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ORIGINAL ARTICLE

Microbial profile and antimicrobial susceptibility pattern in diabetic foot ulcer patients attending a tertiary care hospital.

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ABSTRACT… Objective: To determine the frequency of culture-positive DFUs, identify the microbial profile, and analyze the antimicrobial susceptibility patterns among diabetic patients at Ghulam Muhammad Mahar Medical College (GMMMC) Hospital, Sukkur. **Study Design:** Cross-sectional study. **Setting:** Department of Medicine, Ghulam Muhammad Mahar Medical Teaching Hospital, Sukkur. **Period:** January 2024 to June 2024. **Methods:** 150 diabetic patients with DFUs. A non-probability consecutive sampling technique was used. Culture and sensitivity testing were performed on tissue samples collected from infected ulcers. Frequencies, percentages, means, and standard deviations were calculated. **Results:** Of the 150 DFU cases, 72 (48.0%) were culture-positive. The most commonly isolated organisms were Escherichia coli (29.2%), Klebsiella pneumoniae (25.0%), Staphylococcus aureus (23.6%), and Pseudomonas aeruginosa (22.2%). The highest antibiotic sensitivity was observed for cephalosporins (60.7%), followed by vancomycin (12.7%). The highest resistance was noted against cephalosporins (39.3%) and penicillin (37.5%). **Conclusion:** The study highlights a high burden of MDR infections in DFUs, necessitating improved antibiotic stewardship and infection control measures. Routine culture-based sensitivity testing should be integrated into clinical practice for targeted antibiotic therapy. Further research on biofilm formation and novel antimicrobial therapies is recommended to enhance DFU management and reduce complications.

Key words: Antimicrobial Resistance, Antibiotic Susceptibility, Diabetic Foot Ulcer, Multidrug-resistant Bacteria, Microbiological Profile.

INTRODUCTION

Diabetes mellitus (DM) is a significant metabolic ailment that is distinguished by atypically elevated levels of glucose in the bloodstream. This phenomenon represents a significant contributor to both illness and death on a worldwide scale. The prevalence of diabetes is rapidly increasing worldwide, posing a significant challenge to public health.[1] As per the International Diabetes Federation, the prevailing data indicates that there are 537 million adults globally who are affected by Diabetes mellitus. Individuals afflicted with Diabetes mellitus, particularly those with unregulated glycemic levels, are at a heightened susceptibility to various severe and potentially fatal complications, including but not limited to stroke, Coronary heart disease, diabetic nephropathy, peripheral arterial disease, and Diabetic foot ulcers.[2]

Approximately 50% of individuals with diabetes are uninformed of their condition. It is estimated that approximately 240 million individuals globally are living with undiagnosed diabetes.[3] Almost 463 million adults globally have diabetes, and 90% of them have type 2 diabetes mellitus. Pakistan is placed 3rd globally in diabetes prevalence after China and India, as stated by an article titled “The News”. The ratio of diabetes cases in Pakistan during 2016, 2018, and 2019 was 11.77%, 16.98%, and 17.1%, respectively.[4]

Uncontrolled diabetes causes diabetic foot ulcers. Poor glycemic control, diabetic neuropathy,

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peripheral vascular disease, foot deformities, pressure overload, and foot care cause it. Foot osteomyelitis and lower extremity amputation are also common.[5] Diabetic foot ulcers hospitalize the most diabetics. Diabetes causes most US non-traumatic amputations.[6]

D-Foot International reports that 25% of diabetics with foot numbness will develop an ulcer. If untreated, this can lead to amputation and prevent one from doing their daily duties. Diabetes increases the risk of amputation by 10–20 times. One-third of the half billion diabetics worldwide will develop a DFU: over half of DFUs get infected and 17% need amputation.[7-11]

Among 1813 patients with a foot wound culture collected, 859 (47.4%) had ≥1 positive foot wound culture. S. aureus, the most commonly cultured organism, was isolated in 333/859 (38.8%) of the patients. Enterococcus faecalis, isolated in 149/859 (17.3%) of the patients, was the second most common organism cultured.[12]

The rationale of this is that limited local data of our district and Sindh as a province is available, regarding the culture positive DFUs. In our region, no such study regarding antimicrobial susceptibility is available as well. Hence, there is a critical need to conduct such a study. I have designed this study to determine the frequency of culture positive DFU patients among the diabetes patients presenting in our hospital and to determine the microbial profile and antimicrobial culture sensitivity pattern at GMMMC Hospital Sukkur. There exists a pressing necessity to identify diabetic foot ulcers in the initial stages so as to implement preemptive measures and forestall its associated complications. With better knowledge of culture sensitivity pattern of the infections of DFUs, better targeted treatment can be offered to these patients reducing the morbidity, and cost of treatment.

