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Prevalence and Antimicrobial Resistance of Bacterial Pathogens in Diabetic Foot Infections: A Clinical Microbiological Study

Abdul Raziq[1], Muhammad Mohsin Zahoor[2], Abdul Mannan[3], Saqib Usman[4],

[1]Registrar, Department of General Medicine, North West General Hospital and Research Centre, Peshawar

[2,3]Registrar, Department of Respiratory Medicine, University Hospital Limerick, Ireland

[4]Department of General Internal Medicine, University Hospital Limerick, Ireland

***Corresponding author:**

Muhammad Mohsin Zahoor,

Registrar, Department of Respiratory Medicine, University Hospital Limerick, Ireland

Email ID: drmohsinbajwa481@gmail.com

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ABSTRACT

Background: Diabetic foot infections (DFIs) are a major complication of diabetes mellitus, frequently associated with antimicrobial resistance and poor clinical outcomes.

Objective: To determine the prevalence of bacterial pathogens in diabetic foot infections and assess their antimicrobial susceptibility patterns.

Methodology: This cross-sectional study was conducted at the Medical Unit of Northwest General Hospital and Research Center, Peshawar, from December 2019 to June 2020. A total of 140 adult diabetic patients aged 20–70 years with clinically diagnosed DFIs were included. Specimens were collected from ulcers, processed using standard bacteriological techniques, and antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method according to CLSI guidelines. Data were analyzed with SPSS version 22.0.

Results: The mean age of patients was 60.4 ± 7.8 years, with 70.71% males and 29.29% females. The most frequent ulcer grade was Wagner grade 2 (45.00%). *Escherichia coli* was the most prevalent isolate (53.57%), followed by *Staphylococcus aureus* (21.43%), *Klebsiella* species (18.57%), and *Streptococcus* species (6.43%). Pathogen distribution varied with age and ulcer severity, with *Klebsiella* significantly higher in older patients (23.53% vs. 5.26%, p=0.013) and *Streptococcus* species more common in grade 3 ulcers (11.63%, p=0.000). Patients with a history of toe or foot amputation showed a higher prevalence of *Klebsiella* (22.77% vs. 7.69%, p=0.039). Antimicrobial susceptibility revealed high resistance among Gram-negative organisms to cephalosporins, whereas carbapenems demonstrated comparatively better activity.

Conclusion: *Escherichia coli* was the predominant pathogen in DFIs, and high resistance to commonly used antibiotics highlights the need for ongoing surveillance and rational antibiotic prescribing.

Keywords: *Diabetic foot infections; antimicrobial resistance; bacterial pathogens; Escherichia coli; Staphylococcus aureus; Klebsiella.*

1. INTRODUCTION

Diabetic foot infections (DFIs) represent a significant and increasingly prevalent complication of diabetes mellitus, contributing substantially to morbidity, prolonged hospitalization, and healthcare costs worldwide [1,2]. The complex interplay of peripheral neuropathy, peripheral arterial disease, and impaired immune responses in diabetic patients creates a favorable environment for the colonization and proliferation of pathogenic microorganisms [3]. Infections of the diabetic foot often range from superficial cellulitis to deep tissue involvement, including abscess formation, osteomyelitis, and, in severe cases, gangrene [4]. These infections are not only a frequent cause of lower extremity amputations but also pose a serious public health challenge, particularly in regions with high diabetes prevalence and limited healthcare resources [5].

The microbial etiology of DFIs is diverse and often polymicrobial, encompassing both Gram-positive and Gram-negative bacteria, as well as occasional fungal pathogens [6]. Among the bacterial agents, *Staphylococcus aureus*, including methicillin-resistant strains (MRSA), is frequently reported as the predominant pathogen, followed by *Escherichia coli*, *Pseudomonas aeruginosa*, and other enteric bacteria [7,8]. The increasing emergence of multidrug-resistant (MDR) strains complicates empirical therapy, frequently resulting in treatment failure and extended hospital stays [9]. Inappropriate or delayed antibiotic administration, combined with poor glycemic control and delayed wound care, further exacerbates the risk of chronic infection and amputation [10].

Understanding the local microbial spectrum and resistance patterns is essential for guiding targeted antibiotic therapy and preventing the development of antimicrobial resistance. Continuous surveillance of pathogen prevalence and susceptibility profiles can inform clinical decision-making and optimize patient outcomes. Despite advances in infection control measures and antimicrobial stewardship programs, DFIs remain a persistent clinical challenge, necessitating comprehensive microbiological evaluation to address evolving resistance trends effectively.

