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Microbiological profile of diabetic foot ulcers: A prospective study

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Abstract
Background and Objective: Infection, extended hospital stays, and even amputation of limbs are common outcomes of diabetic foot ulcers (DFUs), a prevalent and serious consequence of diabetes mellitus. Effective management relies on the prompt identification of the bacteria responsible for the infection and their profiles of antibiotic susceptibility. The purpose of this research was to analyze the antibiotic susceptibility patterns and microbiological profiles of bacteria isolated from diseased DFUs.
Material and Methods: A tertiary care hospital was the setting for a 12-month prospective observational study. This study was conducted at the Department of Microbiology, Sree Balaji Medical College and Hospital, Chennai, Tamil Nadu, India from March 2018 to February 2019. We included 60 patients who had diabetic foot ulcers that were clinically infected. Standard microbiological procedures were used to culture wound swabs or tissue samples obtained under aseptic conditions. In order to identify the isolates, biochemical testing, Gram staining, and colony morphology were employed. The Kirby-Bauer disc diffusion method was used to conduct antimicrobial susceptibility testing in accordance with CLSI standards.
Results: Microbiological growth was detected in 54 out of 60 samples, or 90% of the time. Nineteen (35.2%) of the cultures that came back positive had evidence of polymicrobial illness. The bulk of the organisms were Gram-negative, with the most common being Pseudomonas aeruginosa (22.2%), Escherichia coli (18.5%), and Klebsiella pneumoniae (14.8%). Staphylococcus aureus was detected in 16.7% of cases among Gram-positive isolates, with 40% of those cases involving MRSA. Only 7.4 percent of samples did not include anaerobes. A lot of gram-negative bacteria are resistant to ciprofloxacin and ampicillin, however meropenem and amikacin still work rather well. When it came to Gram-positive bacteria, linezolid and vancomycin worked wonders.
Conclusion: The study shows that Gram-negative bacilli and other multidrug-resistant organisms are frequently found in diabetic foot ulcers. The best way to handle DFUs is with a personalized antibiotic strategy that takes culture and sensitivity into account. The most effective way to guide empirical therapy is to periodically monitor microorganism profiles and resistance trends.

Keywords: Diabetic foot ulcer, microbiological profile, antimicrobial susceptibility, gram-negative bacteria, MRSA, multidrug resistance

Introduction
Diabetes mellitus is a prevalent chronic metabolic condition globally, defined by hyperglycemia due to impairments in insulin production, insulin action, or both. The increasing prevalence of diabetes worldwide, especially in low- and middle-income nations, is rendering its consequences a significant public health issue. Diabetic foot ulcers (DFUs) represent one of the most distressing and expensive complications, resulting in considerable morbidity, extended hospitalizations, recurring infections, and, in extreme instances, lower limb amputation [1-3].
Approximately 15-25% of individuals with diabetes are projected to acquire a foot ulcer at some stage in their lives. The etiology of diabetic foot ulcers is multifaceted, encompassing peripheral neuropathy, peripheral vascular disease, immunological dysfunction, and recurrent trauma or pressure. When the skin's integrity is compromised, the ulcer becomes susceptible to microbial colonization and subsequent infection, frequently resulting in rapid wound worsening [4-6].
Infections in diabetic foot ulcers are often polymicrobial, comprising a combination of aerobic Gram-positive cocci, Gram-negative bacilli, and anaerobes. The microbiological profile is known to differ according on geographical area, patient demographics, ulcer

severity, and previous antibiotic exposure. In acute infections, Gram-positive bacteria, particularly Staphylococcus aureus (including methicillin-resistant Staphylococcus aureus [MRSA]), are usually predominate. Conversely, persistent and more profound ulcers typically contain Gram-negative bacteria such as Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, and anaerobes [7-9].

