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Prevalence of the pelA gene in Pseudomonas aeruginosa and serum IL-22 levels in patients with diabetic foot ulcers

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Abstract

Objective: Diabetic foot ulcers (DFUs) are a major complication of diabetes mellitus, often complicated by Pseudomonas aeruginosa, a biofilm-forming pathogen associated with delayed wound healing. This study evaluated the prevalence of the pelA gene, a determinant of biofilm production, in P. aeruginosa isolates from DFU patients and assessed systemic immune response through serum interleukin-22 (IL-22) levels.

Methods: A cross-sectional study was conducted from May 2024 to January 2025, including 48 DFU patients and 48 age- and sex-matched healthy controls. Wound swabs were collected from patients, and P. aeruginosa isolates were identified using the VITEK-2 system or PCR amplification of the 16S rRNA gene. The presence of the pelA gene was confirmed by PCR. Serum IL-22 concentrations were measured using ELISA. Data were analysed with SPSS v26, using t-tests or Mann–Whitney U tests as appropriate, with $P < 0.05$ considered significant.

Results: All 48 P. aeruginosa isolates (100%) carried the pelA gene. Mean serum IL-22 levels were significantly higher in DFU patients compared with healthy controls (31.04 ± 0.22 pg/mL vs. 28.89 ± 0.84 pg/mL; $P = 0.015$).

Conclusion: The universal detection of pelA highlights its potential as a molecular marker of biofilm-forming P. aeruginosa in DFUs. Elevated IL-22 levels indicate an active host immune response in affected patients. Combined microbial and immunological markers may support improved assessment of infection dynamics in diabetic foot ulcers, although further multicentre and longitudinal studies are warranted.

Keywords: Diabetic foot ulcer, Pseudomonas aeruginosa, pelA gene, Biofilm, IL-22, Virulence factor

Plain English Summary

Diabetic foot ulcers are one of the most serious complications of diabetes and often lead to long-lasting infections that are difficult to treat. A common cause of these infections is the bacterium Pseudomonas aeruginosa, which can form a sticky protective layer known as a biofilm. Biofilms make bacteria more resistant to antibiotics and enable them to persist in wounds. In this study, we collected samples from 48 patients with diabetic foot ulcers and compared them with 48 healthy individuals. We searched for a gene called pelA, which helps P. aeruginosa form biofilms, and also measured levels of a blood protein called interleukin-22 (IL-22), a component of the body's immune response. We found that all P. aeruginosa samples carried the pelA gene, suggesting that biofilm-forming strains are very common in these infections. Patients also had higher levels of IL-22 compared with healthy controls, showing that the immune system was actively responding to infection. These findings suggest that combining bacterial and immune markers could help improve how doctors assess and monitor diabetic foot infections.

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Introduction

Diabetic foot ulcers (DFUs) are among the most burdensome complications of diabetes mellitus, affecting an estimated 15–25% of people with diabetes during their lifetime and contributing substantially to lower-limb amputation risk and recurrent morbidity (1). Impaired perfusion, neuropathy, and persistent microbial colonisation converge to delay wound healing, and current international guidance emphasises rigorous diagnosis and targeted treatment of infection to improve outcomes [9]. Against this clinical backdrop, understanding microbe–host interactions that sustain chronicity in DFUs remains a priority.

Pseudomonas aeruginosa is a frequent and clinically important pathogen in DFU infections and has been associated with delayed healing and greater wound chronicity (2). Beyond its broad intrinsic and acquired antimicrobial resistance, P. aeruginosa is adept at forming biofilms within the wound microenvironment, structured microbial communities encased in a self-produced extracellular matrix that shields bacteria from antibiotics and host defences (3). Two exopolysaccharides, Pel and Psl, are central to the P. aeruginosa biofilm architecture and can provide functional redundancy for matrix integrity and adherence (4). Pel, in particular, contributes to biofilm cohesion and interacts with extracellular DNA to enhance matrix cross-linking and tolerance, features that are directly relevant to persistence in chronic wounds (5).