Research Objective

To study the frequency of bacterial pathogens implicated in diabetic foot infections and the antimicrobial susceptibility of these pathogens.

2. METHODOLOGY

Study Design and Setting

This cross-sectional study was conducted at the Medical Unit of Northwest General Hospital and Research Center, Peshawar, from 06 December 2019 to 06 June 2020. The study included adult diabetic patients presenting with diabetic foot infections, either admitted to the hospital or attending the outpatient department.

Inclusion and Exclusion Criteria

Adult patients aged 20 to 70 years of either gender with type 1 or type 2 diabetes mellitus for at least two years, presenting with diabetic foot infections, were included in the study. Patients who had received antibiotics for more than 24 hours within the previous 48 hours, those with a history of trauma to the foot, or patients with critical limb ischemia were excluded from the study.

Sample Size

The sample size was calculated using the World Health Organization (WHO) sample size calculator. Based on a reported prevalence of *Escherichia coli* in diabetic foot infections of 63%, with a 95% confidence interval and a margin of error of 8%, the required sample size was determined to be 140 patients. Participants were recruited using consecutive non-probability sampling [11].

Data Collection

After obtaining approval from the hospital's Ethics and Research Committee, informed consent was taken from all participants. Demographic and clinical data, including age, gender, Wagner's grade of the diabetic foot ulcer, prior treatment history, history of toe or foot amputation, and presence of vascular disease, were recorded on a standardized proforma. Each patient underwent a detailed history and physical examination, and the diabetic foot ulcer was clinically assessed. Specimens, including pus, discharge, or debrided necrotic tissue, were collected and immediately transported to the microbiology laboratory. The specimens were subjected to Gram staining and inoculated on blood agar and MacConkey agar. After 24 hours of incubation at 37°C, bacterial isolates were identified using standard bacteriological methods, and antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion method according to Clinical Laboratory Standards Institute (CLSI) guidelines.

Statistical Analysis

Data analysis was performed using SPSS version 22.0. Numerical variables such as age were expressed as mean ± standard deviation (SD), while categorical variables such as gender, bacterial isolates, and antibiotic sensitivity patterns were presented as frequencies and percentages. Stratification was used to control for effect modifiers including age, gender, Wagner grade, prior treatment history, history of amputation, and vascular disease. Post-stratification, the Chi-square (χ^2) test was applied, and a p-value ≤ 0.05 was considered statistically significant. Results were presented in the form of tables and graphs.

Ethical Approval

The study protocol was approved by the Ethics and Research Committee of Northwest General Hospital and Research Center, Peshawar, and written informed consent was obtained from all participants prior to inclusion in the study.

3. RESULTS

Table 1 summarizes the descriptive characteristics of the study population (n=140). The mean age was 60.43 ± 7.81 years, with 102 patients (72.86%) aged ≥55 years and 38 (27.14%) <55 years. Males comprised 99 (70.71%) cases, while females were 41 (29.29%). Ulcer severity was distributed as Wagner grade 1 in 18 (12.86%), grade 2 in 63 (45.00%), grade 3 in 43 (30.71%), grade 4 in 9 (6.43%), and grade 5 in 7 (5.00%). Past history of diabetic foot treatment was reported in 101 (72.14%), toe/foot amputation in 101 (72.14%), and vascular disease in 96 (68.57%) patients.

Table 1: Descriptive Characteristics of Study Participants (n=140)

Variable	Category	Frequency (n;%)
Age Groups (Years)	< 55 years	38 (27.14%)
	≥ 55 years	102 (72.86%)
	Mean ± SD	60.43 ± 7.81
Gender	Male	99 (70.71%)
	Female	41 (29.29%)
Wagner Grade of Ulcer	Grade 1	18 (12.86%)
	Grade 2	63 (45.00%)
	Grade 3	43 (30.71%)
	Grade 4	9 (6.43%)
	Grade 5	7 (5.00%)
Past History of Treatment for DFI	Yes	101 (72.14%)
	No	39 (27.86%)
Past History of Toe/Foot Amputation	Yes	101 (72.14%)
	No	39 (27.86%)
Vascular Disease Present	Yes	96 (68.57%)
	No	44 (31.43%)
Note: DFI = Diabetic Foot Infection; SD = Standard Deviation.		

Figure 1 shows the distribution of pathogens isolated. *Escherichia coli* was most frequent in 75 patients (53.57%), followed by *Staphylococcus aureus* in 30 (21.43%), *Klebsiella* in 26 (18.57%), and *Streptococcus* species in 9 (6.43%).