The study aimed to determine the frequency of culture-positive diabetic foot ulcer (DFU) patients among diabetes patients presenting at the hospital and to analyze the microbial profile and antimicrobial culture sensitivity pattern in these patients.

METHODS

A cross-sectional study was conducted at the Department of Medicine Unit-II, Ghulam Muhammad Mahar Medical College (GMMMC) Hospital, Sukkur, over a period of six months following the approval of the research synopsis. The sample size was calculated by considering an expected incidence of culture-positive diabetic foot ulcer (DFU) patients of 47.4%, a 95% confidence level, and an absolute precision of 8%, yielding a total sample size of 150 patients. A non-probability consecutive sampling method was used for selecting patients.

The study population included patients aged 18 to 80 years of either sex diagnosed with diabetes mellitus (DM) according to the operational definition or known diabetics with diabetic foot ulcers. Patients were excluded if there were non-diabetic foot ulcers, deep vein thrombosis (DVT), any foot skin pathology, or in case they have taken antibiotics for over 24 hours within the last 72 hours prior to enrollment.

The study was approved by the hospital review board (RTMC No. MED-2021-230-18545) and the College of Physicians and Surgeons of Pakistan (CPSP). Written informed consent was obtained from all enrolled patients before participation. Eligible patients visiting the Department of Medicine Unit-II at GMMMC Hospital, Sukkur, who met the inclusion criteria, were enrolled. A consultant with at least five years of experience supervised the assessment of each diabetic patient for clinical symptoms associated with diabetic foot ulcers. The SINBAD classification system was used to evaluate the ulcer site, ischemia, neuropathy, bacterial infection, and depth.

Laboratory investigations included complete blood count (CBC), random blood sugar (RBS), fasting blood sugar (FBS), urine detailed report (Urine D/R), and glycated hemoglobin (HbA1c). Tissue culture samples were collected from the infected site in two separate containers and sent to the laboratory for microbiological culture and

antimicrobial susceptibility testing, which was conducted by a pathologist with a minimum of five years of experience. Study variables such as age, gender, BMI, type of DM, duration of ulcer, site, neuropathy, ischemia, bacterial infection, depth, ulcer area, HbA1c levels, isolated organism, and antimicrobial susceptibility were recorded in a predesigned proforma.

Data were entered and analyzed using SPSS version 23. Categorical variables such as gender, site of ulcer, ischemia, neuropathy, type of DM, positive culture, isolated organism, and antimicrobial susceptibility were reported as frequencies and percentages. Continuous variables such as age, duration of ulcer, HbA1c, ulcer area, and depth were reported as mean and standard deviation or median (interquartile range), depending on data normality, which was assessed using the Shapiro-Wilk test. Outcome variables were stratified based on age, gender, type of DM, duration of ulcer, site, ischemia, neuropathy, ulcer area, depth, and HbA1c levels.

RESULTS

Baseline Characteristics of Patients with Diabetic Foot Ulcers

Table-I presents the baseline characteristics of patients with diabetic foot ulcers (DFU). The mean age of the study population was 47.97 years with a standard deviation of 17.11 years. The average duration of ulcers was 173.21 days with a standard deviation of 107.69 days, indicating significant variation in ulcer chronicity among patients. The mean ulcer area was 4.92 cm^2 with a standard deviation of 2.78 cm^2, while the mean ulcer depth was 5.46 mm with a standard deviation of 2.74 mm, reflecting differences in ulcer severity. Regarding glycemic control, the mean HbA1c level was 8.53% with a standard deviation of 2.05%, suggesting poor long-term glycemic management among patients. The mean fasting blood sugar level was 191.47 mg/dL with a standard deviation of 66.93 mg/dL, further indicating inadequate diabetes control in the study population.

Clinical and Microbiological Characteristics of Patients with Diabetic Foot Ulcers

Table-II provides the frequency distribution of clinical and microbiological characteristics in patients with DFU. Among the study population, 54.7% were female (n=82) and 45.3% were male (n=68). The majority of patients had type 2 diabetes mellitus (51.3%), while 48.7% had type 1 diabetes mellitus. The most commonly affected ulcer site was the heel (28.0%), followed by the dorsum of the foot (26.0%), toes (24.7%), and plantar surface (21.3%).

Regarding ulcer-associated complications, 44.0% of patients (n=66) had ischemia, while 56.0% (n=84) did not exhibit ischemic changes. Neuropathy was present in 52.0% of cases (n=78), whereas 48.0% (n=72) did not have neuropathic involvement. The presence of bacterial infection was assessed through culture positivity, revealing that 48.0% of patients (n=72) had a positive culture, while 52.0% (n=78) did not show bacterial growth.

