Section: Microbiology

Original Research Article

CLINICO-BACTERIOLOGICAL PROFILE OF DIABETIC FOOT ULCERS IN A TERTIARY CARE HOSPITAL

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Received : 10/07/2026
Received in revised form : 14/06/2026
Accepted : 01/07/2026

Keywords:
Diabetic foot ulcers, multidrug-resistant organisms, risk factors, amputation.

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DOI: 10.47009/jamp.2026.8.4.152

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2026; 8 (4); 898-903

ABSTRACT

Background: Diabetic foot ulcers (DFUs) are a common complication of diabetes mellitus (DM) and are frequently associated with prolonged hospital stay, increased healthcare costs, and an increased risk of amputation. The present study was undertaken to evaluate the clinical characteristics, bacteriological profile, antimicrobial susceptibility patterns and associated risk factors among patients with diabetic foot ulcers. **Materials and Methods:** The study was conducted in the Department of Microbiology at a tertiary care hospital in central Kerala over a one-year period from December 2019 to December 2020. A total 100 patients with DFUs were included in the study. Tissue specimens and aspirates from DFUs were collected and processed under aseptic precautions. Aerobic bacterial isolates recovered on culture were identified by standard biochemical tests, and antimicrobial susceptibility testing was performed. Multidrug resistant (MDR) isolates were identified, and their association with various risk factors was assessed. Statistical analysis was performed using SPSS software. **Results:** A total of 123 bacterial isolates were obtained from 100 samples. Gram-negative isolates (71.5%) predominated over Gram-positive (28.5%) isolates, with *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella spp*. being the predominant isolates. Males were affected (79%) more frequently than females (21%). Previous antibiotic therapy and long-standing ulcer showed a significant association with multi drug resistance. Imipenem and Meropenem were the most effective antibiotic against Gram-negative isolates whereas Vancomycin was the most effective antibiotic against Gram-positive isolates. **Conclusion:** Diabetic foot infections are polymicrobial in nature, and a considerable proportion of isolates exhibit multidrug resistance. Early identification of risk factors, prompt microbiological evaluation, culture-guided antimicrobial therapy, and comprehensive wound care can reduce morbidity and prevent adverse outcomes such as amputations.

INTRODUCTION

"Diabetes mellitus is considered a major global public health challenge of the 21st century.[1] Among individuals with diabetes, approximately 15-25% are at risk of developing diabetic foot infections (DFIs) at some point during their life time.[2] Diabetic foot infections are serious and long-lasting complications of diabetes. Additional factors such as smoking, advanced age, a prolonged history of uncontrolled diabetes, gangrenous infections, and large sized ulcers increase the risk of lower limb amputation.[3] DFIs are often managed inadequately due to lack of understanding of the microbial profile, antimicrobial susceptibility patterns, and appropriate therapeutic approaches.[4] The injudicious use of antibiotics contributes to emergence of multi drug resistant organisms (MDROs) making these infections difficult to treat. Consequently, they prolong hospital stay, increase the cost of treatment and imparts additional morbidity and mortality to the patient.[5,6] This study was undertaken to determine the microbiological profile and antimicrobial susceptibility patterns of diabetic foot infections and to assess the association of various clinical and demographic factors with the outcomes of patients with diabetic foot ulcers (DFUs).

MATERIALS AND METHODS

A hospital-based observational prospective study was conducted in Department of Microbiology in collaboration with Department of General Surgery at Government Medical College, Thrissur, a tertiary

ISSN (O): 2687-5365; ISSN (P): 2753-6556

care referral centre in central Kerala catering to the districts of Thrissur, Palakkad and Malappuram.

Inclusion Criteria

The study included patients with clinically diagnosed diabetes mellitus presenting with infected foot ulcers.

Exclusion Criteria

Non-diabetic patients with foot ulcers due to other causes, those who refuse to give consent and those who are illiterate or otherwise unable to comprehend or respond to the questionnaire were excluded from the study.

