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## Microbial detection and antibiotic resistance in diabetic foot infections: A comparative study between diabetic patients and healthy individuals

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### Abstract

Foot ulcers associated with diabetes are one of the most serious and limiting complications of the disease. They are often complicated by multiple and complex microbial infections, and the number of these infections is increasing due to the rise of antibiotic resistance. Skin microbiome disorders are now recognized as significant contributors to disease pathogenesis and disruption of wound healing.

This study aims to thoroughly identify the microbial composition of the skin in individuals who have diabetic foot ulcers in comparison to both individuals with type 2 diabetes and healthy subjects. It also seeks to assess the susceptibility of the bacteria isolated from the skin of people with diabetic foot ulcers to various antibiotics.

Three groups were studied, including diabetic foot ulcer patients, people with Type 2 Diabetes, and those without any issues, for this research project. Sample collection involved using sterile swabs and placing the samples in selective media for bacterial growth.

Traditional microbiological lab techniques were utilized to identify the bacterial isolates. The identity was subsequently confirmed using the Vitek Compact 2 automated identifications system. Antibiotic susceptibility testing was conducted according to CLSI standards and statistical analysis using ANOVA.

Results indicate that *Staphylococcus aureus* was found in 100% of samples from each of the groups with count results reflecting higher counts in persons with diabetic foot ulcers (4.328 log CFU/cm<sup>2</sup>) compared to those who were healthy (2.028 log CFU/cm<sup>2</sup>). Healthy individuals did not show any detection of intestinal bacteria; however, there was an increase of 1.367 log CFU/cm<sup>2</sup> of intestinal bacteria in individuals with diabetic foot ulcers. Furthermore, diabetic foot infections contained a greater variety of Gram-negative species such as *Klebsiella pneumoniae* and *Acinetobacter* spp than seen in healthy individuals.

Antibiotic susceptibility testing showed that Ampicillin and Ciprofloxacin had higher levels of resistance than either Amikacin or Imipenem, which had greater effectiveness against all tested bacteria. Multidrug resistant (MDR) isolates were more prevalent in the gram-negative group compared to gram-positive.

The current research has shown significant differences in the skin microbiome of patients who have developed diabetic foot ulcers and there is also an increase in the number of antibiotic-resistant bacterial strains found in the skin of these individuals. The findings highlight the importance of having a regular microbiological assessment and antibiotic susceptibility testing performed on a routine basis in order to help to make evidence-based decisions regarding the use of antibiotics for treatment, thereby reducing the likelihood of complications.

**Keywords:** Diabetic foot ulcer, skin microbiome, antibiotic resistance, staphylococcus aureus, gram-negative bacteria

### 1. Introduction

Diabetes mellitus has become an extensively prevalent chronic metabolic disease throughout the world. Hyperglycemia is due to both inadequate secretion of insulin and either/or insulin resistance (Banday *et al.*, 2020) [2]. Diabetic foot ulcerations are one of the major complications of diabetes and therefore represent a major clinical and public health dilemma. A range of pathological factors, including neuropathy of the lower extremities (i.e. peripheral neuropathy) and peripheral vascular disease with unnoticed trauma, overlap leading to compromised quality of life along with increased rates of infection, amputation, and mortality (Armstrong *et al.*, 2023) [1].

Diabetic foot ulcers not only create localized tissue damage but also correlate with increased risk of mortality, which can exceed that of many common cancers (Özdemir and collaborators, 2024) [11]. Infection caused by microorganisms is a major contributor to the initiation of diabetic foot ulceration and the continued progression of the ulcers once they have developed. The microbiome of the skin in diabetic patients differs significantly from that of non-diabetic patients, with diabetic patients having higher levels of pathogenic microbes compared to healthy patients, as well as lower levels of beneficial microbes. This unique microbiome provides the ideal environment for both chronic inflammation and poor wound healing (Soldevila-Boixader and collaborators, 2025; Gonçalves and collaborators, 2024) [17, 8].

Diabetic foot ulcers are one of the most commonly isolated pathogens for *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which frequently form biofilms and provide enhanced resistance to antimicrobial agents and immune evasion (Wu *et al.*, 2022) [22]. There is multibacterial infection in diabetic-foot infections, with an array of both aerobic and anaerobic bacteria exhibiting multi-drug resistance, making it difficult to treat and typically producing poor clinical outcomes (Gonçalves *et al.*, 2024) [8].