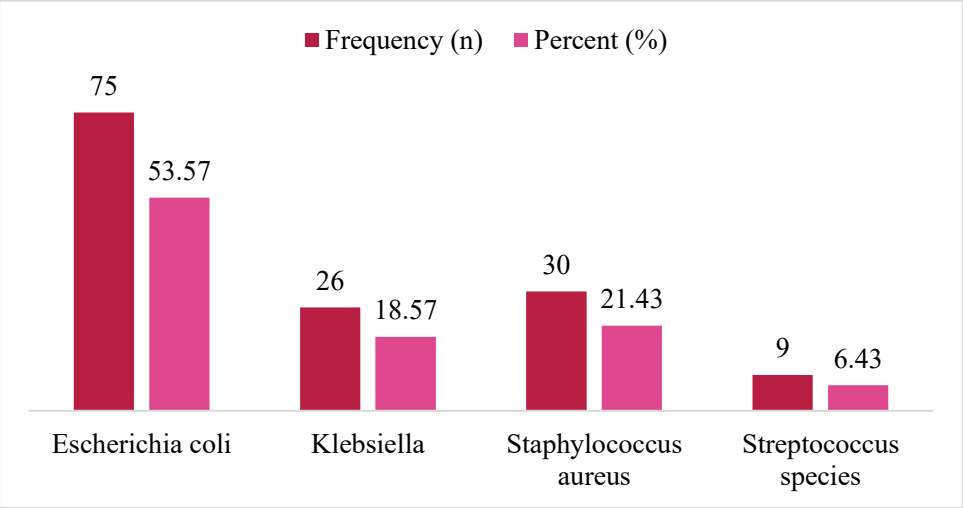

Figure 1: Frequencies and Percentages for Pathogen Isolated (n=140)

Table 2 presents pathogen stratification by age. *E. coli* was isolated in 25 (65.79%) of patients <55 years and 50 (49.02%) of those ≥55 years (p=0.076). *Klebsiella* was significantly more common in older patients, detected in 24 (23.53%) compared to 2 (5.26%) in younger patients (p=0.013). *Staphylococcus aureus* was found in 8 (21.05%) of patients <55 years and 22 (21.57%) of those ≥55 years (p=0.947). *Streptococcus* species were present in 3 (7.89%) younger patients and 6 (5.88%) older patients (p=0.665).

Table 2: Stratification of Pathogen Isolated with Age Groups (n=140)

Pathogen Isolated	< 55 Years n (%)	≥ 55 Years n (%)	P-value
Escherichia coli	25 (65.79)	50 (49.02)	0.076
Klebsiella	2 (5.26)	24 (23.53)	0.013
Staphylococcus aureus	8 (21.05)	22 (21.57)	0.947
Streptococcus species	3 (7.89)	6 (5.88)	0.665

Table 3 shows pathogen distribution by Wagner grade. *E. coli* was isolated in 9 (50.00%) grade 1, 35 (55.56%) grade 2, 21 (48.84%) grade 3, 5 (55.56%) grade 4, and 5 (71.43%) grade 5 cases (p=0.828). *Klebsiella* was detected in 4 (22.22%) grade 1, 12 (19.05%) grade 2, 7 (16.28%) grade 3, 2 (22.22%) grade 4, and 1 (14.29%) grade 5 (p=0.975). *Staphylococcus aureus* occurred in 4 (22.22%) grade 1, 13 (20.63%) grade 2, 10 (23.26%) grade 3, 2 (22.22%) grade 4, and 1 (14.29%) grade 5 (p=0.987). *Streptococcus* species were mainly seen in grade 3 (5 cases, 11.63%), with fewer in grade 1 (1, 5.56%) and grade 2 (3, 4.76%), showing a significant association (p=0.000).

