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الكشف عن بكتيريا *Staphylococcus aureus* وبكتيريا *Pseudomonas aeruginosa* لدى مرضى قرحة القدم السكري في محافظة واسط

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المستخلص:

تُعدّ عدوى قرحة القدم السكرية مشكلة سريرية خطيرة تنشأ عند تداخل الاضطراب الاستقلابي المزمن، واعتلال الأعصاب، وقصور الأوعية الدموية، وتلف الأنسجة، والغزو البكتيري. اجري هذا البحث من بيانات رسالة الماجستير، وركّز فقط على أهمّ ممرضين بكتيريين مُعزولين من مرضى قرحة القدم السكرية في محافظة واسط، وهما المكوّرات العنقودية الذهبية (*Staphylococcus aureus*) والزائفة الزنجارية (*Pseudomonas aeruginosa*). تضمنت الدراسة الأصلية ٩٠ مشاركًا، منهم ٣٥ مريضًا بقرحة القدم السكرية، و٣٥ مريضًا سكريًا دون قرحة، و٢٠ شخصًا سليمًا كمجموعة ضابطة. زُرعت مسحات الجروح المأخوذة من مجموعة قرحة القدم السكرية وتم التعرف عليها باستخدام الطرائق الميكروبيولوجية التقليدية، والأوساط الزرعية الانتقائية، ونظام VITEK٢ الآلي، وتقنية تفاعل البوليميراز المتسلسل (PCR).كشفت النتائج البكتريولوجية أنّ الزائفة الزنجارية شكّلت أعلى نسبة من العزلات، إذ بلغت ١٧ من أصل ٣٥ حالة (٤٨,٦%)، تلتها المكوّرات العنقودية الذهبية في ١٣ حالة (٣٧,١%)، بينما شكّلت بكتريا أخرى الحالات الخمس المتبقية (١٤,٣%).

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وكانت القروح أكثر شيوعًا في القدم اليسرى، وغالبًا ما توضّعت في منطقة أصابع القدم ومقدمتها. وبين التحليل الجزيئي تطابقا كاملا مع التشخيص الظاهري، من خلال تضخيم جين S rRNA١٦ والجينات النوعية لكل نوع: جين nuc للمكوّرات العنقودية الذهبية، وجين oprL للزائفة الزنجارية، في الوقت الذي تباينت بين احتمالية التشخيص بنظام ٢VITEK بين ٩٠ و٩٩%. وأظهر اختبار الحساسية للمضادات الحيوية عن مقاومة الميثيسيلين لدى عزلات المكوّرات العنقودية الذهبية، ومقاومة الفلوروكينولونات والكاربابينيمات لدى عزلات الزائفة الزنجارية، علاوة على ضعف السيطرة على مستوى السكر التراكمي لدى كلتا المجموعتين. وتؤكد النتائج الأهمية السريرية لكلا الممرضين بسبب قدرتهما على الاستمرارفي الجروح المزمنة، وتكوين الأغشية الحيوية، ومقاومة مضادات الميكروبات، وتأخير التئام الجرح. وتدعم النتائج اعتماد الجمع بين الطرائق الزرعية والآلية والجزيئية لتحقيق تشخيص دقيق ومعالجة سريرية مناسبة لعدوى القدم السكرية في محافظة واسط.

الكلمات المفتاحية: قرحة القدم السكرية، المكورات العنقودية الذهبية (*Staphylococcus aureus*)، الزائفة الزنجارية (*Pseudomonas aeruginosa*)، العدوى البكتيرية، ٢VITEK، تفاعل البوليميراز المتسلسل (PCR)، جين nuc، جين oprL، محافظة واسط.