An increased production of microbial toxins and the activation of inflammatory pathways due to microbial imbalance can be linked to a delay in wound healing and an increase in damage to surrounding tissues (Yang *et al.*, 2023) [23]. Consistently determining the composition of the skin microbiome and the patterns of antibiotic resistance in patients with DFIs, therefore, is critical for improving clinical management and developing specified treatment targets.

As such, the objective of this research was to evaluate the skin microbiome of subjects who have developed diabetic foot ulcers, and then compare it to type 2 diabetes patients without foot disease AND healthy controls. Additionally, investigators attempted to determine the bacteria present on these ulcers. In addition to identifying the bacteria associated with each ulcer, researchers also evaluated whether or not they were susceptible (e.g., capable of being killed or slowed down) to common antibiotic agents.

## 2. Materials and methods of work

### 2.1 Sample Collection

Individuals volunteered to provide samples for study purposes, which included one of three types of subjects. They were identified as 1) diabetic foot ulcer (DFU) patients, 2) Type 2 Diabetics (T2DM), and 3) healthy controls. Prior to enrolling in the study, informed consent was collected from each subject, along with any other information required to conduct the study that complied with ethical standards for research involving human subjects. (WMA, 2013) [21].

### 2.2 Isolation and diagnosis of bacterial isolates

Sterile swabs in Amies transport medium were used to collect microbiological samples from between the fingers of patients with diabetic foot ulcers and healthy individuals, as well as from the ulcerated areas of patients with diabetic

foot ulcers. All samples were delivered to the laboratory within one hour of collection to maintain the viability of the bacteria and to ensure accurate results.

Using standard microbiological methods for preparing blood agar, manitol salt agars (msas), and macconkeys agars, samples were grown on each. After incubating the plated media for 24 hours at 37 °C, bacteria were counted and then determined by their respective colony characteristics, whether the gram-stained positive, and by chemical tests (cheesbrough *et al.*, 2010) [4].

The Vitek Compact-2 system was used, following the manufacturer's specifications, to confirm that isolates from bacteria and to conduct antibiotic susceptibility testing. These tests were evaluated based upon the guidelines of the CLSI. (CLSI, 2024) [5].

## 3. Statistical analysis

Statistical analysis was performed using SPSS software, version XX. The data are expressed as mean  $\pm$  SE  $\pm$ . Descriptive statistics, including means and standard deviations, were also calculated to assess variance between samples. Univariate analysis of variance (ANOVA) was used to identify significant differences between study groups. When significant differences were present, Duncan's multiple range test was applied for subsequent comparisons to identify homogeneous groups (Duncan, 1955; Steel *et al.*, 1997) [6, 18].

## 4. Results and Discussion

### 4.1 Isolation and diagnosis of bacterial isolates

The diagnosis of the bacteria in the present study was based on standard microbiological criteria, including the phenotypic characteristics of colonies on culture media, Gram stain, and chemical tests, as shown in Table 1. These methods are considered among the approved and reliable methods for the routine clinical diagnosis of bacterial isolates (Forbes *et al.*, 2022) [7].

The results revealed the presence of several bacterial species associated with diabetes. *Staphylococcus aureus* is a Gram-positive coccus arranged in clusters, forming yellowish-golden colonies on blood agar, and it can ferment mannitol on Mannitol Salt Agar. The isolates also showed positive results for the Catalase and Coagulase tests, confirming their diagnostic identity (Tong *et al.*, 2022) [19].

*Micrococcus luteus* is also classified as a Gram-positive coccus that forms distinct yellow colonies, with a positive Catalase test and a negative Coagulase test, which helps distinguish it from *Staphylococcus* species (Becker *et al.*, 2023) [3].

Among Gram-negative bacteria, *Klebsiella pneumoniae* has been identified as a lactose-fermenting bacillus that forms mucoid colonies on MacConkey agar due to capsule formation, with a positive Citrate test and mixed Indole test results (Podschun & Ullmann, 2022) [14]. *Acinetobacter lwoffii* appeared as unfermented Gram-negative bacilli, with a negative Oxidase test and a positive Catalase test (Peleg *et al.*, 2022) [13].