Within the host, epithelial repair programs and immune pathways are activated in response to infection and injury. Interleukin-22 (IL-22), produced by T helper 22 (Th22) cells and innate lymphoid cells, acts primarily on epithelial and stromal cells to promote proliferation, barrier restoration, and antimicrobial defence (6, 7, 8). Experimental models of diabetes support pro-regenerative roles for IL-22 in cutaneous repair, yet IL-22 can also participate in inflammatory circuits whose net effect in chronic infection may depend on context, magnitude, and timing of the response (6, 7). Clarifying how IL-22 behaves in the setting of DFU infection, particularly where biofilm-forming P. aeruginosa predominates, could yield insight into wound chronicity and potential biomarkers of disease activity.

Despite the recognised importance of biofilms in DFUs and the emerging interest in IL-22 biology, there are limited data that concurrently examine P. aeruginosa biofilm determinants and systemic IL-22 responses in patients with DFUs. The Pel biosynthetic locus offers a tractable molecular handle for such inquiry: genes within the pel operon are essential for Pel production and biofilm structural stability, and their detection in clinical isolates provides a pragmatic surrogate for Pel-mediated biofilm capacity (4, 5).

Accordingly, this study aimed (i) to determine the prevalence of the pelA gene among P. aeruginosa isolates recovered from DFU patients and (ii) to compare circulating IL-22 levels between DFU patients and healthy controls. By pairing a molecular marker of biofilm potential with a host cytokine implicated in epithelial repair, we sought to generate integrative data that illuminate pathogen, host dynamics in DFU infection and may guide future hypothesis-driven work on prognostic markers or therapeutic targets (1, 2, 3, 4, 5, 6, 7, 8, 9). These aims align with current recommendations to refine infection assessment in DFUs while avoiding claims beyond the scope of our cross-sectional design.

Materials and Methods

Study Design

A cross-sectional study was conducted between May 2024 and January 2025 at the University of Al-Qadisiyah, Iraq.

Study Population and Sample Size

The study included 48 patients with clinically diagnosed diabetic foot ulcers (DFUs) and 48 healthy controls. The sample size was determined pragmatically based on the expected number of patients presenting to the diabetic foot clinic during the study period, previous similar studies reporting sample sizes ranging from 30–60 patients (2), and resource availability. Power calculations suggested that this sample size would provide >80% power to detect a mean difference of 2 pg/mL in IL-22 levels at $\alpha = 0.05$.

Inclusion Criteria for DFU Patients

1. Adults (≥18 years) with type 2 diabetes mellitus.
2. Presence of a DFU confirmed by a diabetologist, using the International Working Group on the Diabetic Foot (IWGDF) diagnostic criteria (9).
3. No systemic antibiotic therapy within the preceding two weeks.
4. Ability to provide informed consent.

Exclusion Criteria for DFU Patients

1. Presence of non-diabetic chronic wounds (e.g., venous or arterial ulcers, pressure ulcers).
2. Immunosuppressive therapy or known immunodeficiency disorders.
3. Concurrent systemic infections unrelated to the foot ulcer.

Control Group Selection

Healthy controls (n = 48) were recruited from hospital staff and patient attendants. They were age- and sex-matched to the patient group, non-

diabetic (fasting blood glucose <100 mg/dL), without any acute or chronic infections, and not receiving antibiotics or immunosuppressants within the previous two weeks.

Sample Collection
For each DFU patient, two types of samples were collected:
1. Wound swabs (n = 48): Obtained after debridement and cleansing of the ulcer with sterile saline, before initiation of any new antibiotic treatment.
2. Venous blood samples (n = 48): Collected for immunological assessment.
3. Additionally, venous blood samples from the 48 healthy controls were obtained under aseptic conditions.