Detection of *Staphylococcus aureus* and *Pseudomonas aeruginosa* among Diabetic Foot Ulcer Patients in Wasit Province

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Microbiology/ Immunology

Abstract

Diabetic foot ulcer infection is a severe clinical complication determined by neuropathy, vascular impairment, tissue injury, and successful bacterial invasion. Diabetic foot infections are the most common complication

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affecting people with diabetes, and bacterial pathogens play a key role. In this study, 90 subjects, including 35 patients with diabetic foot ulcers (DFU), as well as 35 diabetic patients without ulcers and 20 healthy controls, were enrolled. Methods: Wound swabs from the ulcer group were processed through conventional culture, selective media, VITEK 2 identification, and PCR. Univariate analysis identified isolation of *P. aeruginosa* (17/35;48.6%) being the most frequent isolate followed by *S.aureus* (13/35;37.1%) while other bacteria represented only 14.3%. Molecular confirmation using 16S rRNA, nuc and oprL genes showed complete concordance with phenotypic identification. Identification probabilities of both species on the VITEK 2 were greater than 90%. *P. aeruginosa* and *S. aureus* are clinically significant in diabetic foot ulcers due to their persistence, biofilm-forming ability and antimicrobial resistance; study results highlight concurrent use of culture, automated identification and PCR for accurate diagnosis/management

Keywords: diabetic foot ulcer, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, bacterial infection, VITEK 2, PCR, *nuc* gene, *oprL* gene, Wasit Province.

Introduction

Diabetic foot ulcers are frequent and severe complications of diabetes mellitus, usually related to chronic infection, delayed tissue repair, repeated hospitalization, and higher rates of lower-limb amputation. They are developed by multiple factors such as peripheral neuropathy, decreased circulation, impaired immunity, hyperglycemia, and recurrent mechanical load, over time, loss of protective sensation impairs wound healing, allowing small wounds—particularly on pressure-bearing areas—to develop silently into chronic ulcers that need to be diagnosed and managed promptly (Armstrong et al. 2023; Wang et al. 2022).

Because bacteremia delivers bacteria to the diabetic injury milieu (Necrotic tissue, exudate, reduced oxygen supply, and high glucose levels),

even if bacterial colonization of the wound surface occurs, any consequent invasion into deeper tissue accompanied by an inflammatory response would signal clinical infection. Infection results in more tissue damage, delayed healing, and more difficult treatment (especially if the pathogens are resistant to widely used antibiotics) (Lipsky et al., 2023; Alkhatieb et al., 2024).

Staphylococcus aureus (S. aureus) is a common Gram-positive infection identified as a clinically significant pathogen in diabetic foot infection; it can adhere to host tissues, express virulence factors, evade immune responses, and survive in polymicrobial wound environments, which are reasons for persistence of infection and poor healing. Treatment is further complicated by infections with methicillin-resistant *S. aureus* and underscore the necessity for a precise laboratory diagnosis (Abalkhail & Elbehiry, 2023; Becker *et al.*, 2023; Tong *et al.*, 2023).

Pseudomonas aeruginosa *(P. aeruginosa)*: also a major Gram-negative pathogen encountered in chronic, moist, long-standing, and/or previously treated wounds. It can survive stressful conditions because of its extracellular enzymes, pigments, antimicrobial resistance mechanisms, and biofilm-forming ability. Biofilm formation creates a protective environment around bacterial cells, limiting the efficacy of antibiotics and hampering host immune clearance, which leads to chronicity or recurrence/freezing of wounds as well as treatment failure especially in diabetic patients (Garousi *et al.*, 2023; Pang *et al.*, 2022; Moradali *et al.*, 2021).

Diabetic foot ulcers have a geographical variation in their bacterial profile, hospital setting, patient status, wound duration and previous antibiotic exposure. Our literature shows the predominance of Gram-positive area, mainly *S. aureus*, while other studies show evidence of larger frequency of Gram-negatives (like *P. aeruginosa*). Such data are particularly important in developing the empirical therapy using local bacteriological

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studies to improve infection control strategies, especially in Wasit Province (Norton et al., 2024; Maity et al., 2024).

In diabetic foot infections, accurate bacterial identification is crucial to the effective management, although it has been overshadowed in recent years by molecular techniques, conventional culture still plays an important role because it facilitates bacterial isolation and antimicrobial susceptibility testing; phenotypic methods can be limited by mixed growth phenotypes, atypical biochemical reactions, slow-growing organisms or previous antimicrobial administration, automatized systems including VITEK 2 are able to offer rapid, reproducible identification and susceptibility results for routine diagnosis, while PCR offers confirmatory molecular testing (Nazer *et al.*, 2024; Goh *et al.*, 2020).

