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Prevalence of Microorganisms in Diabetic Foot Patients and their Sensitivity Analysis at Fatima Memorial Hospital Lahore

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ABSTRACT

Background: Diabetic foot infections (DFIs) or ulcers (DFUs) are a major problem among diabetic patients that can result in extended hospital stays and amputations in case of improper treatment. Therapy frequently begins with empirical treatment; however, insights into the local microbial epidemiology, antibiotic resistance, and sensitivity patterns are essential to successful therapy. The purpose of the study was to identify the prevalence of Gram-positive and Gram-negative organisms in DFIs and to assess their antibiotic susceptibility.

Methods: This cross-sectional study was conducted on 77 patients with infected foot ulcer cases at Fatima Memorial Hospital, Lahore. Samples of deep wounds were obtained, and microbiological anaerobic and aerobic culturing was performed. Kirby-Bauer disk diffusion testing was used for antibiotic sensitivity testing with SPSS Statistics version 26.0. Organism prevalence and sensitivity were reported using descriptive statistics, and the relationship between the organism and demographic variables was analyzed using Chi-square and t-tests, with a p-value < 0.05 being significant.

Results: E. coli (16 (21.6%)) and Klebsiella pneumoniae (8(10.8%)) were dominant among gram-negative isolations, whereas MRSA (8 (10.8%)) and Staphylococcus aureus (6 (8.1%)) were the prevalent gram positives. Cefotaxime had the greatest sensitivity (17 (22.9%)), followed by colistin and imipenem. Hypertension and obesity were prevalent, with no significant correlation with antibiotic resistance patterns ($p>0.05$). The results found Gram-negative organisms in DFIs, with their antibiotic sensitivity patterns were inconsistent and requiring local surveillance.

Conclusion: This study concluded that DFIs in this population were caused by Gram-negative organisms, and cefotaxime was highly sensitive, making it a possible addition to empirical therapy.

Keywords: Diabetic Foot, Foot Ulcer, Anti-Bacterial Agents, Wound Infection, Diabetes Mellitus.

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INTRODUCTION

Diabetic foot infections (DFI) or ulcers are a widespread and significant manifestation of diabetes mellitus that is caused by a mixture of peripheral arterial disease, neuropathy, and poor wound healing [1]. Microorganisms colonize or infect these chronic wounds, making them difficult to manage with higher chances of sepsis development, hospital stays, and amputations [2]. The DFI microbiome is heterogeneous, with Gram-positive (Staphylococcus aureus, including MRSA) or Gram-negative (Pseudomonas aeruginosa, Escherichia coli) microorganisms, frequently of a polymicrobial nature [3]. It has been demonstrated that polymicrobial infections, predominantly Gram-negative, are prevalent in Asia, with S. aureus being the most frequent single pathogen [4].

Recent studies also observed an increased Gram-negative dominance and multidrug resistance, especially in low- and middle-income countries [5]. Proper management is necessary for early microbiological diagnosis and individual antibiotic therapy guided by regional susceptibility patterns [6]. Nevertheless, delays in culture results are a reason why empirical treatment is being commonly employed due to the risk of inappropriate use of antibiotics and resistance [7]. The duration of diabetes, peripheral vascular disease, and peripheral neuropathy has been recognized as clinical variables that increase the susceptibility and severity of the infections [8]

Although studies conducted at the global and regional level provide valuable knowledge, there is a need to generate recent and inclusive data on DFI that not only entail microbial profiles but also incorporate antimicrobial susceptibility together with clinical risk characteristics in these conditions. The characterization of the contemporary bacteriological landscape, drug resistance pattern, and the relationship with clinical risk factors may help support empiric-therapy decisions and enhance antibiotic stewardship, and in the long term, decrease morbidity and the potential of amputation [9].

The purpose of the study was to determine the dominant bacterial causative agents of diabetic foot infections. It also aimed to evaluate their antimicrobial susceptibility patterns. It further analyzed relationships between clinical risk factors and the nature of the infection to develop more effective local interventions.