Bacterial Identification
Twenty-four isolates were identified using the VITEK-2 Compact system (bioMérieux, France) (10). The remaining isolates were confirmed via PCR amplification of the 16S rRNA gene using universal primers (10). PCR products were separated on a 1.5% agarose gel stained with ethidium bromide, and bands were visualised under UV transillumination.

Detection of the pelA Gene
DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Germany) (12). PCR was performed using pelA-specific primers [4]. The amplicons were visualised on a 1.5% agarose gel, with a band at 118 bp indicating positivity for pelA.

Measurement of IL-22
Serum IL-22 levels were quantified using a commercial ELISA kit (R&D Systems, USA), following the manufacturer's instructions (13). Absorbance was measured at 450 nm, and concentrations were determined against a standard curve.

Statistical Analysis
Data were analysed using SPSS version 26.0 (IBM, USA). Continuous variables were expressed as mean ± standard error (SE). Normality of distribution was tested using the Shapiro–Wilk test. Independent-samples t-test was applied for normally distributed data, while the Mann–Whitney U test was used for non-parametric comparisons. Categorical variables (e.g., sex distribution) were analysed using the chi-square test. A two-sided P value <0.05 was considered statistically significant.

Results
Patient Demographics
A total of 48 patients with diabetic foot ulcers (DFUs) and 48 healthy controls were included. The mean age of DFU patients was 57.4 ± 9.8 years (range: 42–76 years), compared with 56.2 ± 10.3 years (range: 40–74 years) in controls (P = 0.62). The sex distribution was comparable between groups (patients: 28 males, 20 females; controls: 26 males, 22 females; P = 0.68). The mean duration of diabetes among patients was 11.3 ± 6.4 years. Hypertension was the most common comorbidity (54%), followed by dyslipidaemia (39%). None of the control participants had diabetes or chronic comorbidities.

Molecular Identification of Pseudomonas aeruginosa
PCR amplification of the 16S rRNA gene produced the expected ~1500 bp band in 24 isolates, confirming them as Pseudomonas aeruginosa. The remaining 24 isolates were identified using the VITEK-2 system. Together, these analyses verified all 48 wound isolates as P. aeruginosa.

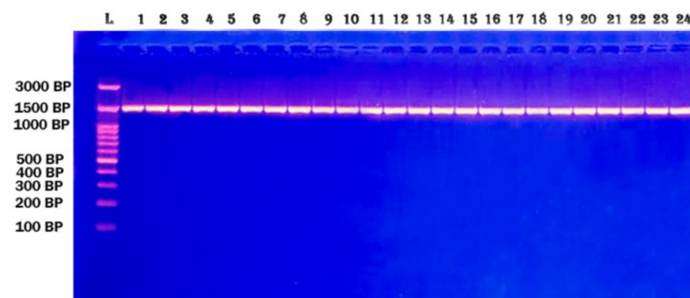

Figure 1: Electrophoresis of PCR-amplified 16S rRNA gene fragments of P. aeruginosa isolates.
DNA was separated on a 1.5% agarose gel run at 80 V for 95 minutes. Lanes correspond to representative isolates (numbered 1–24), each showing the expected 1500 bp band. The molecular ladder ranges from 100 to 3000 bp. (Note: Figure must be submitted in high-resolution, ≥300 dpi, with clear lane demarcations.). Lane M: Molecular weight ladder

Detection of the pelA Gene
All 48/48 isolates (100%) carried the pelA gene, indicated by the expected 118 bp amplicon on agarose gel electrophoresis.