The rise of multidrug-resistant organisms has exacerbated the therapeutic management of diabetic foot ulcers (DFUs). The irrational or empirical administration of antibiotics without appropriate culture and sensitivity testing frequently results in treatment failures and the emergence of resistant bacteria. As a result, there is a growing focus on the necessity for evidence-based antimicrobial therapy customized to local microbial and resistance trends. Precise identification of the causative microorganisms and their antibiotic susceptibility profiles is essential for improving treatment options, minimizing healing time, eliminating complications, and ultimately reducing amputation and fatality rates [8-10].

This prospective study aimed to analyze the microbiological spectrum of diabetic foot ulcers in patients in a tertiary care hospital and to assess the antibiotic susceptibility patterns of the isolated organisms. The findings intend to offer valuable insights to assist doctors in choosing successful empirical therapy and enhancing the overall management of diabetic foot infections.

Materials and Methods

This prospective observational study was carried out over 12 months in the Department of Microbiology, Sree Balaji Medical College and Hospital, Chennai, Tamil Nadu, India from March 2018 to February 2019. Ethical approval was secured from the Institutional Ethics Committee before the initiation of the project. Informed permission in writing was acquired from all subjects. The study comprised 60 participants with clinically confirmed diabetic foot ulcers. A multidisciplinary team of surgeons, diabetologists, and microbiologists conducted the clinical evaluation. The ulcers were evaluated according to the Wagner categorization method to determine their depth and severity.

Sample Collection and Processing

After sterilising the ulcer sites with normal saline, samples were taken using aspirates, deep tissue biopsies, or sterile cotton swabs. Surface pollutants were carefully avoided. In order to conduct culture and sensitivity tests, samples were promptly sent to the microbiology lab. Anaerobic medium, Blood agar, and MacConkey agar were all inoculated with specimens as needed. After 24-48 hours of aerobic incubation at 37°C, the plates were subjected to 48-72 hours of anaerobic incubation. Gram staining, colony morphology, and a battery of routine biochemical tests were used to identify isolates.

Antibiotic Susceptibility Testing

The Kirby-Bauer disc diffusion method was used on Mueller-Hinton agar to conduct antimicrobial susceptibility testing in accordance with Clinical and Laboratory Standards Institute (CLSI) recommendations. Local prescription trends and commonly utilized empirical agents were considered for selecting an antibiotic panel. Discs containing cefoxitin (30 µg) were used to screen for methicillin resistance in Staphylococcus aureus. Standard ATCC strains were used for quality control.

Inclusion Criteria

- Patients aged 18 years and above with diagnosed Type 1 or Type 2 diabetes mellitus.
- Presence of clinically infected foot ulcers as per the Infectious Disease Society of America (IDSA) criteria.
- Patients who had not received antibiotics for at least 48 hours prior to sample collection.
- Willingness to participate and provide informed consent.

Exclusion Criteria

- Non-diabetic patients with foot ulcers.
- Patients who had received systemic antibiotics within 48 hours prior to sample collection.
- Patients with pressure ulcers, traumatic wounds, or ulcers due to other causes.
- Patients who refused or were unable to provide informed consent.

Results

The study included 60 diabetic individuals who had foot ulcers that were clinically infected. Here are the results of the analysis of the demographic and clinical variables, microbiological profile, and patterns of antibiotic susceptibility:

Table 1: Demographic and Clinical Characteristics of Patients

Parameter	Number (%)
Age (Mean ±SD)	56.3 ± 10.5 years
Gender (Male: Female)	38:22
Duration of Diabetes >10 years	34 (56.7%)
Wagner Grade 1-2	21 (35%)
Wagner Grade 3-5	39 (65%)
History of Previous Ulcer	26 (43.3%)
Prior Hospitalization	18 (30%)
Peripheral Neuropathy	41 (68.3%)
Peripheral Vascular Disease	16 (26.7%)

The majority of patients were between the ages of 50 and 70. Notably, there was a masculine predominance. The majority of patients had high-grade ulcers (Wagner 3-5) and neuropathy or vascular disease as consequences of their long-term diabetes (>10 years).