Confirmatory properties of plant-based complex solutions for the respective molecular detection. The *16S rRNA* gene is considered a universal bacterial marker, the *nuc* gene is specific for *S. aureus*, and the *oprL* gene can be used as a reliable confirmatory target for *P. aeruginosa*. Our results indicate that culture, automated identification and molecular methods can therefore improve the diagnostic accuracy of diabetic foot ulcer investigations (Kim et al., 2001; Khodabandeh et al., 2021; Chieffi et al., 2024; Gawad & Gharbi, 2022; Gharehaghaji et al., 2021).

The aim of the current study: the present study was a bacteriological detection for *S. aureus* and *P. aeruginosa* in diabetic foot ulcers patients in Wasit Province. These species are significant in the clinical setting due to their association to chronic infection, biofilm formation, antimicrobial resistance and wound healing impairment. The isolates were characterized using conventional culture method, VITEK 2 system as well as polymerase chain reaction (PCR) technique targeting the genes of *16S rRNA*, *nuc* and *oprL*.

Materials and Methods

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This study was adapted from a case-control thesis conducted in Wasit Province (specifically at Al-Numaniyah General Hospital and private clinics). The study participants were adults aged approximately 35–75 years and originally stratified into three groups: diabetic foot ulcer patients (n = 35), diabetic patients without ulcers (n = 35), and healthy controls (n = 20). For bacteriological analysis, wound swabs were taken only from the diabetic foot ulcer group.

Aseptic swab samples were taken after cleaning the ulcer surface with sterile normal saline to minimize superficial contamination and debris from necrotic tissue. Material from the infected wound area was collected by rotating a sterile swab over the ulcer bed, and then placed in transport medium and transferred to the microbiology laboratory.

Samples were then enriched in Brain Heart Infusion broth and inoculated onto blood agar and MacConkey agar for 24–48 hours of incubation at 37°C under aerobic conditions. Presumptive isolation of *S. aureus* was performed on Mannitol Salt Agar, while selective isolation of *P. aeruginosa* was done on Cetrimide agar. Colonies were morphologically identified, and further details of the preliminary identification were performed by Gram stain, microscopic inspection, and conventional biochemical tests.

The VITEK 2 automated system was used for definitive phenotypic identification and antimicrobial susceptibility testing as per the manufacturer's instructions. From each sample, bacterial suspensions of microbial culture were processed by using standard operation procedures with appropriate source identification and susceptibility cards from a fresh colony.

Genomic DNA was extracted from the confirmed bacterial isolates for molecular confirmation. New bacterial cultures were grown at 37°C for 18–24 hours, centrifuged and washed before processing depending on the type

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of bacteria. Lysozyme and proteinase K treatment were used for supporting *S. aureus* lysis of Gram-positive cells, while *P. aeruginosa* isolates were treated with Gram-negative lysis buffer and proteinase K (24). Elution of purified DNA was performed according to (Total DNA Mini Kit Gene aid) and stored at −20°C until PCR analysis.

Concentration and purity of the isolated DNA samples were measured by a NanoDrop spectrophotometer. PCR was performed in a final volume of 25 µL containing master mix, forward and reverse primers for target gene amplification, nuclease-free water, and template DNA. The universal bacterial marker was the 16S rRNA gene, while confirmation of S. aureus was performed using the nuc gene and P. aeruginosa using the oprL gene; the thermal cycling conditions were optimized for each bacterial group as shown in the following tables. The thermal cycling conditions were optimized for each bacterial group as shown in the following tables.

Table (1): The optimum condition of detection *16SrRNA* of *S. aureus, nuc*

No.	Phase	Tm (°C)	Time	No. of cycle
1-	Initial Denaturation	95°C	3 min	1 cycle
2-	Denaturation -2	95°C	30 Sec	35 cycle
3-	Annealing	59°C	30Sec	
4-	Extension-1	72°C	30 Sec	
5-	Extension -2	72°C	7 min.	1 cycle

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Table (2): The optimum condition of detection *16SrRNA* of *P. aeruginosa*, oprl

No.	Phase	Tm (°C)	Time	No. of cycle
1-	Initial Denaturation	95°C	3 min	1 cycle
2-	Denaturation -2	95°C	30 Sec	35 cycle
3-	Annealing	66°C	30Sec	
4-	Extension-1	72°C	30 Sec	
5-	Extension -2	72°C	7 min.	1 cycle