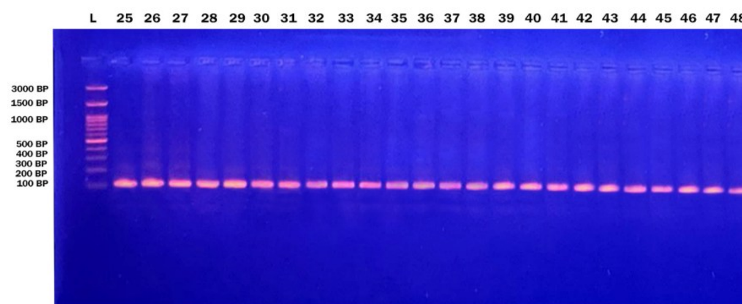

Figure 2: PCR amplification of the pelA gene from P. aeruginosa isolates.
DNA fragments were visualised on a 1.5% agarose gel stained with ethidium bromide, and the gel was run at 80 V for 95 minutes. All 48 isolates showed the expected 118 bp band, confirming the universal presence of the pelA gene. Lane M: Molecular weight ladder

Serum IL-22 Levels
Mean serum IL-22 levels were significantly higher in DFU patients compared with healthy controls (31.04 ± 0.22 pg/mL vs. 28.89 ± 0.84 pg/mL; P = 0.015).

Table 1: Serum interleukin-22 (IL-22) levels in patients and controls

Parameter	Patients (n = 48)	Controls (n = 48)	Test statistic	P value
IL-22 (pg/mL), mean ± SE	31.04 ± 0.22	28.89 ± 0.84	t = 2.53	0.015*
Range (min–max)	29.8 – 34.5	26.2 – 30.5	—	—

* Significant at P < 0.05.

Summary of Key Findings
All P. aeruginosa isolates from DFUs were confirmed to carry the pelA gene (100%). Serum IL-22 levels were significantly elevated in DFU patients compared with healthy controls. Demographic characteristics were balanced between groups, reducing confounding by age or sex.

Discussion
This study investigated the presence of the pelA gene in Pseudomonas aeruginosa isolates from diabetic foot ulcer (DFU) patients and examined systemic immune response through circulating IL-22 levels. All isolates (100%) harboured the pelA gene, and IL-22 concentrations were significantly higher in DFU patients than in healthy controls. These findings highlight the concurrent presence of microbial virulence determinants and host immunological responses in the chronic wound setting.

Prevalence of pelA and Implications
The universal detection of pelA in our isolates contrasts with earlier studies that reported variable prevalence rates. For example, Colvin et al. demonstrated pel-dependent structural redundancy within the P. aeruginosa biofilm matrix, though not all isolates expressed Pel polysaccharide (4). A recent report found pelA positivity in approximately two-thirds of clinical isolates (14), indicating heterogeneity across populations and sampling contexts. The 100% prevalence observed in our cohort may suggest strong selective pressure for biofilm-forming strains in the DFU microenvironment, which is characterised by chronic inflammation, impaired vascularisation, and repeated antimicrobial exposure. While these findings underscore the pathogenic potential of P. aeruginosa in DFUs, larger multicentre studies are required to determine whether this reflects regional epidemiology or sampling limitations.

Comparison with African and LMIC Data
The predominance of P. aeruginosa in DFUs has been reported globally, but its clinical impact may be especially significant in low- and middle-income countries (LMICs), where delayed presentation, limited access to wound care, and constrained antimicrobial options contribute to poorer outcomes. A systematic review reported wide variability in P. aeruginosa prevalence in DFUs, with particularly high rates documented in Middle Eastern and African cohorts (2). In Nigeria, for example, DFU pathogens commonly include Staphylococcus aureus, Escherichia coli, and P. aeruginosa, with the latter often linked to

multidrug resistance and prolonged healing (15). Our finding of universal pelA positivity is therefore clinically relevant in the LMIC context, where biofilm-associated resistance may further limit therapeutic effectiveness.

IL-22 Levels and Host Response

IL-22 plays a dual role in tissue homeostasis. Experimental models have shown their capacity to enhance epithelial proliferation and wound repair in diabetic settings (6), while other studies suggest that uncontrolled IL-22 signalling may contribute to persistent inflammation (7). In this study, serum IL-22 was significantly elevated in DFU patients compared to controls. This finding is consistent with its known role in epithelial defence and repair, although the cross-sectional design precludes conclusions about causality or long-term effects. Importantly, our data should be interpreted as reflecting an active immune response to chronic infection, rather than speculating on detrimental roles of IL-22 in this context. Longitudinal studies tracking IL-22 dynamics during treatment could provide more definitive insights into its prognostic utility.