METHODS

This cross-sectional study was conducted at the Department of Surgery in Fatima Memorial Hospital, Lahore, a tertiary care teaching hospital, from May 2023 to November 2023 (FMH-15/06/2023-1222). A non-probability consecutive sampling method was used in recruiting the participants within six months. The OpenEpi version 3.0.0 (released 2013, Atlanta, GA, USA) was used to calculate the required sample size. Using a finite population of 95 patients, the sample size required to estimate a

population proportion with a 95% confidence level and a margin of error of 5% was calculated as 77 patients [10]. The inclusion criteria were the age of patients between 18 to 80 years, both genders, diagnosis with Type 2 Diabetes Mellitus (DM), which was verified by HbA1c above 7%, fasting, or random blood glucose monitoring. Patients with clinically infected DFU that were graded Wagner Grade 2 to 5 and were not on systemic antibiotic treatment in the previous week of time were also included. Patients below 18 years of age and above 80 years, non-diabetics, and those who were taking antibiotics were excluded.

Deep wound specimens were obtained with a sterile curette at the base of the ulcer after it was cleaned with sterile normal saline and debrided of superficial and necrotic tissue. Samples were re-processed in an accredited pathology laboratory with standard microbiological techniques under aerobic and anaerobic conditions. The cultured organisms were identified and tested for sensitivity to commonly used antibiotics through the Kirby-Bauer disk diffusion technique. SPSS version 26.0 (released 2019, IBM Corp., Armonk, NY) was used to analyze data. The Chi-square test was used to compare the categorical variables, whereas independent t-tests evaluated the difference between the means (statistical significance as $p < 0.05$).

RESULTS

Table 1. Demographic and Clinical Characteristics of Study Participants (n = 77)

Variable	Value	Statistical Test	Test Value	Significance (p-value)
Age (years) Mean ± SD	55.4 ± 10.8	Independent t-test	t = 1.31	0.194
BMI (kg/m²) Mean ± SD	28.6 ± 4.2	Independent t-test	t = 0.87	0.387
Gender	Male = 50 (64.9%) Female = 27 (35.1%)	Chi-square	χ^2 = 3.12	0.077
Hypertension	Yes = 40 (51.9%) No = 37 (48.1%)	Chi-square	χ^2 = 0.12	0.730
Smoking status	Smoker = 26 (33.8%) Non-smoker = 51 (66.2%)	Chi-square	χ^2 = 1.21	0.270
Foot infection severity	Mild = 76 (98.7%) Severe = 1 (1.3%)	Chi-square	χ^2 = 74.51	< 0.001

BMI = Basal Metabolic Index, DM = Diabetes Mellitus, SD = Standard Deviation, N = Number of participants, % = Percentage, p< 0.05 is considered significant.

This study enrolled 77 DFI patients to discover the prevalence of bacterial organisms originating in DFU and assess their antibiotic sensitivity results. The most identified organism in the study was E. coli, followed by other gram-negative and gram-positive bacteria. Patients were sensitive to at least one antibiotic, and cefotaxime was found to be the popular choice. There was no significant relationship between antibiotic resistance and comorbidity with hypertension, obesity, or smoking. Demographic and Clinical Characteristics of Study Participants are presented in **Table 1.**

Table 2. Frequency and Percentage of Isolated Organisms in DFI

Organism	Frequency N (%)	Statistical Test
E. coli	16 (21.6%)	Descriptive frequency
Klebsiella pneumonia	8 (10.8%)	Descriptive frequency
MRSA	8 (10.8%)	Descriptive frequency
Pseudomonas aeruginosa	7 (9.5%)	Descriptive frequency
Staphylococcus aureus	6 (8.1%)	Descriptive frequency

E. coli = Escherichia coli, MRSA = Methicillin-resistant Staphylococcus aureus, N = Number of participants, % = Percentage, p< 0.05 is considered significant.

Of the 77 diabetic foot infections, the average age was 55.4 ± 10.8 years, indicating the dominance of middle-aged patients. The average BMI of the population was 28.6 ± 4.2 kg/m^2, demonstrating an almost overweight population. A majority (50 (64.9%)) of the sample was comprised of males. Marked comorbidities were the presence of hypertension (40 (51.9%)) and smoking (26 (33.8%)). Infection severity was found to be highly significant, with 76 (98.7%) infections being mild, whereas 1 (1.3%) case was severe ($p < 0.001$). The high proportion of less severe cases implies early presentation in the hospital or good outpatient care, although comorbid risk factors such as obesity and hypertension were common. **Table 2** represents the frequency of isolated microbes from DFI patients.