Agarose gel electrophoresis of PCR products, followed by ethidium bromide staining and ultraviolet light imaging. For categorical variables, frequencies and percentages (n, %) are reported and analyzed using the Chi-square or Fisher's exact test when applicable. Continuous variables with normal distribution are presented as means ± standard deviation and compared using unpaired t-test or Welch's t-test. The associations between continuous clinical variables and systemic immunological markers were assessed with Spearman's rank correlation. Statistical Analysis: $p < 0.05$ was considered statistically significant, and all analyses were performed using GraphPad Prism 11.

Results and Discussion

A total of 35 swab specimens were collected from patients with diabetic foot ulcers for bacteriological analysis. Four bacterial groups were identified; the most recovered was ***P. aeruginosa*** with 17 isolates (48.6%), and second was ***S. aureus*** with 13 cases (37.1%). Other isolates included *Klebsiella spp. and Proteus spp.* The prevalence of *P. aeruginosa* in our cohort and *S. aureus* indicates that diabetic foot ulcer infections were related to both Gram-negative and Gram-positive bacteria in the patients studied.

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Table (3): Distribution of bacterial isolates among diabetic foot ulcer patients.

Bacterial isolate	Number (n)	Percentage (%)
Pseudomonas aeruginosa	17	48.6
Staphylococcus aureus	13	37.1
Other bacteria	5	14.3
Total	35	100

The high levels of *P. aeruginosa* that we detected in the environment and in diabetic foot wounds may be explained by the occurrence of chronic low-grade inflammation, remnant wound moisture, exposure to antibiotics (which *P. aeruginosa* can survive under), or even its inherent biofilm-forming ability (Garousi et al., 2023). *P. aeruginosa* is renowned as an opportunistic pathogen in chronic wounds, with its survival being mediated by biofilm formation, multidrug resistance, and virulence factor production underpinning tissue damage and impaired healing. Based on these properties, once established in the diabetic wound niche, *P. aeruginosa* becomes recalcitrant towards elimination (Pang et al., 2022; Darwitz et al., 2025).

S. aureus was the second most isolated organism, this is a clinically relevant finding as *S. aureus* is one of the most prevalent pathogens in skin and soft tissue infections (SSTI) and has been implicated in diabetic foot lesions , the fact that it adheres to damaged tissue, is capable of producing extracellular enzymes and forms biofilm-like communities may all result in bacterial persistence, furthermore, the possible presence of methicillin-resistant strains adds to challenges in treatment and underscores the need for susceptibility testing prior to commencing antimicrobial therapy (Abalkhail & Elbehiry, 2023; Becker et al., 2023; Tong et al., 2023).

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Ulcer Anatomic Distribution: Ulcers were more common on the left foot than the right (25 vs 16 ulcers; p = 0.018). The majority of ulcers were in the toes and forefoot region, heel and midfoot or lateral edges. The characteristic pattern can be attributed to increased pressure of the forefoot against the ground during walking, especially in diabetic patients with neuropathy and consequent decrease in protective sensation. This unnoticed repeated trauma in these ways could allow the entry of bacteria leading to wound chronicity (Wang et al., 2022; Parveen et al., 2025).

Ulcer sites were not statistically correlated to bacterial type. This suggests that *S. aureus* and *P. aeruginosa* were not confined to a particular anatomic site on the foot. The lack of a preference for specific sites probably indicates that factors such as wound duration, local tissue oxygenation, previous antibiotic exposure, ulcer depth, glycemic control, and host variables affect bacterial colonization more than an anatomical site alone. This interpretation is in line with the notion that chronic diabetic foot infection results more from the wound environment than its site of occurrence alone (Senneville et al., 2024; Norton et al., 2024).

This helped reinforce the reliability of bacteriological findings, with strong support from PCR confirmation. The *16S rRNA* gene, and the genes corresponding to each species (*P. aeruginosa: oprL; S. aureus: nuc*) were positive in all 17 *P. aeruginosa* isolates and all 13 *S. aureus* isolates. The fragment *16S rRNA from S. aureus* it was of 228 bp and for *P. aeruginosa* it was of 956 bp. The amplification of our selected genes according to species showed the expected gene product for *nuc* (279 bp) in *S. aureus* and *oprL* (504 bp) in *P. aeruginosa*. Such strong concordance between phenotypic and molecular findings lends support to the validity of our identification strategy. This result validates the reliability of the nuc gene, a stable and diagnostic molecular marker.