The distribution of isolated organisms from culture-positive DFU cases showed that Escherichia coli (E. coli) was the most frequently isolated pathogen (29.2%), followed by Klebsiella pneumoniae (25.0%), Staphylococcus aureus (23.6%), and Pseudomonas aeruginosa (22.2%). Antimicrobial susceptibility testing revealed that cephalosporins were the most effective antibiotics, with 60.7% sensitivity, followed by vancomycin (12.7%), gentamicin (10.0%), carbapenems (9.3%), and ciprofloxacin (7.3%). Conversely, antibiotic resistance patterns indicated that cephalosporins showed the highest resistance (39.3%), followed by penicillin (37.5%) and aminoglycosides (23.2%).

These findings highlight the clinical and microbiological burden of diabetic foot ulcers, emphasizing the need for targeted antimicrobial therapy and improved management strategies to reduce infection-related complications.

Variable	Mean	Std Dev
Age	47.97	17.11
Duration of Ulcer (days)	173.21	107.69
Ulcer Area (cm^2)	4.92	2.78
Ulcer Depth (mm)	5.46	2.74
HbA1c Level (%)	8.53	2.05
Fasting Blood Sugar (mg/dL)	191.47	66.93

Table-I. Baseline characteristics of patients with diabetic foot ulcers (n=150)

Variable	Category	Count	Percent
Gender	Female	82	54.7%
	Male	68	45.3%
Diabetes Type	Type 2	77	51.3%
	Type 1	73	48.7%
Site	Heel	42	28.0%
	Dorsum	39	26.0%
Toe 37			24.7%
	Plantar	32	21.3%
Ischemia	Yes	66	44.0%
	No	84	56.0%
Neuropathy	Yes	78	52.0%
	No	72	48.0%
Positive Culture	Yes	72	48.0%
	No	78	52.0%
Organism Isolated	E. coli	21	29.2%
	Klebsiella pneumoniae	18	25.0%
	Staphylococcus aureus	17	23.6%
	Pseudomonas aeruginosa	16	22.2%
Antibiotic Sensitivity	Cephalosporins	91	60.7%
	Vancomycin	19	12.7%
	Gentamicin	15	10.0%
	Carbapenems	14	9.3%
	Ciprofloxacin	11	7.3%
Antibiotic Resistance	Cephalo sporins	22	39.3%
	Penicillin	21	37.5%
	Aminogly cosides	13	23.2%

Table-II. Frequency distribution of clinical and microbiological characteristics in patients with diabetic foot ulcers (n=150)

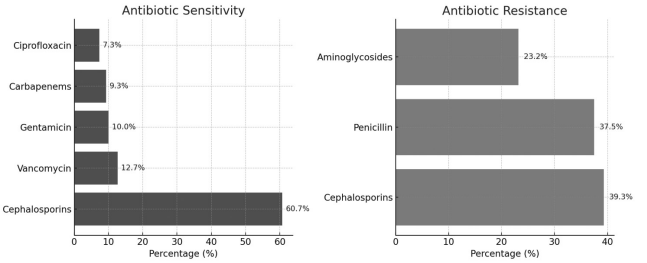

Figure-1. Antimicrobial susceptibility pattern in diabetic foot ulcer patients

DISCUSSION

Diabetic foot ulcers (DFUs) are a major healthcare problem because of their high frequency, delayed healing, and the development of severe complications, such as amputations. Our objective was to identify the prevalence of culture-positive DFUs, microbial profile, and antimicrobial susceptibility patterns among diabetic patients presenting at Ghulam Muhammad Mahar Medical College (GMMMC) Hospital, Sukkur. Our study's results are in agreement with some of the earlier published work but differ in some respects, pointing to regional differences in microbial occurrence and resistance to antibiotics.

Our study found that 48% of DFU cases were culture-positive, which is lower than the 69.6% reported by Taki et al[13] (2022) in Tehran and the 66.2% found by Ahmad et al[14] (2022) in a tertiary care hospital in Peshawar. This discrepancy could be due to variations in sampling methods, prior antibiotic use, or differences in laboratory culture techniques. Additionally, some DFUs may have been infected with anaerobic bacteria, which were not adequately assessed in our study but were considered in studies like that of Taki et al[13] (2022), where both aerobic and anaerobic pathogens were identified.

The most frequently isolated organisms in our study were Escherichia coli (29.2%), Klebsiella pneumoniae (25.0%), Staphylococcus aureus (23.6%), and Pseudomonas aeruginosa (22.2%). These findings are comparable to the results of Muhammad Sami et al[15] (2024), who reported E. coli (28%) and Klebsiella pneumoniae (22%) as dominant gram-negative bacteria in Peshawar. However, studies conducted in Tehran (Taki et al.,

2022)[13] and Saudi Arabia by Orfali et al[16] identified Staphylococcus spp. as the leading pathogen, with a prevalence of 52.2% and 40%, respectively. The differences in microbial distribution could be attributed to geographical variations in healthcare practices, infection control measures, and antibiotic prescription patterns.

