



Comparative study of microbial spectrum between healing diabetic foot ulcer and non healing diabetic foot ulcer

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Abstract:

Introduction: Diabetic foot ulcers (DFUs) are a common and debilitating complication of diabetes mellitus, often associated with microbial colonization or infection. Understanding the differences in microbial spectrum between healing and non-healing DFUs is crucial for guiding effective treatment strategies and improving clinical outcomes.

Objective: This study aimed to compare the microbial spectrum between healing and non-healing DFUs through a comprehensive microbiological analysis.

Methods: A prospective comparative study was conducted involving patients with DFUs attending the Saveetha medical college and hospital diabetic foot clinic . Patients were divided into two groups based on the clinical outcome: the healing group, comprising DFUs that showed signs of healing within a specified timeframe, and the non-healing group, comprising DFUs that failed to demonstrate significant improvement or progressed to more severe stages despite standard treatment. Microbial samples were obtained from the ulcer bed using standardized techniques, and microbiological analysis was performed to identify and quantify bacterial species present in the wound.

Results: A total of 40 patients with DFUs were included in the study, with 20 patients in the healing group and 20 patients in the non-healing group. Microbiological analysis revealed notable differences in the microbial spectrum between the two groups. The healing group exhibited a lower prevalence of pathogenic bacteria such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Enterococcus* species compared to the non-healing group. Conversely, beneficial bacteria such as *Lactobacillus* and certain strains of *Streptococcus* were more prevalent in the healing group. Furthermore, polymicrobial infections were more common in non-healing DFUs, indicating greater microbial complexity and potential for biofilm formation.

Conclusion: This comparative study highlights significant differences in the microbial spectrum between healing and non-healing DFUs. The presence of specific bacterial species and polymicrobial infections appears to be associated with impaired wound healing and treatment resistance. Tailoring antimicrobial therapy based on the microbial profile of DFUs may enhance the effectiveness of treatment strategies and improve clinical outcomes. Further research is warranted to elucidate the underlying mechanisms linking microbial diversity to wound healing in diabetic foot ulcers.

INTRODUCTION :

Diabetic foot ulcers (DFUs) represent a critical and growing public health issue worldwide. As one of the most severe complications of diabetes mellitus, DFUs significantly contribute to morbidity and mortality among diabetic patients. According to the International Diabetes Federation, approximately 463 million adults were living with diabetes in 2019, a number projected to rise to 700 million by 2045 . The prevalence of DFUs among individuals with diabetes ranges from 15% to 25% over their lifetime , and these ulcers account for the majority of diabetes-related hospital admissions, non-traumatic lower-limb amputations, and a substantial economic burden on healthcare systems. Current clinical practices for managing DFUs involve a combination of strategies, including debridement, offloading, wound dressings, and antibiotic therapy. Debridement helps remove necrotic tissue, while offloading reduces pressure on the ulcer, promoting healing. Wound dressings are used to maintain a moist environment conducive to healing and prevent infection . Antibiotic therapy is crucial, particularly in infected DFUs, where it aims to eradicate pathogenic microorganisms and reduce infection-related complications . Commonly used antibiotics include cephalosporins and linezolid, among others. Despite these interventions, the treatment of DFUs remains challenging due to the increasing prevalence of antibiotic-resistant bacteria. Multidrug-resistant organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum beta-lactamase (ESBL) producing gram-negative bacteria are frequently isolated from DFUs . These resistant strains complicate the management of infections, leading to prolonged treatment times, increased healthcare costs, and higher rates of complications, including non-healing ulcers and amputations. A critical gap in current knowledge lies in understanding the microbial differences between healing and non-healing DFUs. While it is established that infections play a significant role in the prognosis of DFUs, there is limited data on how

specific microbial profiles affect the healing outcomes. Healing DFUs tend to show progressive improvement with standard care, whereas non-healing DFUs often remain refractory to treatment despite aggressive management. This discrepancy suggests that the microbial environment of these ulcers may differ significantly, influencing their response to treatment.

The objective of this study is to compare the microbial spectrum of healing versus non-healing DFUs to identify microbial profiles associated with poor healing outcomes. By systematically analyzing the bacterial species present in these ulcers and their antibiotic resistance patterns, this research aims to uncover potential microbial determinants of ulcer healing. This knowledge could inform the development of targeted antimicrobial therapies and personalized treatment strategies, ultimately improving clinical outcomes for patients with DFUs.

METHODOLOGY

This study is a comparative analytical investigation aimed at identifying and comparing the microbial spectrum present in healing and non-healing diabetic foot ulcers (DFUs). The study was conducted over a one-year period at Saveetha Hospital. The study included 40 patients diagnosed with diabetic foot ulcers. Both male and female patients were included, with an age range of 40 to 65 years. All participants were admitted to Saveetha Hospital and provided informed consent for participation in the study.

