.68
IQ.HASS-4	PQ650124.1	*P. aeruginosa*	India	MW073526.1	98.40
IQ.HASS-5	PQ650125.1	*P. aeruginosa*	India	MW073526.1	98.32
IQ.HASS-6	PQ650126.1	*P. aeruginosa*	India	MW073526.1	99.15

The 16S rRNA gene has an essential foundation for conducting *Pseudomonas aeruginosa* research because it facilitates evolutionary analysis of the organism [19]. The highly conserved genetic marker provides researchers with sequence polymorphisms and mutations data to reveal *P. aeruginosa* isolates genetic differences when sampled between clinical sites and environments [20]. The study of *Pseudomonas aeruginosa* at the molecular level helps explain immune evasion and antimicrobial resistance mechanisms so researchers can create targeted treatments and new drug candidates [6, 21].

The research of *P. aeruginosa* faces a continuous challenge regarding the exact classification and identification of multiple strain types. The intricate nature of strain differentiation results in variable laboratory reporting and endangers epidemiological tracking because it reduces the ability to compare research center findings [22].

The absence of suitable rapid diagnostic tools for detecting *P. aeruginosa* infections produces delayed appropriate treatment initiation that worsens patient outcomes [23].

The analysis through 16S rRNA gene sequencing showed that *P. aeruginosa* existed in 18.89 % of patients with diabetic foot ulcers. This observation matches previous studies that determined *P. aeruginosa* occurrence in various geographic regions such as India (17.42 %) [24], Egypt (19.4 %) [25]. The United States (4.5 %) [26], Turkey (25.8 %) [27], China (10.5 %) [28], Iraq (34.4 %) [16], and Iran (16.6 %) [29]. The diverse rates observed for diabetic foot ulcer cases stem from different diagnostic practices and selection methods for patients, varying population sizes together with healthcare development levels and the frequency of patients with diabetes mellitus that contributes substantially to hospital admissions as well as limb amputation.

The management of DFUs becomes challenging because diabetes-related physiological complications prevent normal wound healing according to Moore *et al*. [30]. For optimal patient results one must adopt a wide-ranging clinical method that integrates care for wounds alongside offloading strategies and infection prevention and preventive teaching [31]. Healing rates become improved and overall treatment expenses decrease while risk of amputation reduces substantially through complete treatment methods which consist of surgical debridement combined with targeted antimicrobial therapy and advanced biologic treatments after wound bed preparation when ischemia or osteomyelitis are absent [32].

Preventing DFU recurrence stands as the essential priority due to its critical importance. Research shows that the recurrence rates of DFUs remain incredibly high after wound healing because approximately 40 % of patients experience reoccurrences during their first year and 65% suffer recurrences over a three-year healing period [33]. Molecular tracking and typifying new multidrug-resistant *P. aeruginosa* DFU patient strains must remain an ongoing field priority. The simultaneous reduction of infection occurrence and pathogen resistance needs these initiatives because they restrain the

way resistance-driving genetic adaptations develop in resistant microorganisms [34].

Table 6: Phylogenetic Relationships Between Local and NCBI-BLAST *P. aeruginosa* Isolates.

Isolate Name	Accession Number	Country	Bootstrap Value (%)	Relationship to Local Isolates
Pseudomonas aeruginosa strain PSA01/India	MH128359.1	India	90 %	Distantly related
Pseudomonas aeruginosa strain CIFRI.B-10/India	OP554286.1	India	94 %	Distantly related
Pseudomonas aeruginosa strain RA5/India	MH160762.1	India	93 %	Moderately related
Pseudomonas aeruginosa strain FB3/Pakistan	HQ658764.1	Pakistan	97 %	Moderately related
Pseudomonas aeruginosa strain DM23/China	OR574359.1	China	94 %	Moderately related
Pseudomonas aeruginosa strain S2/India	PQ579840.1	India	96 %	Closely related
Pseudomonas aeruginosa strain TEN01/India	KP282446.1	India	91 %	Closely related
Pseudomonas aeruginosa strain MK13/India	KF530282.1	India	94 %	Closely related
Pseudomonas aeruginosa strain RB4/Nigeria	MK271756.1	Nigeria	91 %	Moderately related
Pseudomonas aeruginosa strain lyt/China	PQ632476.1	China	87 %	Moderately related
Pseudomonas aeruginosa SN2/Thailand	LC485221.1	Thailand	92 %	Moderately related
Pseudomonas aeruginosa strain S-M-01/Indonesia	PQ573347.1	Indonesia	91 %	Moderately related
Pseudomonas aeruginosa strain IIHR-BDM6/India	KP403282.1	India	89 %	Moderately related

Table 7: Phylogenetic Relationships Between Local and NCBI-BLAST *P. aeruginosa* Isolates.

Isolate Name	Accession Number	Country	Bootstrap Value (%)	Relationship to Local Isolates
Pseudomonas aeruginosa strain RPB21/India	MT772196.1	India	89 %	Moderately related
Pseudomonas aeruginosa strain ArASP187T/Iran	MH1489017.1	Iran	89 %	Moderately related
Pseudomonas aeruginosa strain EN18/Iran	MH142640.1	Iran	89 %	Moderately related
Pseudomonas aeruginosa strain VETSOU/India	MT889999.1	India	89 %	Moderately related
Pseudomonas aeruginosa strain SOU01/India	MT860290.1	India	89 %	Moderately related
Pseudomonas aeruginosa strain KDWS03/India	MW073526.1	India	89 %	Closely related (NCBI-BLAST isolate)
Seq5 *Pseudomonas aeruginosa* IQ.HASS isolate No. 5	PQ650125.1	Iraq	82 %	Closely related (Local isolate)
Seq6 *Pseudomonas aeruginosa* IQ.HASS isolate No. 6	PQ650126.1	Iraq	82 %	Closely related (Local isolate)
Seq1 *Pseudomonas aeruginosa* IQ.HASS isolate No. 1	PQ650121.1	Iraq	89 %	Closely related (Local isolate)
Seq2 *Pseudomonas aeruginosa* IQ.HASS isolate No. 2	PQ650122.1	Iraq	82 %	Closely related (Local isolate)
Seq3 *Pseudomonas aeruginosa* IQ.HASS isolate No. 3	PQ650123.1	Iraq	82 %	Closely related (Local isolate)
Seq4 *Pseudomonas aeruginosa* IQ.HASS isolate No. 4	PQ650124.1	Iraq	81 %	Closely related (Local isolate)

4. Conclusion

The research results demonstrate that the 16S rRNA gene provides exceptional molecular methods to precisely detect *Pseudomonas aeruginosa* infecting diabetic foot ulcer patients. The research demonstrates that this gene shows great potential for detecting and identifying multiple bacterial pathogens within different infectious medical environments. The 16S rRNA gene maintains extraordinary conservation properties which makes it an important molecular approach for understanding *P. aeruginosa* biology combined with evolutionary processes and pathogenic characteristics along with other significant human disease-causing bacteria. Further research that studies 16S rRNA gene sequence variability together with its functional significance remains imperative for future investigations. Research into microbial behavior and evolution through such investigations will help scientists create more precise, along with efficient sustainable methods to prevent and manage *P. aeruginosa* infections.

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