Table 2: Culture Positivity and Pattern of Microbial Growth

Culture Result	Number of Samples (%)
Positive Culture	54 (90%)
Monomicrobial Infection	35 (64.8%)
Polymicrobial Infection	19 (35.2%)
No Growth	6 (10%)

Positive microbial growth was observed in 54 out of 60 samples. The intricacy of diabetic foot infections was highlighted by the fact that almost a third of the positive cultures showed evidence of polymicrobial infections.

Table 3: Distribution of Isolated Microorganisms

Organism	Frequency (%)
Pseudomonas aeruginosa	17 (21.8%)
Escherichia coli	14 (17.9%)
Klebsiella pneumoniae	11 (14.1%)
Staphylococcus aureus	13 (16.7%)
MRSA (among *S. aureus*)	5/13 (38.5%)
Proteus spp.	7 (9.0%)
Enterococcus faecalis	6 (7.7%)
Anaerobes	6 (7.7%)
Others (e.g., *Citrobacter spp.*)	4 (5.1%)

Pseudomonas, Escherichia coli, and Klebsiella were the most common gram-negative bacilli in the sample. A considerable amount of the Gram-positive cocci were Staphylococcus aureus, which includes MRSA. Chronic, deep ulcers were found to be anaerobic.

Table 4: Antibiotic Susceptibility Pattern of Gram-Negative Isolates

Antibiotic	Sensitive (%)	Resistant (%)
Amikacin	40 (81.6%)	9 (18.4%)
Meropenem	44 (89.8%)	5 (10.2%)
Piperacillin-Tazobactam	38 (77.6%)	11 (22.4%)
Ceftriaxone	21 (42.9%)	28 (57.1%)
Ciprofloxacin	18 (36.7%)	31 (63.3%)
Cotrimoxazole	23 (46.9%)	26 (53.1%)

The resistance of gram-negative bacteria and other microbes to fluoroquinolones and cephalosporins is quite strong. No changes were made to the top three agents: amikacin, piperacillin-tazobactam, and meropenem.

Table 5: Antibiotic Susceptibility Pattern of Gram-Positive Isolates

Antibiotic	Sensitive (%)	Resistant (%)
Vancomycin	23 (100%)	0
Linezolid	23 (100%)	0
Clindamycin	16 (69.6%)	7 (30.4%)
Erythromycin	13 (56.5%)	10 (43.5%)
Ciprofloxacin	10 (43.5%)	13 (56.5%)
Penicillin	6 (26.1%)	17 (73.9%)

Vancomycin and linezolid were effective against all Gram-positive bacteria. The penicillin and ciprofloxacin resistance levels, however, were alarmingly high. Clindamycin and erythromycin were less effective against MRSA strains.

Discussion

Amputation risk and patient morbidity are greatly increased by diabetic foot ulcers (DFUs), which continue to rank among the most difficult and expensive consequences of diabetes mellitus. This work provides valuable information about the prevalent bacteria and their drug susceptibility patterns by analyzing the microbiological landscape of infected diabetic foot ulcers in a tertiary care setting [11, 12].

With a mean age of 56.3 years and a male predominance, the study sample is consistent with prior research showing that outdoor activity, delayed care-seeking behavior, and poor foot hygiene make males more likely to acquire foot ulcers. Many of the patients who arrived with high-grade (Wagner grade 3-5) ulcers had diabetes for more than a decade. These results highlight the fact that foot issues caused by diabetes are long-lasting and worsen with time [13, 14]. Similar studies have shown a positive cultural impact in 85-95% of cases, so our 90% success rate is in line with that. Using deep tissue specimens and avoiding surface contamination were effective sampling strategies, as suggested by the high yield. The fact that 35.2% of infections were polymicrobial lends credence to the idea that neglected and chronic ulcers, which frequently have inadequate blood flow and are subject to repetitive stress, sometimes harbor several organisms [15-17].