A total 100 patients admitted to the Department of General Surgery, Government medical College, Thrissur, with diabetic foot ulcer during 2019 - 2020 were included in the study. The clinical history from the patients were elicited using a structured questionnaire that included age, sex, ulcer characteristics, status of glycemic control at admission and predisposing factors. Detailed examination of diabetic foot including examination of the ulcer, evaluation of the foot vascularity and presence of neuropathy. Peripheral vascular disease was assessed by palpating peripheral pulses. Patients with absent or feeble peripheral pulses underwent doppler examination to confirm vascular occlusion. Normal vascularity in Doppler is indicated by a triphasic flow pattern. Biphasic and monophasic flow patterns were considered abnormal. Neuropathy was assessed by sensory examination of foot, including assessment of touch and pain sensations. Ulcers were graded according to the Wagner's classification from 0 to 5.[7,8]

Sample collection

Samples were collected after explaining the purpose of the study and obtaining informed consent from the patients. Following thorough debridement and cleansing of the ulcer, pus aspirates or tissue specimens were collected aseptically from the ulcer base in sterile universal containers. The samples were immediately transported to the Microbiology laboratory and processed within 30 minutes. Direct Gram staining was prepared from the samples to assess the presence of pus cells and organisms. The specimens were cultured on appropriate culture media and the isolates were identified based on colony morphology, microscopic characteristics, and standard biochemical tests.[9,10]

Antimicrobial susceptibility testing: Antibiotic susceptibility testing of the isolates was performed using the standard Kirby-Bauer disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines, and the results were interpreted accordingly.[11] Methicillin resistance in *Staphylococcus aureus* was determined using Cefoxitin (30µg) disc. Isolates with zone of inhibition ≤ 21 mm around the Cefoxitin (30µg) disc were considered as *Methicillin resistance Staphylococcus aureus (MRSA*). Vancomycin minimum inhibitory concentration (MIC) for *Staphylococcus aureus* isolates was determined using E-test (E-strip), which has been shown to provide results comparable to the broth microdilution method.[12,13] Multidrug resistant organisms included *MRSA*, and isolates that showed resistance to at least one agent in three or more antimicrobial classes, according to CDC guidelines.[14]

Statistical Analysis: All categorical variables were expressed as frequencies and percentages. The chi-square test was used to compare the study variables. Data were entered into Microsoft Excel and analysed using IBM Statistical Package for Social Sciences (SPSS) version 25.0. A P value is ≤ 0.05 was considered statistically significant.

RESULTS

A total of 100 patients with diabetic foot ulcers were included in the study. The patient's ages ranged from 38 to 85 years, with a mean age of 56.6 ± 9.1 years. The majority of patients belonged to the 41–60 years age group (63%), followed by the 61–80 years age group (31%). Five patients (5%) were aged 20 - 40 years, while only one patient (1%) was older than 80 years. There was a marked male predominance, with 79 males (79%) and 21 females (21%), resulting in a male to female ratio of 3.8:1. Regarding the type of diabetes, 99 patients (99%) had Type 2 diabetes mellitus, while only one patient (1%) had Type 1 diabetes mellitus. The duration of diabetes was more than 10 years in 55 patients (55%), whereas 45 patients (45%) had diabetes for 10 years or less. The onset of ulcer was spontaneous in 63 patients (63%), while 37 patients (37%) developed ulcers following trauma. The duration of the ulcer was ≤2 weeks in 51 patients (51%) and >2 weeks in 49 patients (49%).

Ulcers were graded according to Wagner's grading system. Grade IV ulcers were the most common, occurring in 60 patients (60%), followed by Grade III ulcers in 22 patients (22%) and Grade II ulcers in 17 patients (17%). Only one patient (1%) had a Grade V ulcer. Poor glycemic control was the most common risk factor, present in 65 patients (65%), followed by recurrent ulceration in 48 patients (48%), neuropathy in 45 patients (45%), smoking in 42 patients (42%), prior foot surgery in 42 patients (42%), and peripheral vascular disease in 35 patients (35%). A history of prior antibiotic use was present in 52 patients (52%), while 48 patients (48%) had no previous antibiotic exposure [Figure 1].