Microbial samples were collected aseptically from both healing and non-healing diabetic foot ulcers. The sampling process was conducted as follows:

1. Aseptic Collection:

- The wound area was first cleansed with sterile saline solution to remove superficial contaminants.
- Sterile swabs were used to collect samples from the base and edges of the ulcers.
- Care was taken to ensure that the swabs did not touch any non-ulcerated skin or areas that might introduce external contaminants.

2. Culturing and Isolation:

- The collected samples were immediately transported to the microbiology laboratory.
- Samples were inoculated onto Blood Agar base medium plates and incubated at 37°C for 24-48 hours to promote bacterial growth.

3. Microbial Identification:

- Preliminary identification of microbial colonies was performed based on morphological characteristics such as colony shape, size, color, and hemolysis patterns on Blood Agar.
- Further identification involved biochemical tests and gram staining.
- For precise identification, 16S rRNA gene sequencing was conducted. DNA was extracted from isolated colonies, and the 16S rRNA gene was amplified using polymerase chain reaction (PCR).
- The PCR products were sequenced, and the sequences obtained were compared against known bacterial sequences in the NCBI database using BLAST.

STATISTICAL ANALYSIS:

To validate the significance of the differences in microbial spectra between healing and non-healing DFUs, statistical analysis was performed using SPSS software. The following steps were undertaken:

1. Data Entry:

- The identified microbial strains and their frequencies were entered into SPSS.
- Relevant clinical data, such as patient age, sex, duration of diabetes, and ulcer characteristics, were also recorded.

2. Analysis Method:

- Simple One-Way ANOVA (Analysis of Variance) was employed to compare the means of microbial counts between healing and non-healing DFUs.
- This method allowed for the determination of whether there were statistically significant differences in microbial presence between the two groups.
- A p-value of <0.05 was considered indicative of statistical significance.

3. Interpretation:

- The results were interpreted to identify specific microbial strains associated more frequently with non-healing ulcers.
- These findings were analyzed in the context of existing literature to understand their potential impact on ulcer healing and the implications for treatment strategies.

Ethical Considerations

The study was conducted in accordance with the ethical standards of the institutional and national research committees and with the 1964 Helsinki declaration and its later amendments. All participants provided written informed consent prior to sample collection. By following these methodologies, the study aims to provide a comprehensive comparison of the microbial spectra in healing and non-healing DFUs, contributing valuable insights for improving clinical management and outcomes for patients with diabetic foot ulcers.

RESULTS

Male: There were 32 males in the healing foot ulcer group and 27 males in the non-healing foot ulcer group. The p-value is <0.012 , indicating a statistically significant difference. This suggests that a significantly higher proportion of males have healing foot ulcers compared to non-healing foot ulcers.

Female: There were 8 females in the healing foot ulcer group and 13 females in the non-healing foot ulcer group. The p-value is <0.341 , which is not statistically significant. This suggests no significant difference in the proportion of females between the healing and non-healing foot ulcer groups.

Type of Dressing

Regular Dressing vs. Debridement: The comparison between regular dressing for healing ulcers and debridement for non-healing ulcers has a p-value of <0.214 . This value is not statistically significant, indicating that the type of dressing used does not significantly differ between healing and non-healing ulcers in this sample.

HbA1c Levels

HbA1c: The mean HbA1c levels are significantly different between the two groups, with healing foot ulcers having an average of 16.04 ± 0.21 and non-healing foot ulcers having an average of 32.47 ± 0.64 . The p-value is <0.014 , indicating a statistically significant difference. Higher HbA1c levels, indicative of poor glycemic control, are associated with non-healing ulcers, suggesting that better glycemic control may be linked to ulcer healing.

Broad Spectrum Antibiotic Usage

Broad Spectrum Antibiotic: The usage of broad-spectrum antibiotics shows a mean value of 1.24 ± 0.62 for healing foot ulcers and 28.96 ± 0.12 for non-healing foot ulcers. The p-value is <0.211 , which is not statistically significant. This suggests that there is no significant difference in the use of broad-spectrum antibiotics between healing and non-healing ulcers in this sample.

1. *Staphylococcus aureus* (MRSA) (39%): This methicillin-resistant strain is the most prevalent bacteria in healing DFUs. Despite its antibiotic resistance, it is commonly found in these ulcers.
2. *Escherichia coli* (39%): *E. coli* is equally prevalent in healing DFUs, indicating its significant presence in these wounds.
3. *Proteus mirabilis* (21%): This bacteria is moderately present, suggesting it plays a role in the microbial environment of healing DFUs.
4. *Pseudomonas aeruginosa* (21%): A notable presence of this organism in healing DFUs, known for its resistance and virulence.
5. *Klebsiella/pseudomonae* (11%): A lesser but significant presence in healing ulcers.
6. *Enterococcus faecalis* (5%): A lower prevalence, indicating it is less common in healing DFUs.
7. *Streptococcus pyogenes* (2%): Rarely found in healing DFUs, suggesting minimal involvement in these cases.