The majority of the isolates were Gram-negative bacilli, according to the microbiological analysis. The most common species found were Pseudomonas aeruginosa, Escherichia coli, and Klebsiella pneumoniae. This data is in line with regional and worldwide trends, showing that the majority of DFU infections, whether they are chronic or hospital-associated, are caused by Gram-negative organisms. There is still cause for concern about the prevalence of antibiotic resistance in these types of environments, since Staphylococcus aureus was the most common Gram-positive isolate and a large percentage was methicillin-resistant Staphylococcus aureus [18-20].

There were alarming trends found in the antimicrobial susceptibility tests. Due to their excessive usage as empirical treatment in outpatient settings, ciprofloxacin and third-generation cephalosporins have shown substantial resistance among Gram-negative isolates. Meropenem and amikacin, on the other hand, maintained a high level of effectiveness, which could make them useful in cases that are particularly severe or resistant. It was comforting to see that all Gram-positive isolates were completely susceptible to linezolid and vancomycin, although clindamycin and erythromycin exhibited moderate to high resistance, especially in MRSA strains [21-23].

The significance of regular microbiological testing and personalized antibiotic treatment is highlighted by these findings. Because incorrect use may increase resistance, local antibiograms, and not assumptions, should direct empirical therapies. To maximize results, it is essential to have a multidisciplinary team that includes surgeons, microbiologists, and diabetologists. It is important not to underestimate the importance of anaerobes, particularly in long-standing or non-healing wounds, because they are present in 7.4% of cases, mostly in deep or necrotic ulcers. Hence, in some cases, anaerobic covering would be necessary [22-24].

Conclusion

Diabetic foot ulcers are characterized by a polymicrobial composition and changing patterns of antibiotic resistance, as shown in this prospective investigation. The majority of the pathogens were Gram-negative bacilli, such as Pseudomonas aeruginosa, Escherichia coli, and Klebsiella

pneumoniae. The most prevalent Gram-positive isolate was Staphylococcus aureus, which includes MRSA. Due to the significant prevalence of bacteria resistant to ciprofloxacin and other third-generation cephalosporins, culture-guided therapy has become increasingly important. If we want to stop the spread of antibiotic-resistant bacteria and make sure that people get the therapy they need, we need to use empirical antibiotic selection based on local microbiological trends and susceptibility profiles. Managing diabetic foot infections and minimizing catastrophic outcomes like amputation requires early diagnosis, proper antibiotic medication, surgical intervention when needed, and a multidisciplinary approach.