Regarding comorbidities, anemia was present in 59 patients (59%), followed by hypertension (HTN) in 33 patients (33%) and coronary artery disease in 21 patients (21%). The major diabetes-related complications were nephropathy in 31 patients (31%) and osteomyelitis in 15 patients (15%).

ISSN (O): 2687-5365; ISSN (P): 2753-6556

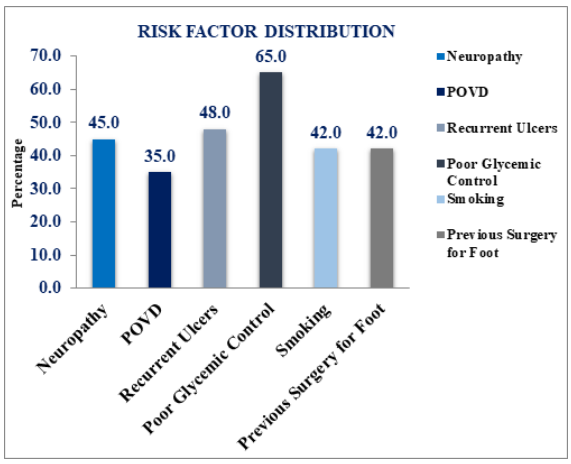

Figure 1: Frequency distribution of risk factors among study population

A total of 123 bacterial isolates were recovered from 100 cases. Among these, 23 (23%) cases had polymicrobial infections, while 77 (77%) cases had monomicrobial infections. Gram-negative isolates predominated, accounting for 88 (71.5%) isolates, whereas Gram-positive bacteria constituted 35 (28.5%) isolates. *Escherichia coli* 35 (28.5%) was the predominant Gram-negative isolate, followed by *Klebsiella pneumonia* 22 (17.9%), *Pseudomonas aeruginosa* 15(12.2%), *Acinetobacter baumannii* 5 (4.1%), *Proteus mirabilis* 5 (4.1%), *Proteus vulgaris* 3 (2.4%), *Citrobacter freundii* 2(1.6%) and *Enterobacter cloacae* 1 (0.8%). Among Gram-positive isolates *Staphylococcus aureus* 24 (19.5%) was the predominant organism, followed by *Streptococcus pyogenes* 5 (4.1%), *Enterococcus faecalis* 3 (2.4%), and other *Streptococcus spp*. 3 (2.4%) [Table 1 & Figure 2].

In the present study, among Enterobacteriaceae isolates complete resistance to Ampicillin was observed. Carbapenems (Imipenem and Meropenem) showed the highest susceptibility across most species, followed by Piperacillin-tazobactam and Amikacin. Lower Susceptibility to Cephalosporins, Ciprofloxacin and Cotrimoxazole was observed among *E. coli* and *K. pneumoniae* isolates. Among 15 *Pseudomonas aeruginosa* isolates, all (100%) were susceptible to Carbapenems. Susceptibility to Amikacin and Piperacillin-tazobactam was 73.3% each, followed by Ceftazidime (60%), Gentamicin (53.3%). Ciprofloxacin showed the highest resistance rate (60%) with only 40% of isolates remaining susceptible. All *Acinetobacter baumannii* isolates (100%) were susceptibility to Meropenem, while 60% were susceptible to Amikacin and Imipenem. Susceptibility to Piperacillin-tazobactam and Gentamicin was 40% each (Table 2). All Gram-positive isolates, including *MRSA*, were 100 % susceptible to Vancomycin. Furthermore, all Streptococcal and Enterococcal isolates were 100 % susceptible to Penicillin and Ampicillin [Table 3].