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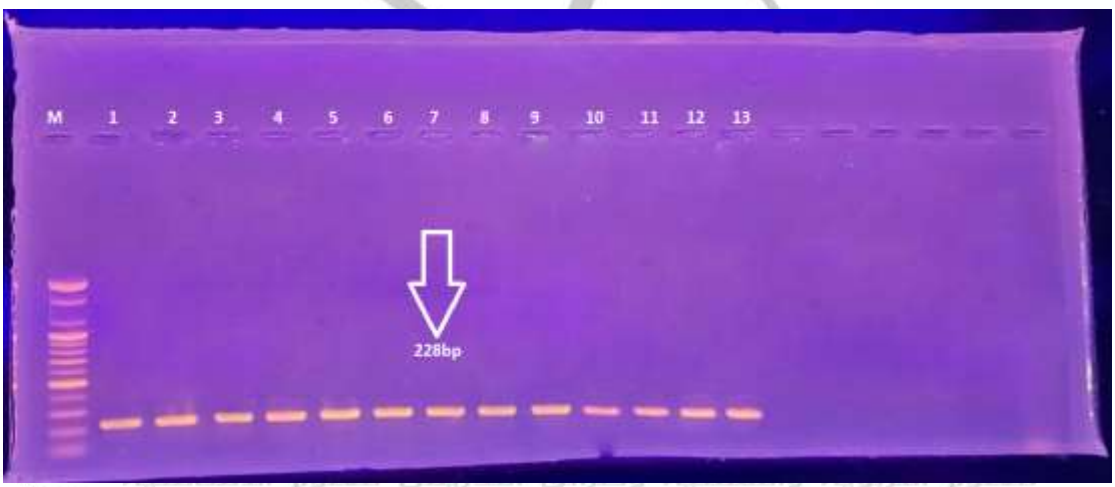
Table (5): Molecular confirmation of the main bacterial isolates.

Bacterial species	Sample size (n)	16S rRNA result	Species-specific gene	PCR-confirmed isolates	Confirmation (%)
P. aeruginosa	17	17 positive	*oprL*	17	100
S. aureus	13	13 positive	*nuc*	13	100
Total	30	30 positive	-	30	100



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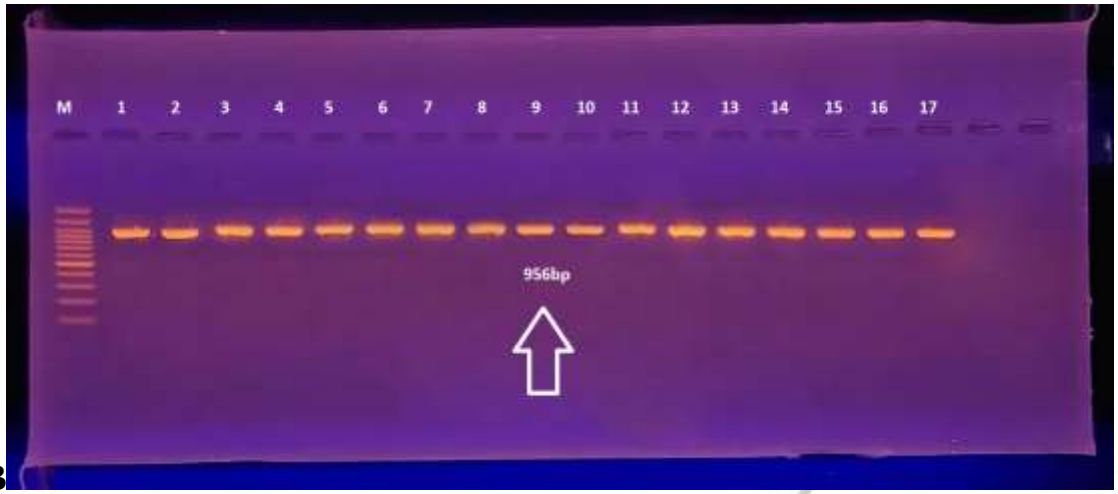