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References

1. Boulton AJM, Vileikyte L, Ragnarson-Tennvall G, *et al.* The global burden of diabetic foot disease. Lancet. 2005;366(9498):1719-1724. doi:10.1016/S0140-6736(05)67698-2.
2. Lipsky BA, Berendt AR, Cornia PB, *et al.* 2012 Infectious Diseases Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. Clin Infect Dis. 2012;54(12):e132-73. doi:10.1093/cid/cis346.
3. Armstrong DG, Boulton AJ, Bus SA. Diabetic foot ulcers and their recurrence. N Engl J Med. 2017;376(24):2367-2375. doi:10.1056/NEJMra1615439.
4. Zubair M, Abbasi M, Ghaffar A, *et al.* The microbiology of diabetic foot infections and the pattern of antimicrobial resistance. J Diabetol. 2017;8(1):13-18.
5. O'Neill E, O'Connell M, Dempsey E, *et al.* The management of diabetic foot infections. Diabetes Care. 2016;39(4):529-36. doi:10.2337/dc15-2023.
6. Schaper NC, van Netten JJ, Apelqvist J, *et al.* Practical guidelines on the management and prevention of diabetic foot disease (IWGDF 2015). Diabetes Metab Res Rev. 2016;32(S1):11-17. doi:10.1002/dmrr.2694.
7. Singh N, Armstrong DG, Lipsky BA. Preventing foot infections in patients with diabetes. JAMA. 2005;293(2):217-228. doi:10.1001/jama.293.2.217.
8. Aslam S, Shukla SK, Khurshid S, *et al.* Microbiological profile of diabetic foot infections and their antimicrobial susceptibility pattern. J Diabetes Metab Disord. 2015;14:46. doi:10.1186/s40200-015-0177-5.
9. Ghanem-Zoubi N, Zohar E, Zimhony O, *et al.* The microbiological profile of diabetic foot infections in Israel. Infect Dis. 2017;49(8):632-639. doi:10.1080/23744235.2017.1364694.
10. Monego C, Silva MS, Lemos JC, *et al.* Microbial patterns in diabetic foot infections: A study of 105 cases. Rev Soc Bras Med Trop. 2014;47(1):65-68. doi:10.1590/0037-8682-0230-2013.
11. Nakao S, Iwashita Y, Kubo Y, *et al.* Clinical and microbiological features of diabetic foot infections: A retrospective study. J Diabetes Investig. 2015;6(1):65-72. doi:10.1111/jdi.12224.
12. Yilmaz S, Uzun O, Gursoy M, *et al.* Antimicrobial resistance of pathogens in diabetic foot infections: A report from a tertiary hospital. Infect Control Hosp Epidemiol. 2016;37(4):472-478. doi:10.1017/ice.2016.9.
13. Sotto A, Lavigne JP, de Lencastre H, *et al.* The role of antibiotic resistance in the microbiology of diabetic foot infections: A review. Diabetologia. 2007;50(11):2244-2253. doi:10.1007/s00125-007-0730-3.
14. Fernandes R, Silva I, Ribeiro O, *et al.* Diabetic foot infection: A review of the microbiology and therapeutic strategies. Diabetes Res Clin Pract. 2013;99(3):204-212. doi:10.1016/j.diabres.2012.12.005.
15. Patel S, Mistry N, Patel D, *et al.* The microbiology of diabetic foot infections: A comprehensive review. J Diabetol. 2018;9(2):125-129.
16. Pozo JC, Soriano A, García-Álvarez M, *et al.* Microbiology of diabetic foot infections: A hospital-based study. Diabet Med. 2003;20(7):530-536. doi:10.1046/j.1464-5491.2003.01083.x.
17. Tiwari S, Pathak S, Sharma R. Microbiological profile of diabetic foot ulcers in a tertiary care hospital. Int J Med Sci Public Health. 2017;6(2):347-350.
18. Kumar A, Rani K, Sharma D, *et al.* Microbial profile and antibiotic resistance patterns of diabetic foot infections. Int J Pharmacol Res. 2016;8(3):35-40.
19. Mohapatra P, Panigrahi M, Sahoo P. Bacterial profile and antimicrobial susceptibility pattern in diabetic foot infections. J Clin Diagn Res. 2015;9(10):DC01-4. doi:10.7860/JCDR/2015/14427.6570.
20. McDonald H, MacPhee M, Smith C, *et al.* Review of diabetic foot infections: Etiology, microbiology, and management strategies. J Diabetes Complications. 2016;30(4):691-697. doi:10.1016/j.jdiacomp.2015.12.002.
21. Kalanuria AA, Zodpey SP. The microbiology of diabetic foot infections: Insights and epidemiology. J Clin Infect Dis. 2014;7(3):164-168. doi:10.1016/j.jcln.2014.07.013.
22. Saran S, Bansal P, Sharma P, *et al.* Antimicrobial resistance and risk factors for diabetic foot infection in an Indian population. Diabet Foot Ankle. 2015;6:26835. doi:10.3402/dfa.v6.26835.
23. Bhandari R, Yadav H, Kumar M, *et al.* Microbiological profile and drug resistance pattern of diabetic foot infections in a tertiary hospital. J Infect Dis Immun. 2015;7(4):65-72.
24. Singh P, Kumar P, Ahuja S. Diabetic foot infections: A study of microbial pathogens and their antibiotic susceptibility patterns. J Clin Microbiol. 2014;52(7):1157-1161. doi:10.1128/JCM.00310-14.