Multidrug resistance was observed in 30 of 35 *E. coli* isolates, 16 of 22 *Klebsiella* isolates and 5 of 15 *Pseudomonas aeruginosa* isolates. Of the 24 *Staphylococcus aureus* isolates, 7 were identified as *MRSA* [Table 4].

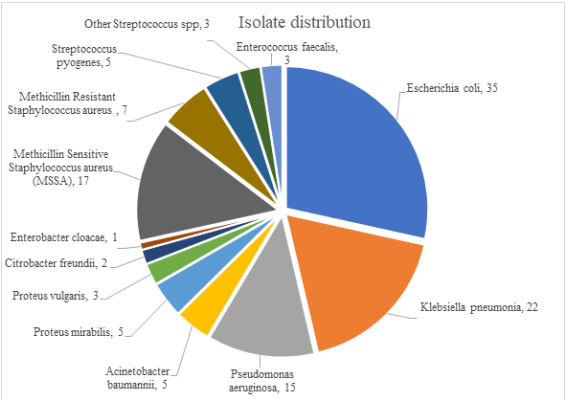

Figure 2: Distribution of various isolates obtained from foot ulcer.

Table 1: Distribution of various isolates obtained from foot ulcer

Organism		Frequency	Percentage
Gram-positive isolates (Total = 35) 28.5%			
Staphylococcus aureus	*Methicillin Sensitive Staphylococcus aureus (MSSA)*	17	13.8
	Methicillin Resistant Staphylococcus aureus (MRSA)	7	5.7
Streptococcus pyogenes		5	4.1
Other *Streptococcus spp,*		3	2.4
Enterococcus faecalis		3	2.4
Gram-negative isolates (Total = 88) 71.5%			
Escherichia coli		35	28.5
Klebsiella pneumoniae		22	17.9
Pseudomonas aeruginosa		15	12.2
Acinetobacter baumannii		5	4.1
Proteus mirabilis		5	4.1
Proteus vulgaris		3	2.4
Citrobacter freundii		2	1.6
Enterobacter cloacae		1	0.8
Total		123	100.0

ISSN (O): 2687-5365; ISSN (P): 2753-6556

Table 2: Antimicrobial susceptibility pattern of Gram-negative isolates

Antibiotics	Number of sensitive strains (Percentage)							
	E.coli N=35	***K.pneumoniae N=22***	***P. mirabilis N=5***	***P. vulgaris N=3***	***C.freundii N=2***	***E.cloacae N=1***	***P.aeruginosa N=15***	***A.baumannii N=5***
Ampicillin	0(0 %)	0 (0 %)	0(0 %)	0(0 %)	0(0 %)	0(0 %)	-	-
Cefazolin	4(11.4 %)	2(9.1%)	1(20%)	0(0%)	0(0%)	0(0%)	-	-
Ceftriaxone	5(14.3 %)	6(27.3%)	1(20%)	1(33.3%)	0(0%)	0(0%)	-	-
Ceftazidime	-	-	-	-	-	-	9(60%)	1(20%)
Gentamicin	15(42.9 %)	11(50%)	3(60%)	1(33.3%)	2(100%)	1(100%)	8(53.3%)	2(40%)
Amikacin	25(71.4 %)	16(72.7%)	4(80%)	1(33.3%)	2(100%)	1(100%)	11(73.3%)	3(60 %)
Cotrimoxazole	11(31.4 %)	7(31.8%)	2(40%)	1(33.3%)	1(50%)	0(0%)	-	0 (0%)
Ciprofloxacin	12(34.3 %)	6(27.3%)	3(60.0%)	1(33.3%)	0(0%)	0(0%)	6(40%)	1(20%)
Piperacillin-tazobactam	29(82.9 %)	15 (68.2%)	5(100%)	3(100%)	2(100%)	0(0%)	11(73.3%))	2(40%)
Imipenem	35(100 %)	19 (86.4%)	3(60%)	2(66.7%)	2 (100%)	1(100%)	15 (100%)	3(60%)
Meropenem	31(88.6 %)	20(90.9%)	4(80%)	3(100%)	2 (100%)	1(100%	15 (100%)	5(100%)