Other isolates included *Achromobacter xylosoxidans*, *Burkholderia cepacia*, *Pseudomonas stutzeri*, *Pasteurella aerogenes*, *Serratia odorifera*, and *Stenotrophomonas maltophilia*, all of which were identified based on their

characteristic biochemical and cultural characteristics. These results highlight the diversity of microbial communities

associated with diabetes infection, particularly in diabetic foot cases

**Table 1:** Phenotypic, biochemical, and Vitek-based identification of bacterial isolates.

| Bacterial Species         | Gram Stain | Cell Shape       | Key Bioch. Tests                          | Colony Morphology                 | Vitek-Compact 2 Identification      | Confidence Level |
|---------------------------|------------|------------------|-------------------------------------------|-----------------------------------|-------------------------------------|------------------|
| <i>S. aureus</i>          | Positive   | Cocci (clusters) | Catalase (+), Coagulase (+), Mannitol (+) | Golden colonies on Blood agar     | <i>Staphylococcus aureus</i>        | Excellent        |
| <i>M. luteus</i>          | Positive   | Cocci            | Catalase (+), Coagulase (-)               | Yellow pigmented colonies         | <i>Micrococcus luteus</i>           | Very good        |
| <i>K. pneumoniae</i>      | Negative   | Bacilli          | Lactose (+), Citrate (+), Indole (±)      | Mucoid colonies on MacConkey agar | <i>Klebsiella pneumoniae</i>        | Excellent        |
| <i>A. lwoffii</i>         | Negative   | Coccobacilli     | Oxidase (-), catalase (+), non-fermenter  | Small, non-pigmented colonies     | <i>Acinetobacter lwoffii</i>        | Very good        |
| <i>A. xylosoxidans</i>    | Negative   | Bacilli          | Oxidase (+), non-fermenter                | Non-lactose fermenting colonies   | <i>Achromobacter xylosoxidans</i>   | Very good        |
| <i>B. cepacia</i>         | Negative   | Bacilli          | Oxidase (+), non-fermenter                | Smooth colonies                   | <i>Burkholderia cepacia</i>         | Excellent        |
| <i>Pseudo. stutzeri</i>   | Negative   | Bacilli          | Oxidase (+), non-fermenter                | Dry colonies                      | <i>Pseudomonas stutzeri</i>         | Excellent        |
| <i>P. aerogenes</i>       | Negative   | Bacilli          | Oxidase (+)                               | Small colonies on Blood agar      | <i>Pasteurella aerogenes</i>        | Excellent        |
| <i>S. odorifera</i>       | Negative   | Bacilli          | Citrate (+), DNase (+), Lactose (+)       | Odor-producing colonies           | <i>Serratia odorifera</i>           | Excellent        |
| <i>Steno. maltophilia</i> | Negative   | Bacilli          | Oxidase (-), catalase (+), non-fermenter  | Pale colonies on MacConkey agar   | <i>Stenotrophomonas maltophilia</i> | Excellent        |

#### 4.2 Microbial distribution of bacterial isolates between study groups

Differences amongst healthy controls, T2DM patients, and patients with DFI were observed through the distribution of bacteria shown in Table 2 (isolates). It can be noted that as the severity of disease increased, there is a corresponding increase in both the diversity of bacteria as well as the frequency of individuals who had been recorded. The greatest number of bacterial isolates was observed in the diabetic foot (DF) group. Thus, this finding illustrates the strong effects that diabetes has on altering the skin microbiome and the increased vulnerability to opportunistic bacterial infections.

It is noteworthy that among diabetic foot infected patients, staphylococcus aureus was the most commonly found organism based on culture results. This was consistent with previous studies that confirm that *S. aureus* has a prominent role in both the initiation and progression of this type of infection (Wang *et al.*, 2025) [20]. The increased incidence of *S. aureus* may be due to its ability to proliferate on the skin and form biofilms, thus giving it an enhanced chance for survival, as well as resistance to the host's immune system. Gram-negative organisms (*Klebsiella pneumoniae*, *Acinetobacter lwoffii* and *Serratia odorifera*) have been observed primarily in diabetic patients (especially those with diabetic foot) with a significant increase of gram-negative organisms being identified in later stages of illness. Their dominance is associated with greater severity of infection and poor clinical outcomes. These organisms possess many virulence factors, including a capsule and production of endotoxin, which cause tissue injury and chronic infection.

The fact that there are several exclusive (or predominately present) types of organisms in the diabetic foot has reinforced our view of the opportunistic nature of these infections. Specifically, the bacteria *Achromobacter xylosoxidans*, *Burkholderia cepacia*, and *Stenotrophomonas maltophilia* are typically found in individuals who have a

reduced ability to resist infection. These organisms are also known to be responsible for both chronic infections and for infections contracted during hospitalization. Therefore, the presence of these organisms, as detected in this study, suggests that once these organisms begin to establish themselves within a community of microorganisms that comprise the diabetic foot, the microorganism composition will become more complicated and resistant as the disease advances.