Clinical and Research Implications

The consistent detection of pelA in DFU-associated P. aeruginosa underscores the potential of this gene as a molecular marker for biofilm-forming strains in chronic wound infections. At the same time, the measurable elevation of IL-22 suggests possible value in incorporating immunological profiling into DFU research and management. Together, microbial and host biomarkers may help refine diagnostic and prognostic strategies, particularly in LMIC settings where empirical treatment remains the norm.

Study limitations

This study has several important limitations that should be considered when interpreting the findings. First, the relatively small sample size restricts the statistical power and limits the generalizability of the results to broader patient populations. Second, because the study was conducted at a single centre, the observed patterns may reflect local microbial ecology and may not be representative of other geographic regions or healthcare settings. Third, the cross-sectional design does not allow for causal inference or for tracking changes in IL-22 levels over time, particularly in response to treatment or clinical outcomes. Finally, the absence of antibiotic susceptibility testing in this study prevents us from linking the universal presence of the pelA gene to actual patterns of antimicrobial resistance. These limitations highlight the need for larger, multicentre, and longitudinal studies to validate our findings and to explore their clinical significance more comprehensively.

Conclusion

In this study, Pseudomonas aeruginosa isolated from diabetic foot ulcers consistently harboured the pelA gene, underscoring its potential role as a molecular marker of biofilm-forming strains in this setting. Circulating IL-22 levels were significantly elevated in affected patients compared with healthy controls, reflecting an active host immune response. Taken together, these findings suggest that combining genetic and immunological markers may enhance the assessment of infection dynamics in diabetic foot ulcers. Further multicentre and longitudinal research is needed to validate these observations and explore their clinical utility in guiding diagnosis and management.

List of Abbreviations

DFU: Diabetic Foot Ulcer
IL-22: Interleukin-22
LMIC: Low- and Middle-Income Country
PCR: Polymerase Chain Reaction
Bp: Base Pairs
SE: Standard Error
SPSS: Statistical Package for the Social Sciences
Th22: T Helper 22 Cells
IWGDF: International Working Group on the Diabetic Foot

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of the College of Education, University of Al-Qadisiyah (Approval No: EDU-BIO-2024-11). Written informed consent was obtained from all participants before enrolment.

Consent for Publication

All the authors gave consent for the publication of the work under the Creative Commons Attribution Non-Commercial 4.0 license.

Availability of Data

Data for this work is available from the authors and may be provided upon reasonable request.

Conflicts of Interest

The authors declare that there are no conflicts of interest related to this study.

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Authors' contributions

AMRK conceived and designed the study, supervised sample collection, and contributed to drafting the manuscript. KHM performed the laboratory analyses, statistical interpretation, and contributed to manuscript writing. Both authors reviewed and approved the final version of the manuscript.