Table 3: Antimicrobial susceptibility pattern of Gram positive isolates

Antibiotic	Number of sensitive strains (Percentage)				
	MSSA N=17	***MRSA N=7***	***S.pyogenes N=5***	***Other Steptococcus spp N=3***	***E.faecalis N=3***
Ampicillin	-	-	5(100%)	3(100%)	3(100%)
Ceftriaxone	-	-	5(100%)	3 (100%)	-
High Level Gentamicin	-	-	-	-	2(66.7%)
Cotrimoxazole	15(88.2%)	4(57.1%)	0 (0%)	0 (0%)	-
Penicillin	0 (0%)	0 (0%)	5 (100%)	3 (100%)	3 (100%)
Erythromycin	2(11.8%)	0 (0%)	4 (80%)	2 (66.7%)	0 (0%)
Clindamycin	9(52.9%)	5(71.4%)	3 (60%)	1 (33.3%)	-
Cefoxitin	17(100%)	0 (0%)	-	-	-
Vancomycin	17(100%)	7 (100%)	5 (100%)	3 (100%)	3 (100%)

Table 4: Distribution of multidrug resistant isolates

Organism	No. of isolates	MDR no.	MDR %
Staphylococcus aureus	24	7	10.0
E.coli	35	30	42.86
Klebsiella pneumoniae	22	16	22.86
Pseudomonas aeruginosa	15	5	7.14
Proteus mirabilis	5	4	5.71
Proteus vulgaris	3	2	2.86
Enterobacter cloacae	1	1	1.43
Citrobacter freundii	2	1	1.43
Acinetobacter baumannii	5	4	5.71
Total		70	100.0

DISCUSSION

Diabetes mellitus is a rapidly expanding health problem worldwide. Infected foot ulcers are among the most chronic and debilitating complications of diabetes and can eventually lead to foot gangrene and lower-limb amputation. Infection with multidrug-resistant organisms (MDROs) further worsen the outcomes. The emergence of antimicrobial resistance is attributed to frequent hospitalizations, repeated exposure to broad-spectrum antibiotics, and inadequate surgical source reduction. Assessment of the magnitude of infection by microbiological analysis and its timely management will prevent complications.

A marked male predominance was observed in the present study, which is consistent with findings of previous studies by Gadepalli et al. and M Zubair et al.[6,15] The higher prevalence among males may be attributed to greater occupational exposure, increased risk of foot trauma, and life style factors such as smoking and alcohol consumption, which adversely affect ulcer healing.

The majority patients in the present study belonging to the 41-60 years age group which is comparable to the findings reported by Gadepalli et al.[15] Furthermore, the majority of cases presented to the hospital at an advanced stage of ulcer, mainly Wagener's grade 4 (60%), followed by grade 3 (22%) which is in agreement with the observations made by Tjokorda et al.[16] Peripheral neuropathy and

ISSN (O): 2687-5365; ISSN (P): 2753-6556

peripheral occlusive vascular disease (POVD) together create a high-risk environment for ulcer formation and delayed healing. In the present study, 45 patients had peripheral neuropathy and 35 had peripheral vascular diseases, both of which showed a statistically significant association with amputation. Similar findings were reported by Vijay et al.[17] However, poor glycemic control on admission (65%) was the predominant risk factor and was also significantly associated with amputation. Similar observations were reported by Gadepalli et al. and Lehto et al.[15,18] The higher prevalence of DFIs among older adults may be attributed to long-standing diabetes, peripheral neuropathy and peripheral vascular disease. Advanced-grade ulcers at presentation may reflect delayed health seeking behaviour, likely influenced by low socioeconomic status of study population, financial constraints and limited awareness.