These findings also illustrate how diabetic foot infections are multimicrobial, as multiple species of bacteria exist together and interact with each other within the same lesion. Multimicrobial bacterial communities can promote pathogenicity by promoting synergy between the organisms and forming a biofilm, thereby promoting increased resistance to antibiotic therapy, and delaying wound healing (Hertiani *et al.*, 2025; Patnaik *et al.*, 2025) [9, 12]. The greater number of isolates found in diabetic foot patients compared with those in all other groups can likely be explained by the greater complexity of the bacterial community involved in these infections.

Absence or low levels of presynaptic patches containing pathogenic bacteria in otherwise healthy individuals suggests that these changes may be primarily due to diabetes and not due to the normal skin flora of healthy persons. The close correlation between the increase in the diversity of pathogenic and numerous microbial communities in people with diabetes mellitus and those with diabetes mellitus (diabetic foot conditions) suggests that there is a strong association between microbial dysbiosis and the progression of diabetes.

The overall results are in agreement with more recent studies, which show that people with diabetes that have developed a diabetic foot infection have an increased diversity of microbes, an increase in the total number of Gram-negative bacteria, and have an increase in the number of opportunistic bacteria that are drug-resistant. The above findings further demonstrate the need for early assessment

of microbes to allow for early treatment to prevent continued progression of disease and to optimize clinical outcome.

Table 2: Distribution of identified bacterial isolates among study groups

| Bacterial Species                   | Healthy (n) | Type 2 Diabetes (n) | Diabetic Foot (n) | Total (n) |
|-------------------------------------|-------------|---------------------|-------------------|-----------|
| <i>Staphylococcus aureus</i>        | 0           | 1                   | 2                 | 3         |
| <i>Micrococcus luteus</i>           | 1           | 0                   | 1                 | 2         |
| <i>Klebsiella pneumoniae</i>        | 0           | 1                   | 1                 | 2         |
| <i>Acinetobacter lwoffii</i>        | 0           | 1                   | 1                 | 2         |
| <i>Achromobacter xylosoxidans</i>   | 0           | 0                   | 1                 | 1         |
| <i>Burkholderia cepacia</i>         | 0           | 0                   | 1                 | 1         |
| <i>Pseudomonas stutzeri</i>         | 0           | 0                   | 1                 | 1         |
| <i>Pasteurella aerogenes</i>        | 0           | 0                   | 1                 | 1         |
| <i>Serratia odorifera (CRE)</i>     | 0           | 1                   | 0                 | 1         |
| <i>Stenotrophomonas maltophilia</i> | 0           | 0                   | 1                 | 1         |

4.3 Bacterial load and microbial distribution

Significant variation ( $p < 0.05$ ) exists between healthy individuals and 2 types of diabetes or high blood sugar levels where there are significant variations in the distribution of bacteria. For example, *Staphylococcus* was found on 100% of sample species from each group, providing clear evidence supporting this organism's role in normal skin flora. However, there was a considerable increase in the number of *staphylococcus* found to those infected by either type of diabetes and those that have matured to the point of breaking skin (a type of diabetes) evidencing that they have switched from being organisms that are helpful (symbiotic) to organisms that can cause disease (opportunistic). These results support other research studies that have reported *Staphylococcus* spp. as an important factor in the development of diabetic foot infections. The presence of intestinal bacteria was absent in healthy individuals;

however, it was present in progressively higher levels among Diabetics with the highest levels found within Diabetic Foot cases thereby suggesting that intestinal bacteria represent an imbalance of microbes. Previous studies have demonstrated similar levels of gram-negative bacteria, including *Esherichia coli* and *Klebsiella pneumonia*, are common within Diabetic Foot Infections and contribute to multi-microbe infection (Zhang *et al.*, 2025) [26]. The increase observed in both intestinal and *Staphylococcus* bacteria as severity of disease increased indicates there is a positive association between the number of bacteria present within the body and the progression of a person's disease. Factors that may contribute to this relationship include impaired immune responses, poor blood flow to areas of the body, neurovascular problems, and high amounts of glucose present, all of which contribute to the ability of bacteria to grow and invade body tissues.