Table 3 Frequency and Percentage of First-Line Sensitive Antibiotics

Antibiotic	Frequency N (%)	Statistical Test
Cefotaxime	17 (22.9%)	Descriptive frequency
Colistin	11 (14.9%)	Descriptive frequency
Imipenem	11 (14.9%)	Descriptive frequency

Linezolid	5 (6.8%)	Descriptive frequency
Meropenem	4 (5.4%)	Descriptive frequency

N = Number of participants, % = Percentage, p< 0.05 is considered significant.

E. coli was the most highly isolated organism (16 (21.6%)), followed by Klebsiella pneumonia (8 (10.8%)), MRSA (8 (10.8%)), and Pseudomonas aeruginosa (7 (9.5%)). These results indicate the polymicrobial and Gram-negative character of DFUs in this group. The prevalence rates of E. coli and Gram-negative bacteria characterize the necessity of broad-spectrum initial coverage, especially against Gram-negative flora. Frequency of sensitive antibiotics against microbes of DFU are listed in **Table 3**.

Table 4. Gender-wise Distribution of Common Organisms in DFI

Organism	Male (n = 46)	Female (n = 31)	Test Value	p-value
E. coli	9 (19.6%)	7 (22.6%)	$\chi^2 = 0.10$	0.752
Klebsiella pneumonia	6 (13.0%)	2 (6.5%)	$\chi^2 = 0.87$	0.351
MRSA	5 (10.9%)	3 (9.7%)	$\chi^2 = 0.03$	0.861

E. coli = Escherichia coli, N = Number of participants, MRSA = Methicillin-resistant Staphylococcus aureus % = Percentage, p< 0.05 is considered significant.

The best general antibiotic was cefotaxime (17 (22.9%) sensitivity), followed by Colistin and Imipenem (11 (14.9%)). Lower sensitivity to Linezolid 5 (6.8%) and Meropenem 4 (5.4%) was observed. The cefotaxime had high sensitivity, indicating the potential first-line empirical choice, although its resistance trend has been increasing; susceptibility must be continually monitored. Common organisms distribution in gender is illustrated in **Table 4.**

Table 5. Comparison Between the Severity of Foot Infection and Antibiotic Sensitivity

Foot Infection	Sensitive to Antibiotics	Test Value	p-value
Mild	76 (98.7%)	0.00	1.000
Severe	1 (1.3%)	0.00	1.000

N = Number of participants, % = Percentage, p< 0.05 is considered significant.

Microorganism distribution was not significantly different between genders. Isolation of E. coli was found in 9 (19.6%) of men and 7 (22.6%) of women (p = 0.752); no significant results were observed in Klebsiella, pneumonia, and MRSA. No gender effect on the microbiological profile of DFUs was observed, indicating a combination of similar empirical treatment options for both sexes. **Table 5** shows the comparison between the severity of DFU and antibiotic sensitivity.

Table 6. Comparison between Comorbidities and Antibiotic Sensitivity

Variable	Sensitive (n=48)	Resistant (n=29)	Test Value	p-value
Hypertension (%)	28 (58.3%)	12 (41.4%)	$\chi^2 = 2.07$	0.150
Obese (BMI ≥30) (%)	20 (41.7%)	12 (41.4%)	$\chi^2 = 0.00$	0.989
Smoker (%)	16 (33.3%)	10 (34.5%)	$\chi^2 = 0.01$	0.923

BMI = Basal Metabolic Index, N = Number of participants, % = Percentage, p< 0.05 is considered significant.

76 (98.7%) of 77 cases were antibiotic sensitive, with all the cases being mild. Sensitivity was seen in only one severe case, although this association was not significant (p = 1.000). However, it suggests that the severity of infections did not influence antibiotic sensitivity in this cohort, although there are a few severe cases that inhibit generalization. The comparison between comorbidities and antibiotic sensitivity is given in **Table 6**.