Comorbidities and complications often coexist in patients with diabetes and require close monitoring and correction while managing DFUs. In the present study, 59 patients had anemia, many of whom required correction with blood transfusions. Blood loss during the wound debridement may also have contributed to anemia. Hypertension (HTN) was present in 33% of cases. Zubair et al. identified HTN as a risk factor for lower extremity amputation in DFUs.[19] Similarly, Tracey et al. reported that arterial HTN is associated with an increased risk of diabetic microvascular complications.[20] HTN also accelerates the development of diabetic retinopathy, nephropathy and peripheral vascular disease.

In the present study, a total of 123 organisms were isolated from 100 patients averaging 1.23 isolates per patient and the report is consistent with the observations made by Bansal et al. and M Zubair et al.[6,21] Out of the 100 patients, 23% suffered polymicrobial infection and rest 77% showed monomicrobial infection. Gram-negative isolates (88 %) were predominant than Gram-positive isolates (35%) similar to studies by Ramani et al., Daniel M Mutonga et al.[22,23] The predominant Gram negatives isolates were *Escherichia coli* (28.5%) followed by *Klebsiella spp* (17.9%) and *Pseudomonas aeruginosa* 12.2% and predominant Gram-positive isolate is *Staphylococcus aureus* 24 (19.5 %) followed by *Streptococcus pyogenes* 5(4%). The reason for Gram-negative prevalence might be the chronicity of infection and environmental factors like contamination by faecal flora while cleaning. In contrast, several other studies have documented a predominance of Gram-positive organisms in diabetic foot infections.[24-26] Regional variation in the bacterial flora and other environmental factors contribute to this difference. Consistent finding was also found in study by Ramkanth et al.[27]

The antimicrobial susceptibility pattern of Enterobacteriaceae observed in the present study was comparable to that reported by Sudhir K Jain et al. and Mojtaba Anvarinejad et al.[28,29] Among non-fermenters, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, both showed 100% susceptibility to Meropenem. These finding suggest Carbapenems remain an effective therapeutic option in DFIs.

Among the Gram-positive isolates, all were susceptible to Vancomycin. Additionally, all isolates of *Streptococcus pyogenes* and *Enterococcus faecalis* were susceptible to both Penicillin and Ampicillin [Table 3]. This suggests Vancomycin remain an effective option for treating DFIs by Gram-positive pathogens. However, empiric antibiotic therapy also depends on various other factors like severity of infection, likelihood of *MRSA* and local antibiogram data. β-lactam antibiotics still remain effective for susceptible Streptococcal and Enterococcal strains.

High prevalence of multidrug resistance observed in the study is likely attributed to recurrent hospital admissions and frequent exposure to multiple courses of antimicrobials during the same. In the present study, factors like history of prior antibiotic use, recurrent as well as long standing ulcers and previous foot surgeries showed significant association (p value < 0.05) with multidrug resistance. Therefore, antimicrobial therapy must be optimised based on culture and antimicrobial susceptibility results, as targeted therapy is more effective than prolonged empirical therapy in improving clinical outcome and minimizing the emergence of drug resistance.

Amputation was the predominant clinical outcome obtained in the study, which may be attributed to the high proportion (60%) of patients with Wagener's grade 4 ulcers with partial foot gangrene. Findings of the study highlights the importance of educating diabetic population regarding the nature of their illness, importance of maintaining optimal glycaemic control, potential complications and the need for early recognition of warning signs and prompt medical consultation.

CONCLUSION

Diabetic foot infections are among the most serious long-term complications of diabetes mellitus and constitutes a major public health concern. Effective management of DFUs requires a multidisciplinary approach involving thorough wound debridement, appropriate antimicrobial therapy and culture-guided treatment based on antimicrobial susceptibility testing and local antibiogram data. Debridement accelerates wound healing and prevents bacterial colonisation. Antimicrobial agents must be used judiciously to limit the emergence of MDROs. Empirical antimicrobial therapy should be initiated promptly and subsequently modified according to culture report. Early identification of risk factors and timely intervention can improve wound healing, reduce complications and enhance patient outcomes.

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ISSN (O): 2687-5365; ISSN (P): 2753-6556