B

Figure (١): Agarose gel electrophoresis of PCR amplification products for the 16S rRNA gene.The DNA samples were fractionated on a 1% agarose gel prepared with 1x TBE buffer. Electrophoresis was completed at 80V and 65 mA for 1 hour. Upper Image (A): Positive amplification of 16S rRNA for Staphylococcus aureus at the expected product size of 228 bp. Lane M: 100 bp DNA ladder; Lanes 1-13: Bacterial isolates. Lower Image (B): Positive amplification of 16S rRNA for Pseudomonas aeruginosa at the expected product size of 956 bp. Lane M: 100 bp DNA ladder; Lanes 1-17: Bacterial isolates. The DNA bands were stained with red stain and visualized under a UV transilluminator.

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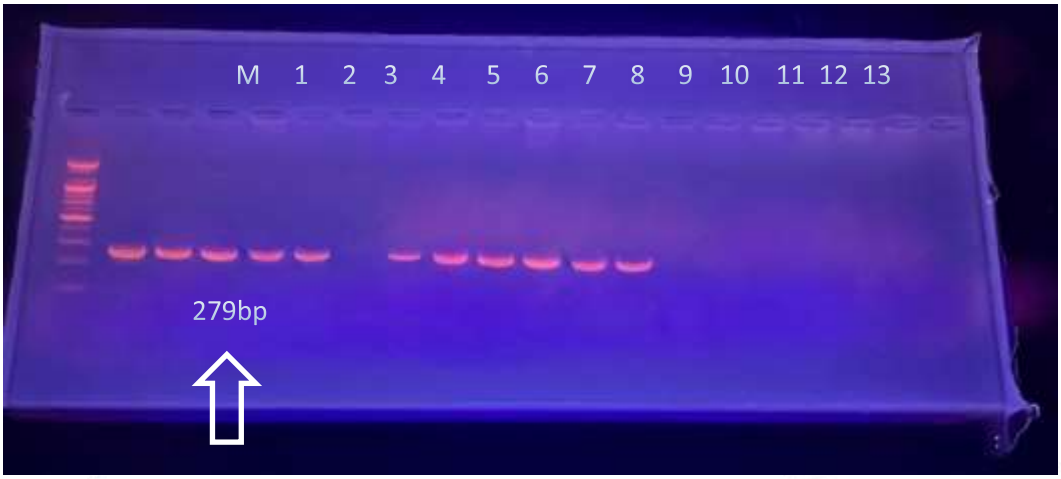

Figure (٢): Agarose gel electrophoresis of PCR amplification products for the *nuc gene*. The DNA samples were fractionated on a 1% agarose gel prepared with 1x TBE buffer. Electrophoresis was completed at 80V and 65 mA for 1 hour. The image shows positive amplification of the *nuc gene* for *Staphylococcus aureus* at the expected product size of 279 bp. Lane M: 100 bp DNA ladder; Lanes 1-13: Bacterial isolates. The DNA bands were stained with red stain and visualized under a UV transilluminator.