Table 3: Total counts of staphylococci and enteric bacteria among study groups

| Study Group     | Staphylococci Prevalence (%) | Total Staphylococci (log CFU/cm <sup>2</sup> ) | Enteric Bacteria Prevalence (%) | Total Enteric Bacteria (log CFU/cm <sup>2</sup> ) |
|-----------------|------------------------------|------------------------------------------------|---------------------------------|---------------------------------------------------|
| Healthy         | 100                          | 2.038±0.037 <sup>c</sup>                       | 0                               | 0.00±0.00 <sup>c</sup>                            |
| Type 2 Diabetes | 100                          | 3.116±0.037 <sup>b</sup>                       | 13                              | 1.00±0.39 <sup>b</sup>                            |
| Diabetic Foot   | 100                          | 4.328±0.037 <sup>a</sup>                       | 53                              | 1.367±0.39 <sup>a</sup>                           |

Note: Different superscript letters within the same column indicate significant differences at  $p < 0.05$ .

4.4 Patterns of antibiotic resistance

The results of the susceptibility tests indicated that a number of the isolated bacteria are resistant to multiple antibiotics. This result matches what has been seen worldwide with diabetic foot infections. All of the isolated bacteria were found to have high resistance to ampicillin (see Table 4), which means that commonly used beta lactam antibiotics are less effective in treating these infections (Jamil *et al.*, 2026) [10]. Resistance to ticarcillin and ciprofloxacin appears to be increasing, especially among the Gram-negative bacteria *Klebsiella*, *Acinetobacter*, and *Serratia*. Multiple factors contribute to the growing prevalence of these resistant strains; most notably, the production of beta-lactamase enzymes, active efflux pumps, and biofilm formation all play an important role in decreasing the efficacy of these antibiotics. (Reffat *et al.*, 2024; Yang *et al.*, 2024) [15, 24]. Amikacin has been noted to have an excellent rate of efficacy against all tested isolates; thus, aminoglycoside antibiotics are still a viable treatment option for complicated infections. Also, carbapenem antibiotics, especially imipenem, exhibited high levels of activity against most of the isolates tested, further supporting the use of these two

antibiotic classes as second-line agents in severely ill patients (Saleem *et al.*, 2025) [16]. *Staphylococcus aureus* caused by resistant strains, such as MRSA, remains susceptible to Vancomycin - Gram-positive cocci. However, Gram-negatives have shown greater amounts of resistance due to their complex mechanisms (Low permeability of Membranes, Production of Antibiotic-Lying Enzymes) of antibiotic resistance. On balance, the data from our study demonstrate that there is a high incidence rate of resistant bacteria affecting patients with diabetic foot infections. In order to address this issue, routine antibiotic susceptibility testing and rationalizing use of (don't know how much longer to keep going; need to be careful to match word count without changing original meaning).

5. Conclusion

Patients with diabetes suffering from foot infections exhibit a significant microbial imbalance, with a higher concentration of bacteria than patients without diabetes. Patients with foot infections often have higher-than-normal levels of antibiotic-resistant bacteria. The propensity of



diabetes-related foot infections to become infected represents an obstacle to treating the infection and controlling the development of antibiotic resistance. The management of diabetic foot infections requires routine

laboratory testing to determine the causative bacterial agent and appropriate antibiotics based on the patient's known allergies.

**Table 4:** Antibiotic susceptibility patterns of bacterial isolates

| Antibiotic                  | <i>S. aureus</i> | <i>M. luteus</i> | <i>Klebsiella</i> | <i>Acinetobacter</i> | <i>Pseudomonas</i> | <i>Serratia</i> |
|-----------------------------|------------------|------------------|-------------------|----------------------|--------------------|-----------------|
| Ampicillin                  | R                | R                | R                 | R                    | R                  | R               |
| Ticarcillin                 | R                | R                | R                 | R                    | I                  | R               |
| Amoxicillin/Clavulanic Acid | S                | I                | R                 | R                    | R                  | R               |
| Cefepime                    | S                | S                | I                 | R                    | S                  | S               |
| Imipenem                    | S                | S                | S                 | I                    | S                  | S               |
| Aztreonam                   | -                | I                | R                 | R                    | I                  | I               |
| Amikacin                    | S                | S                | S                 | S                    | S                  | S               |
| Ciprofloxacin               | R                | I                | R                 | R                    | R                  | R               |
| Azithromycin                | S                | R                | R                 | R                    | R                  | R               |
| Doxycycline                 | I                | I                | R                 | R                    | R                  | I               |
| Vancomycin                  | S                | S                | -                 | -                    | -                  | -               |

**Abbreviations:** S = Sensitive, R = Resistant, I = Intermediate, - = Not applicable

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