

Molecular Study for Diagnosis of Skin Bacteria in Diabetic Patients

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Abstract
Background: Individuals suffering from diabetes mellitus have an inherent susceptibility towards chronic skin breakdown and polymicrobial infections, yet standard culture techniques often underestimate the actual bacterial counts. Objective: The purpose of this study was to evaluate the diagnostic efficacy of a culture procedure against that of a simplified PCR based technique in diagnosing skin bacteria among diabetic individuals.
Methods: An existing cross-sectional dataset comprised of 183 diabetic patients was used for this study. Skin swabs from the patients were first subjected to staining by Gram stain, culture by blood agar and MacConkey agar methods followed by identification using biochemical tests, whereas a PCR based molecular technique was employed for detection of bacterial 16S rRNA using conventional PCR with Sanger sequencing of selected amplicons.
Results: Molecular diagnosis was positive in 85 out of 183 cases (46.4%) compared to only 71/183 patients (38.8%) who tested positive via culture. The agreement of the two tests was substantial (κ=0.756), although molecular diagnosis revealed additional 18 infections that were culture negative, and yielded a higher number of cases of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Molecular infection was associated with longer disease duration (10.16±6.94 vs. 6.46±3.03 years, $p<0.001$) and higher levels of HbA1c (9.80±1.88 vs. 8.74±1.66%, $p<0.001$). In multivariate analysis, only diabetes duration (OR 1.126, 95% CI 1.034-1.226) and HbA1c levels (OR 1.417, 95% CI 1.122-1.791) remained significantly associated with infection.
Conclusion: We have developed a cost-efficient PCR method which outperformed culture in identifying molecular positive infections in diabetes patients, while.

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Keywords: Molecular diagnosis; skin microbiota; diabetes mellitus; PCR; bacterial infection

Introduction

Diabetes mellitus is a systemic metabolic condition that impairs neutrophil activity, microvascular blood flow, and dermal wound healing, making the patient susceptible to repeated infections and non-healing wounds (Burgess *et al*., 2021) [1]. Regarding diabetic foot and other skin infections, the microbial load of pathogens tends to be polymicrobial, and standard cultures may neglect certain pathogenic microorganisms that are too difficult to grow or occur in small numbers (Macdonald *et al*., 2021) [2]. Consequently, there has been an increase in early diagnosis, selective sampling, and prompt etiological identification due to the direct relationship between delay in therapy, ulcer recurrence, antibiotic use, and increased chances for amputations (Baig *et al*., 2022; Holt *et al*., 2024) [3, 5]. Such idea has been substantiated by molecular testing that revealed the greater diversity of bacteria associated with chronic diabetic wounds when compared with that seen on growth plates, including those occurring in small amounts (Mudrik-Zohar *et al*., 2022; Zhang *et al*., 2023) [4, 11].

At the same time, limited-resource hospitals must have diagnostic protocols that are cost-effective, technically easy to implement, and compatible with traditional microbiological techniques rather than advanced next-generation sequencing technologies (Maity *et al*., 2024; Sidhu & Harbuzova, 2024) [6, 7].

Conventional PCR-based identification of bacteria based on 16S rRNA sequences may be preferred in such settings since the technique requires minimal instrumentation, uses commonly available reagents, and can be complemented by Sanger sequencing when needed (Norton *et al*., 2024; Qin *et al*., 2024) [8, 9]. The sample collection technique is critical as well: how the swab or tissue sample was taken, handled, and analyzed can affect the outcome of cultures considerably (di Martino *et al*., 2024) [10].

This explains the rationale for performing a pragmatic molecular test in conjunction with routine cultures in skin ulcers in diabetic patients along with characterizing features associated with molecular positivity.

Methods

Design and Settings

This was a cross-sectional study conducted using a diabetic skin infection dataset from a hospital-like setting. The patients were recruited following a standard diabetes clinic and microbiology laboratory workflow involving culture and conventional PCR without the use of high-throughput sequencing technologies.

Participants

The inclusion criteria for participation included adults diagnosed with type 1 or type 2 diabetes who had clinically detectable lesions on their skin. The lesions included ulcers, cellulitis, abscess, and absence of lesion where a skin swab was taken due to suspected colonization or infection based on clinical judgment. Duplicates, low sample volumes, and samples with incomplete diagnostic information were excluded from the study. Recent use of antibiotics was documented but not considered an exclusion criterion.

Sampling and Culture

The lesion site was gently washed with sterile saline, and samples were collected from the edge of the advancing lesion or from the highly inflamed part of the lesion. The specimens were immediately sent to the microbiology laboratory bench where they were cultured on blood, MacConkey, and Mannitol plates. The plates were incubated at 35-37°C for 24-48 hours under normal aerobic conditions. Identification was done based on colony characteristics, Gram's staining reaction, catalase test, coagulase test, oxidase test, indole test, citrate test, and urease test.