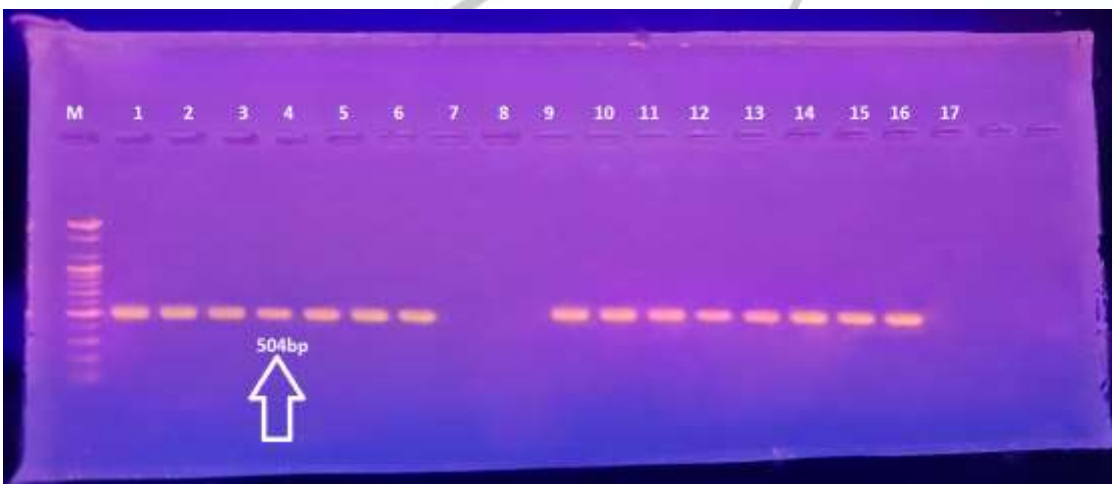

Figure (٣): Agarose gel electrophoresis of PCR amplification products for the *oprL gene.* The DNA samples were fractionated on a 1% agarose gel prepared with 1x TBE buffer.

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Electrophoresis was completed at 80V and 65 mA for 1 hour. The image shows positive amplification of the oprL gene for *Pseudomonas aeruginosa* at the expected product size of 504 bp. Lane M: 100 bp DNA ladder; Lanes 1-17: Bacterial isolates. The DNA bands were stained with red stain and visualized under a UV transilluminator.

The species-level confirmation of *S. aureus* was done by the identification of *nuc* gene detection. MRSA causes skin and soft tissue infections Lead As Barry comes up for Air this marker is used in most laboratories since it has a strong association with *S. aureus* respectively, and as such can distinguish between *S. aureus* and other coagulase-positive or coagulase-negative staphylococci that may be isolated from clinical specimens. This division is particularly relevant in diabetic foot ulcers where superficial sampling might occur, and *S. aureus* has to be confirmed as a true pathogen of infection (Kim et al., 2001; Khodabandeh et al., 2021; Chieffi et al., 2024).

The identification of *P. aeruginosa* was further confirmed by amplification of the *oprL* gene. The *oprL* gene, which encodes an outer membrane lipoprotein, is regarded as a good identifier for this species. This reduces the chance of misidentifying P. aeruginosa, as other Gram-negative non-fermenting bacteria are also present in specimens. This concept is important because *P. aeruginosa* are often long-term colonisers, form biofilms and can be resistant to many anti-microbials (Gawad & Gharbi,2022; Gharehaghaji et al., 2021; Wang et al., 2022), thus the confirmation of oprL is beneficial in studies involving diabetic foot ulcers.

The VITEK 2 automated platform supported the diagnosis of these two key isolates. Excellent identification was classified as 90 to 95% for S. aureus, and 95–99% for *P. aeruginosa* while the possibility of read across relied on the experimental results as well, this study highlights the utility of VITEK 2 in day-to-day diagnostics, especially when performed in conjunction with PCR. Standardized identification and antimicrobial susceptibility results

were available with automated systems, while molecular testing gives living confirmations of genetic species identity.

Antimicrobial susceptibility testing demonstrated clinically relevant resistance patterns. All tested beta-lactam antibiotics (including amoxicillin, benzylpenicillin, cloxacillin, and oxacillin) were resisted by the *S. aureus* isolates, so these isolates could be reported as methicillin resistant staphylococcus aureus. This is a serious finding, as diabetic foot ulcer MRSA may restrict options for empirical treatment, and careful selection of antibiotic agents based on susceptibility results will be required (Moradali *et al.,* 2021). The study noted lower sensitivity to certain therapeutic agents than previously observed in clinical studies, indicating an aggressive local resistance pattern.

The susceptibility profile for *P. aeruginosa* revealed resistance against critical antimicrobial classes, such as first-line fluoroquinolones and carbapenems. Clinically, this is worrisome because we use these agents frequently for Gram-negative infections. *P. aeruginosa* can become resistant after a number of mechanisms such as efflux pumps, enzymatic degradation, altered outer membrane permeability and biofilm associated tolerance. Consequently, the incidence of their resistant *P. aeruginosa* in diabetic foot ulcers needs to be diagnosed promptly at the laboratory alongside a unique antimicrobial treatment adapted to these specific cases (Moradali et al., 2021; Barrigah-Benissan et al., 2023; Darwitz et al., 2025).