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References

1. Armstrong DG, Boulton AJ, Bus SA. Diabetic foot ulcers and their recurrence. New England Journal of Medicine. 2017 Jun 15;376(24):2367-75. https://doi.org/10.1056/NEJMra1615439
2. Garousi M, MonazamiTabar S, Mirazi H, Farrokhi Z, Khaledi A, Shakerimoghaddam A. Epidemiology of Pseudomonas aeruginosa in diabetic foot infections: a global systematic review and meta-analysis. Germs. 2023 Dec 31;13(4):362. https://doi.org/10.18683/germs.2023.1406
3. Flemming HC, van Hullebusch ED, Neu TR, Nielsen PH, Seviour T, Stoodley P, Wingender J, Wuertz S. The biofilm matrix: multitasking in a shared space. Nature Reviews Microbiology. 2023 Feb;21(2):70-86. https://doi.org/10.1038/s41579-022-00791-0
4. Colvin KM, Irie Y, Tart CS, Urbano R, Whitney JC, Ryder C, Howell PL, Wozniak DJ, Parsek MR. The Pel and Psl polysaccharides provide Pseudomonas aeruginosa structural redundancy within the biofilm matrix. Environmental microbiology. 2012 Aug;14(8):1913-28. https://doi.org/10.1111/j.1462-2920.2011.02657.x
5. Jennings LK, Storek KM, Ledvina HE, Coulon C, Marmont LS, Sadovskaya I, Secor PR, Tseng BS, Scian M, Filloux A, Wozniak DJ. Pel is a cationic exopolysaccharide that cross-links extracellular DNA in the Pseudomonas aeruginosa biofilm matrix. Proceedings of the National Academy of Sciences. 2015 Sep 8;112(36):11353-8. https://doi.org/10.1073/pnas.1503058112
6. Kolumam G, Wu X, Lee WP, Hackney JA, Zavala-Solorio J, Gandham V, Danilenko DM, Arora P, Wang X, Ouyang W. IL-22R ligands IL-20, IL-22, and IL-24 promote wound healing in diabetic db/db mice. PloS one. 2017 Jan 26;12(1):e0170639. https://doi.org/10.1371/journal.pone.0170639
7. Alabbas SY, Begun J, Florin TH, Oancea I. The role of IL-22 in the resolution of sterile and nonsterile inflammation. Clinical & translational immunology. 2018;7(4):e1017. https://doi.org/10.1002/cti2.1017
8. Barnes JL, Plank MW, Asquith K, Maltby S, Sabino LR, Kaiko GE, Lochrin A, Horvat JC, Mayall JR, Kim RY, Hansbro PM. T-helper 22 cells develop as a distinct lineage from Th17 cells during bacterial infection and phenotypic stability is regulated by T-bet. Mucosal immunology. 2021 Sep;14(5):1077-87. https://doi.org/10.1038/s41385-021-00414-6
9. Lipsky BA, Senneville É, Abbas ZG, Aragón-Sánchez J, Diggle M, Embil JM, Kono S, Lavery LA, Malone M, Van Asten SA, Urbančič-Rovan V. Guidelines on the diagnosis and treatment of foot infection in persons with diabetes (IWGDF 2019 update). Diabetes/metabolism research and reviews. 2020 Mar;36:e3280. https://doi.org/10.1002/dmrr.3280
10. Csiki-Fejer E, Traczewski M, Procop GW, Davis TE, Hackel M, Zambardi G. Performance evaluation of plazomicin susceptibility testing of Enterobacterales on VITEK 2 and VITEK 2 Compact Systems. Journal of Clinical Microbiology. 2025 Aug 1:e00449-25. https://doi.org/10.1128/jcm.00449-25
11. Tlou M, Ndou B, Mabona N, Khwathisi A, Ateba C, Madala N, Serepa-Dlamini MH. Next generation sequencing-aided screening, isolation, molecular identification, and antimicrobial potential for bacterial endophytes from the medicinal plant, Elephantorrhiza elephantina. Frontiers in microbiology. 2024 May 22;15:1383854. https://doi.org/10.3389/fmicb.2024.1383854
12. Qiagen. QIAamp DNA Mini Kit Handbook. 2022.
13. R&D Systems. Human IL-22 Quantikine ELISA Kit. 2023.
14. Subai ZO, Al-Galebi AA. Molecular detection of virulence and carbapenem resistance genes in clinical isolates of Pseudomonas Aeruginosa from Iraqi hospitals. Babcock Univ. Med. J.2025 8(2):226-233.
15. Anyim O, Okafor C, Young E, Obumneme-Anyim I, Nwatu C. Pattern and microbiological characteristics of diabetic foot ulcers in a Nigerian tertiary hospital. African health sciences. 2019 Apr 18;19(1):1617-27. https://doi.org/10.4314/ahs.v19i1.37