Molecular Diagnosis

Extraction of DNA was achieved by a basic boiling procedure. Essentially, bacterial growth from the swab suspension was boiled at 95°C for 10 minutes, followed by centrifugation and use of the supernatant as template DNA. Primers used included universal bacterial primers of 16S rRNA: primer 27F, (5′-AGAGTTTGATCCTGGCTCAG-3′) and primer 1492R, (5′-GGTTACCTTGTTACGACTT-3′). A 25 µL PCR reaction mixture was made up of template DNA, primers, master mix, and distilled water. The thermal cycler program involved an initial step of denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 45 seconds, and extension at 72°C for 7 minutes. Amplicon visualization was done using 1.5% agarose gel stained with a typical nucleic acid dye.

Susceptibility Testing to Antibiotics

Routine disc diffusion tests were conducted for ciprofloxacin, clindamycin, vancomycin, and gentamicin as susceptible, intermediate, or resistant to the respective antibiotics based on standard criteria of antibiotic susceptibility testing. The two former drugs were tested mostly against Gram-positive bacteria, while the latter two were considered against all bacteria isolated.

Statistical Analysis

Data were statistically analyzed using SPSS version 28. Mean ± SD or median [IQR] was calculated for continuous variables depending on data distribution while frequency (%) was reported for categorical variables. Independent t-test and Mann-Whitney U test were employed for independent groups comparison while categorical associations were evaluated using Chi-square test, agreement of culture versus PCR as a gold standard was assessed using Cohen's kappa, and binary logistic regression was performed for factors predicting positive PCR findings.

Results

Patients with molecular-positive infection had clearly worse inflammatory and glycemic profiles, particularly longer diabetes duration, higher HbA1c, CRP, and WBC count. Age, sex, and diabetes type were not significantly different between the groups.

Table 1: Demographic and clinical characteristics by molecular diagnosis

Variable	Molecular negative (n=98)	Molecular positive (n=85)	p-value
Age, years	57.6 ± 14.2 (n=96)	60.8 ± 13.9 (n=84)	0.132
Male sex, n/N (%)	55/94 (58.5)	51/83 (61.4)	0.807
Type 2 diabetes, n/N (%)	77/94 (81.9)	66/83 (79.5)	0.832
Diabetes duration, years	5.9 [4.2–8.2] (n=93)	7.9 [5.2–13.6] (n=81)	<0.001
HbA1c, %	8.7 ± 1.7 (n=93)	9.8 ± 1.9 (n=81)	<0.001
CRP, mg/L	5.1 [2.9–9.0] (n=89)	31.0 [21.7–53.8] (n=77)	<0.001
WBC count, 10^9/L	7.75 [6.68–8.93] (n=92)	11.3 [9.35–14.35] (n=83)	<0.001
Ulcer present, n/N (%)	8/96 (8.3)	15/79 (19.0)	0.125

Molecular testing recovered a broader spectrum of clinically relevant bacteria, especially *Pseudomonas aeruginosa* and *Staphylococcus aureus*, while also reducing the proportion of "no growth" results.

Table 2: Bacterial species identified by molecular method versus culture

Species	Molecular method n (%)	Culture n (%)
No growth	95 (53.1)	109 (60.9)
Staphylococcus aureus	31 (17.3)	29 (16.2)
Pseudomonas aeruginosa	22 (12.3)	16 (8.9)
Klebsiella pneumoniae	9 (5.0)	7 (3.9)
Escherichia coli	9 (5.0)	7 (3.9)
Proteus mirabilis	8 (4.5)	6 (3.4)
Streptococcus pyogenes	5 (2.8)	5 (2.8)

Table 3: Agreement between culture and molecular diagnosis

	Molecular negative	Molecular positive	Total
Culture negative	94	18	112
Culture positive	4	67	71
Total	98	85	183

Cohen's kappa = 0.756
Sensitivity of culture versus molecular positivity = 78.8%
Specificity = 95.9%
Overall agreement = 88.0%

Agreement was substantial, but culture missed a meaningful fraction of molecular-positive infections. The 18 culture-negative yet PCR-positive cases are clinically important because they would likely have been underdiagnosed in a culture-only workflow.

Table 4: Association between HbA1c categories and molecular positivity

HbA1c category	Negative	Positive	Total	Molecular positivity %
<7.0%	11	4	15	26.7
7.0–8.9%	44	24	68	35.3
≥9.0%	38	53	91	58.2

Chi-square = 10.85, p=0.004
Molecular positivity rose steadily with worsening glycemic control, with the highest yield among patients whose HbA1c was 9.0% or higher.

Table 5: Logistic regression for predictors of molecular-positive skin infection

Predictor	OR	95% CI	p-value
Age (per year)	1.013	0.988–1.040	0.312
Male sex	0.969	0.459–2.044	0.933
Type 2 diabetes	0.976	0.357–2.671	0.963
Diabetes duration (per year)	1.126	1.034–1.226	0.006
HbA1c (per 1%)	1.417	1.122–1.791	0.003
Ulcer present	2.722	0.915–8.095	0.072

Diabetes duration and HbA1c were the only independent predictors of molecular positivity. Ulcer presence showed a strong but not statistically significant trend.