The association between comorbidities and antibiotic resistance was not significant. Hypertension was observed in 28 (58.3%) cases in the sensitive group compared to 12 (41.4%) in the resistant group (p = 0.150). Similarly, 20 (41.7%) cases exhibited obesity in the sensitive group compared to 12 (41.4%) cases in the resistant group (p = 0.989). Moreover, smoking was observed in 16 (33.3%) cases in the sensitive group compared to the 10 (34.5%) cases in the resistant group (p = 0.923). No strong associations suggest that antibiotic resistance may be linked to factors related to microbes and therapy rather than the patient's comorbidity.

DISCUSSION

This study aimed to determine the prevalence of the most frequently isolated Gram-positive and Gram-negative organisms in diabetic foot infections and to determine their pattern of antibiotic sensitivity at a tertiary care hospital. The findings of this study suggest that Gram-negative bacteria are the most common organisms that infect the diabetic foot. It also showed that there is regional variation in the antimicrobial sensitivity that affects the treatment options.

The socio-demographic factors were found to have high prevalence in middle-aged men with hypertension and overweight conditions. It is consistent with the studies that found neuropathy and poor glucose control in middle-aged men predispose them to diabetic foot ulcers [11,12]. It was also indicated that hypertension and obesity are associated with slow wound healing [13]. Moreover, adding to the risk of infection by these comorbidities was also demonstrated by another study [14].

Microbiological evidence showed that the most common Gram-negative organisms isolated were E. coli and Klebsiella pneumoniae, whereas MRSA and Staphylococcus aureus were prominent in

Gram-positive isolation, with high sensitivity shown to cefotaxime. This is consistent with the findings of the study, which stated that diabetic foot infections in low- and middle-income countries were predominantly Gram-negative [15,16]. Similar trends have been described by previous literature, which included E. coli and Klebsiella among the most frequent pathogens, with an increasing rate of multidrug resistance [17,18]. Furthermore, a study showed that MRSA prevalence was not associated with worse prognosis in patients with DFU [19].

We also found that cefotaxime had the highest sensitivity, which is consistent with other findings that reported cefotaxime was effective in treating Gram-negative isolates; however, another study reported emerging patterns of resistance that need close monitoring [20,21]. Additionally, other studies explained the drift towards carbapenem usage owing to the presence of ESBL-producing organisms in DFIs [22].

The outcomes of the study revealed no significant correlation between comorbidities and antibiotic resistance, which aligns with studies, highlighting factors other than comorbidities, such as poor hygiene or neuropathy, that influence the complexity of the infections [23,24]. These results indicate that empirical therapy of diabetic foot infections should focus on Gram-negative coverage [25]. Moreover, cefotaxime may be considered as a first-line agent, although culture-based modification is necessary [26].

The study limitations are a single-center design and small sample size, which does not provide the possibility of generalizing. The results may have been affected by confounding factors in the form of undocumented use of antibiotics and variations in wound care. Molecular resistance profiling, multicenter, and longitudinal research design should be used in future research to inform antibiotic stewardship and enhance positive diabetic foot outcomes.

CONCLUSION

This study demonstrated that Gram-negative microorganisms, especially E. coli and Klebsiella pneumoniae, were the most common in diabetic foot infections, and cefotaxime exhibited the greatest sensitivity among the confirmed antibiotics. These results confirm the initial purpose of determining the most prevalent Gram-positive and Gram-negative isolates and antibiotic resistance profiles in a tertiary care facility.

These results suggest that Gram-negative coverage should be a priority of empirical antibiotic treatment of diabetic foot infection, where cefotaxime may become a first-line therapy, depending

on clinic-specific antibiotic sensitivities. Integration of culture and sensitivity testing into routine practice is critical to the optimal treatment and avoidance of antibiotic resistance.

LIST OF ABBREVIATIONS

DFI: Diabetic Foot Infection

DFU: Diabetic Foot Ulcer

DM: Diabetes Mellitus

ESBL: Extended Spectrum Beta Lactamase

MRSA: Methicillin-Resistant Staphylococcus aureus

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CONFLICT OF INTEREST

None

ETHICAL APPROVAL

This cross-sectional study was conducted at the Department of Surgery in Fatima Memorial Hospital, Lahore, a tertiary care teaching hospital, from May 2023 to November 2023 (FMH-15/06/2023-1222).

AUTHORS' CONTRIBUTION

All authors contributed equally as per ICMJE policy

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