Our study found high resistance rates to cephalosporins (39.3%), penicillin (37.5%), and aminoglycosides (23.2%), which is consistent with the findings of Ahmad et al[14] (2022) and Muhammad Sami et al (2024)[15], who reported that gram-negative bacteria exhibited strong resistance to beta-lactam antibiotics, including cephalosporins and penicillins. However, Taki et al[13] (2022) observed notably higher resistance to ciprofloxacin (70.6%) and clindamycin (73.5%) in Staphylococcus aureus, whereas our study reported only 7.3% ciprofloxacin resistance. This suggests that fluoroquinolones may still be effective in our population, though the increasing resistance trend in other regions highlights the need for careful monitoring.

One of the significant concerns highlighted in our study is the prevalence of multidrug-resistant (MDR) organisms, particularly among gram-negative bacteria. The detection of MDR pathogens is in line with the findings of Raha Orfali et al (2024)[16], who reported that biofilm-forming bacteria in DFUs exhibited increased antibiotic resistance, complicating treatment. The presence of biofilms can reduce antibiotic penetration, leading to persistent infections and delayed wound healing. Future studies should explore the role of biofilm formation in DFUs in our population.

While our study provides valuable insights into the microbial landscape and antimicrobial susceptibility of DFUs, several limitations must be acknowledged. First, our study did not assess anaerobic bacteria, which have been reported in studies such as those by Taki et al (2022)[13] and Ahmad et al (2022)[14] to play a significant role in DFU infections. The exclusion of anaerobic cultures may have led to an underestimation of the actual microbial diversity present in DFUs.

Another limitation is the lack of molecular characterization of resistant strains. Studies like those conducted by Taki et al. (2022)[13] have identified ESBL genes, mecA genes, and metallo-beta-lactamases (MBL) in DFU isolates, which provide critical insights into antibiotic resistance mechanisms. Without molecular analysis, our study could not determine whether resistance was due to genetic mutations or acquired resistance mechanisms.

The cross-sectional study design presents another limitation. Since data were collected at a single time point, we could not evaluate longitudinal changes in microbial profiles or resistance trends over time. A prospective study design with follow-up cultures could provide a better understanding of antibiotic resistance evolution in DFU infections.

Furthermore, our study did not assess clinical outcomes, wound healing rates, or patient response to different antibiotic regimens. Future studies should correlate microbial findings with treatment success, recurrence rates, and amputation rates to provide a more comprehensive picture of DFU management.

Given the increasing prevalence of MDR infections in DFUs, future research should focus on comprehensive microbial surveillance programs to track resistance patterns and inform antibiotic stewardship programs. Implementing routine culture-based antimicrobial sensitivity testing could help tailor patient-specific antibiotic regimens, reducing the risk of treatment failure and unnecessary antibiotic exposure.

The role of biofilm formation in DFU infections warrants further investigation. Studies such as that of Raha Orfali et al (2024)[16] have shown that biofilms contribute significantly to antibiotic resistance. Future research should assess anti-biofilm treatment strategies, such as enzyme-based therapies or combination antibiotic approaches, to enhance treatment efficacy.

Another promising area of research is the use

of novel antimicrobial agents, such as phage therapy, antimicrobial peptides, and silver-based dressings, which have shown potential against MDR pathogens. Investigating alternative therapies and personalized medicine approaches could lead to better DFU management strategies.

Finally, patient education and preventive strategies must be emphasized in future studies. Early intervention through foot care education, routine screening for neuropathy and ischemia, and aggressive glycemic control can significantly reduce the incidence and severity of DFUs. Future studies should explore the effectiveness of community-based intervention programs in reducing DFU-related complications.

CONCLUSION

Our study provides crucial insights into the microbial profile and antibiotic resistance patterns in DFUs in a regional hospital in Pakistan. While our findings align with previous studies in terms of high rates of MDR infections and the predominance of gram-negative bacteria, regional differences in microbial prevalence and antibiotic resistance trends underscore the need for location-specific treatment protocols. The high resistance to cephalosporins and penicillins reinforces the importance of antibiotic stewardship programs in DFU management.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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1	**Muhammad Faheemullah Kamboh:** Data collection, analysis, paper writing.
2	**Iftikhar Ali Shah:** Discussion writing, review of manuscript.
3	**Bakhtawar Rafique:** Data collection, paper writing.
4	**Saleh Muhammad Channa:** Discussion writing, review of manuscript.
5	**Bashir Ahmed Chandio:** Data analysis, manuscript writing.
6	**Umair Ali Shah:** Review of manuscript.