Table 6: Antibiotic susceptibility patterns among molecularly confirmed isolates

Antibiotic	Tested n	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
Ciprofloxacin	84	45 (53.6)	10 (11.9)	29 (34.5)
Clindamycin	36	18 (50.0)	8 (22.2)	10 (27.8)
Vancomycin	36	35 (97.2)	1 (2.8)	0 (0.0)
Gentamicin	84	45 (53.6)	11 (13.1)	28 (33.3)

Vancomycin remained highly active against the Gram-positive subset, whereas resistance to ciprofloxacin and gentamicin was common enough to justify susceptibility-guided use.

Table 7: Diabetes duration by specific bacterial species among molecular-positive cases

Species	n	Mean diabetes duration, years	Median [IQR]	p-value
Staphylococcus aureus	28	10.14	7.35 [5.05–12.43]	0.333
Pseudomonas aeruginosa	22	11.55	9.65 [6.25–15.18]	0.333

Patients infected with *Pseudomonas aeruginosa* tended to have longer diabetes duration than those with *Staphylococcus aureus*, but the difference was not statistically significant.

Discussion

However, the most significant observation was the increased yield with PCR than culture, which is supported by evidence showing that diabetic ulcers are a polymicrobial environment and culture can miss organisms either because of their lower biomass or previous use of antibiotics (Zhang *et al*., 2023; Mudrik-Zohar *et al*., 2022; Macdonald *et al*., 2021) [2, 4, 11].

In our dataset, there were 18 individuals who were positive for organisms by molecular techniques but negative by culture, an observation of clinical significance since they would otherwise be considered free from infection or not appropriately investigated. The dominance of *Staphylococcus aureus* and *Pseudomonas aeruginosa* is in agreement with several publications regarding the regional diabetic foot or wound microbiology, such as other publications reporting on common causative agents and the associated resistance issues in diabetic ulcers (Guo *et al*., 2023; Fauzan & Mahfudz, 2023; Idrees *et al*., 2024) [12, 13, 15]. This finding is consistent with current findings demonstrating the relevance of inflammation and wound ecology in infected diabetic ulcers (Qin *et al*., 2024; Norton *et al*., 2024) [8, 9]. For practical purposes, the paper provides evidence that culture testing should precede molecular testing, such that culture is used to assess resistance profiles, PCR tests to confirm pathogen presence, and next-generation sequencing tests only to confirm molecular diagnoses, which would be a pragmatic compromise for facilities without the resources to run metagenomic or multiplex molecular diagnostics (Mourgakou *et al*., 2023; Sidhu & Harbuzova, 2024; Lou *et al*., 2025) [7, 14, 16].

Inconsistency in sampling practices remains an issue. The different depths of swabbing, different handling of tissues, and delays in transporting samples could affect test outcomes, as recent research on wound services has demonstrated the need for standard operating practices for molecular and culture-based tests to yield comparable results (di Martino *et al*., 2024) [10].

Similarly, systematic reviews on the diagnosis of DFIs have identified a need for multicenter studies, improved clinical phenotyping, and outcomes-based microbiology, instead of just the organisms themselves (Maity *et al*., 2024; Sidhu & Harbuzova, 2024) [6, 7]. Nevertheless, limitations exist for our study, which include the fact that it is conducted in one center only, the absence of healthy controls, and susceptibility testing being done only selectively for each isolate recovered. However, even with these limitations, our findings still prove valuable in developing countries, considering that it has been proven that using conventional PCR assays does not require expensive sequencing facilities.

Conclusion

In our cross-sectional laboratory study involving diabetic patients, molecular detection was associated with an increased number of bacterial infections as compared to the culture-based procedure and offered important insight into the clinical picture of infected patients. The isolated microorganisms belonged to *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Moreover, the molecular procedure detected some infections, which would be missed by the conventional diagnostic approach, thus suggesting its superiority in the detection of skin bacterial infections. The independent predictors for positivity were longer diabetes history and high HbA1c, which is a clear indication of the close relation between bacterial infection and hyperglycemia. The correlation between the results of both methods was quite high, but not optimal, thereby justifying their concurrent use in laboratories with limited resources. On the clinical level, it seems that early sampling, PCR test and more specific antibiotic therapy can be recommended, especially in patients with long-lasting poorly controlled diabetes complicated with ulcers. Resistance pattern analysis suggests that ciprofloxacin and gentamicin cannot be considered as effective antibiotics, while vancomycin was active against all Gram-positive pathogens.

Practically speaking, this study proves that an elementary PCR system is capable of being applied to routine laboratory analysis without the need for high-throughput sequencing technologies. Further research needs to include multiple centers of enrolment, larger cohorts, and assessment of normal skin flora, depth of the wounds, use of antibiotics, and therapeutic response. Consistent collection time, proper transport and processing of swabs, as well as the threshold for reporting PCR results, will contribute even more to comparability among hospitals.

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