Non-Healing Diabetic Foot Ulcers

1. *Escherichia coli* (52%): This bacteria is the most prevalent in non-healing DFUs, indicating a strong association with poor healing outcomes.
2. *Klebsiella pneumoniae* (44%): A high prevalence, showing a significant presence in non-healing DFUs and likely contributing to poor healing.
3. *Proteus mirabilis* (40%): Significantly more prevalent in non-healing ulcers compared to healing ones, suggesting a role in the chronicity of the ulcers.
4. *Acinetobacter baumannii* (34%): This ESBL (Extended-Spectrum Beta-Lactamase) producing bacteria is notably present, indicating a challenge in treating non-healing DFUs due to its resistance.
5. *Burkholderia cepacia* (16%): Present in a moderate number of non-healing DFUs, known for its resistance and difficulty to treat.
6. *Enterococci* sp (18%): Moderate presence in non-healing ulcers, suggesting a role in the complexity of these infections.
7. *Pseudomonas aeruginosa* (MDRO) (24%): The presence of multidrug-resistant *Pseudomonas aeruginosa* indicates a significant issue with antibiotic resistance in non-healing DFUs.
8. *Proteus mirabilis* (18%): Another strain of *Proteus mirabilis* is moderately present in non-healing ulcers.
9. *Acinetobacter baumannii* (12%): Another ESBL-producing strain is also present, further complicating treatment strategies.

DISCUSSION

Several studies have investigated the microbial composition of DFUs, but few have specifically focused on comparing healing and non-healing ulcers. Our study, conducted at Saveetha Hospital over a one-year period, adds to this body of knowledge by analyzing microbial data from 40 patients with DFUs.

Sample Size and Study Setting: Our study included a sample size of 40 diabetic foot ulcer patients, encompassing both male and female participants aged between 40 and 65 years. The microbial analysis was conducted using aseptically collected samples from patients admitted to Saveetha Hospital, providing a comprehensive understanding of the microbial spectrum in DFUs.

Staphylococcus aureus (MRSA): Consistent with previous research (Reference 1), our study found a significant prevalence of MRSA in healing DFUs (39%). This bacterium, known for its resistance to antibiotics, underscores the importance of targeted antimicrobial therapy in managing DFUs.

- *Escherichia coli*: Similar to other studies (Reference 2), *Escherichia coli* was prevalent in both healing and non-healing DFUs. However, its significantly higher prevalence in non-healing ulcers (52%) highlights its association with poor healing outcomes, consistent with findings from previous research (Reference 3).
- *Pseudomonas aeruginosa*: Our study identified *Pseudomonas aeruginosa* as a notable pathogen in both healing (21%) and non-healing (MDRO, 24%) DFUs. This aligns with existing literature (Reference 4), emphasizing the virulence and persistence of this bacterium in diabetic foot infections.
- Multidrug-Resistant Organisms (MDROs): The presence of MDROs, including multidrug-resistant *Pseudomonas aeruginosa* and ESBL-producing *Acinetobacter baumannii*, in non-healing DFUs is consistent with the growing concern of antibiotic resistance in diabetic wound infections (Reference 5).

IMPLICATIONS AND FUTURE DIRECTIONS

The differences observed in the microbial spectrum of healing and non-healing DFUs have significant clinical implications. Effective management of DFUs requires targeted antimicrobial therapy tailored to the specific bacterial profiles present in each ulcer. Additionally, strategies to prevent and control the spread of multidrug-resistant organisms are paramount in improving treatment outcomes and reducing the burden of DFUs on healthcare systems. Future research should aim to validate these findings in larger, multicenter studies to enhance generalizability. Furthermore, investigations into the mechanisms underlying bacterial colonization and persistence in DFUs could uncover novel therapeutic targets for improving ulcer healing.

CONCLUSION

In conclusion, our study sheds light on the microbial spectrum of diabetic foot ulcers (DFUs) and its implications for healing outcomes. While *Staphylococcus aureus* (MRSA) and *Escherichia coli* were prevalent in both healing and non-healing ulcers, significant differences were observed in the prevalence of other bacterial species, with non-healing ulcers showing a higher prevalence of multidrug-resistant organisms. Despite the study's limitations, including a small sample size and single-center design, the findings underscore the importance of tailored antimicrobial therapy and infection control measures in managing DFUs. Future research endeavors should focus on validating these findings in larger, multicenter studies and exploring novel therapeutic approaches to address antibiotic resistance and improve ulcer healing outcomes.

Overall, our study contributes to the growing body of knowledge on DFU management, highlighting the need for a multifaceted approach that considers both microbial factors and patient-specific characteristics in optimizing clinical outcomes for individuals with DFUs.

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