The differences between the two bacteriological groups showed that both organisms had been recovered from patients with poor long-term glycemic control. Mean HbA1c values were elevated in both study groups: 9.87 ± 1.96% for S. aureus-infected patients and 10.27 ± 1.81% for *P. aeruginosa*-infected patients, Co-circulation of two or more bacterial species in diabetic foot ulcer infection is not necessarily associated with poorer glycemic

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control as changes in HbA1c between the no-bacterial and the two pathogenic groups are insignificant, chronic hyperglycemia can also affect leukocyte function, promote angiogenesis and collagen synthesis, stimulate keratinocyte apoptosis and inhibit epithelial regeneration; different environments in wounds are conducive to bacterial persistence (Burgess et al., 2021; Dawi et al., 2025).

Table (6): Selected clinical comparison between *S. aureus* and *P. aeruginosa* groups.

Variable	*S. aureus* (n=13)	*P. aeruginosa* (n=17)	Interpretation
HbA1c (%)	9.87 ± 1.96	10.27 ± 1.81	High in both groups; no major species-specific difference
WBC (x10^9/L)	8.24 ± 2.61	10.66 ± 5.46	Higher mean in *P. aeruginosa* group but not statistically significant
Granulocytes (%)	47.29 ± 28.10	68.05 ± 11.05	Significantly higher in *P. aeruginosa* group
Lymphocytes (%)	21.40 ± 12.98	20.52 ± 11.23	No significant difference

One possible explanation rest on the finding of more pronounced innate inflammatory responsiveness to infection with *P.aeruginosa*, as demonstrated by a markedly increased proportion of granulocytes in day-7 blood among patients infected compared with non-infected Gram-negative pathogens. This also means that Gram-negative bacterial components

themselves are very powerful local and systemic host defense stimulators, since they induce both innate immune activation and neutrophil recruitment (Versey *et al.*, 2021).

The biofilm factors secreted by the persisting *P. aeruginosa* may also maintain ongoing recruitment of immune-cell types in chronic wounds, thereby driving a delayed healing process that does not resolve cellular inflammation in such sites. This premise is confirmed by the evidence that biofilms associated with diabetic foot ulcers drive chronic inflammation, immune evasion, and limit the efficacy of antimicrobial therapy (Afonso et al., 2021).

The difference in WBC count was higher in the *P. aeruginosa* group but not statistically significant, so it should be interpreted with caution. More extensive comparative studies in well-defined groups will be needed to determine if this reflects a particular systemic hematological response unique to *P. aeruginosa* vs *S.aureus* (Sahu & Ruhal, 2025).

One of the interesting findings was that *P. aeruginosa and S. aureus* were identified as the most dominant isolates from specimens obtained from diabetic foot ulcers in this investigation, which is in accordance with prior literature reporting these pathogens as medically significant organisms that cause infections in diabetic foot patients multidrug resistance, biofilm production, and persistence within the wound environment of *P. aeruginosa* which are associated with high clinical relevance in diabetic foot infections (Garousi et al., 2023).

From a clinical point of view, both pathogens could survive acutely and or chronically due to virulence factors, antimicrobial-resistance patterns and biofilm-associated tolerance, the full agreement between phenotypic identification and PCR imbued the confidence in laboratory results with the strength of accompanying routine to culture, automated identification, antimicrobial susceptibility testing, and molecular confirmation when

feasible in chronic or non-healing diabetic foot ulcers (Senneville *et al.*, 2023).

Conclusion

The present study showed that *Pseudomonas aeruginosa* and *Staphylococcus aureus* were the dominant bacterial pathogens isolated from diabetic foot ulcer patients in Wasit Province. *P. aeruginosa* was the most frequent isolate, followed by *S. aureus*, reflecting the involvement of both Gram-negative and Gram-positive bacteria. PCR results showed complete agreement with phenotypic identification, confirming the reliability of culture, VITEK2, and molecular methods.

The 16S rRNA gene confirmed bacterial identity, while *nuc* and *oprL* genes confirmed *S. aureus* and *P. aeruginosa*, respectively. These findings highlight the importance of accurate identification and antimicrobial susceptibility testing for effective treatment and improved wound healing.

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