Table 3: Stratification of Pathogen Isolated with Wagner Grade of Ulcer (n=140)

Pathogen Isolated	Grade 1 (n;%)	Grade 2 (n;%)	Grade 3 (n;%)	Grade 4 (n;%)	Grade 5 (n;%)	P-value
Escherichia coli	9 (50.00)	35 (55.56)	21 (48.84)	5 (55.56)	5 (71.43)	0.828
Klebsiella	4 (22.22)	12 (19.05)	7 (16.28)	2 (22.22)	1 (14.29)	0.975
Staphylococcus aureus	4 (22.22)	13 (20.63)	10 (23.26)	2 (22.22)	1 (14.29)	0.987
Streptococcus species	1 (5.56)	3 (4.76)	5 (11.63)	0 (0.00)	0 (0.00)	0.000

Table 4 shows the stratification of pathogens with clinical characteristics. *Escherichia coli* was more frequently isolated in males (56, 56.57%) compared to females (19, 46.34%), though not statistically significant (p=0.269). *Klebsiella* was slightly higher among females (9, 21.95%) than males (17, 17.17%) (p=0.508). *Staphylococcus aureus* (19.19% vs. 26.83%, p=0.316) and *Streptococcus* species (7.07% vs. 4.88%, p=0.630) showed no significant gender differences. Patients with prior treatment for DFU showed similar frequencies of *E. coli* (56, 55.45% vs. 19, 48.72%, p=0.474) and *Staphylococcus aureus* (22, 21.78% vs. 8, 20.51%, p=0.869). A significant finding was that *Klebsiella* was more common in patients with a history of toe/foot amputation (23, 22.77% vs. 3, 7.69%, p=0.039). Vascular disease presence showed no significant associations with pathogen type.

Table 4: Stratification of Pathogens with Clinical Characteristics

Variable	Pathogen Isolated	Yes (n;%)	No (n;%)	P-value
Gender (Male=Yes, Female=No)	*Escherichia coli*	56 (56.57)	19 (46.34)	0.269
	Klebsiella	17 (17.17)	9 (21.95)	0.508
	Staphylococcus aureus	19 (19.19)	11 (26.83)	0.316
	Streptococcus species	7 (7.07)	2 (4.88)	0.630
Past History of Treatment for DFU	*Escherichia coli*	56 (55.45)	19 (48.72)	0.474
	Klebsiella	18 (17.82)	8 (20.51)	0.713
	Staphylococcus aureus	22 (21.78)	8 (20.51)	0.869

	Streptococcus species	5 (4.95)	4 (10.26)	0.251
Past History of Toe/Foot Amputation	*Escherichia coli*	51 (50.50)	24 (61.54)	0.240
	Klebsiella	23 (22.77)	3 (7.69)	0.039
	Staphylococcus aureus	21 (20.79)	9 (23.08)	0.767
	Streptococcus species	6 (5.94)	3 (7.69)	0.704
Vascular Disease Present	*Escherichia coli*	54 (56.25)	21 (47.73)	0.347
	Klebsiella	17 (17.71)	9 (20.45)	0.698
	Staphylococcus aureus	20 (20.83)	10 (22.73)	0.799
	Streptococcus species	5 (5.21)	4 (9.09)	0.384

Table 5 presents the antimicrobial susceptibility patterns of isolated pathogens. *Escherichia coli* was the predominant isolate across antibiotics, ranging from 50 (51.02%) with ceftazidime to 62 (53.45%) with co-trimoxazole, though differences by antibiotic exposure were not statistically significant. *Klebsiella* proportions varied between 16 (15.53%) for Tazocin and 24 (20.34%) for Augmentin, with no significant associations. *Staphylococcus aureus* ranged from 19 (17.92%) with ciprofloxacin to 25 (21.55%) with co-trimoxazole, while *Streptococcus* species were less frequent, ranging between 4.17% and 10.81%. No significant differences were observed across antibiotic groups, except for a trend toward higher *Klebsiella* isolation in ciprofloxacin-naïve patients (21.70% vs. 8.82%, p=0.093) and higher *Staphylococcus aureus* isolation in ciprofloxacin-treated patients (32.35% vs. 17.92%, p=0.074).

Table 5: Antimicrobial Susceptibility Patterns of Pathogens

Antibiotic	**Pathogen Isolated**	**Yes n (%)**	**No n (%)**	**P-value**
Augmentin	Escherichia coli	61 (51.69)	14 (63.64)	0.302
	Klebsiella	24 (20.34)	2 (9.09)	0.212
	Staphylococcus aureus	25 (21.19)	5 (22.73)	0.871
	Streptococcus species	8 (6.78)	1 (4.55)	0.694
Co-trimoxazole	Escherichia coli	62 (53.45)	13 (54.17)	0.948
	Klebsiella	21 (18.10)	5 (20.83)	0.754
	Staphylococcus aureus	25 (21.55)	5 (20.83)	0.937
	Streptococcus species	8 (6.90)	1 (4.17)	0.619
Ciprofloxacin	Escherichia coli	57 (53.77)	18 (52.94)	0.932
	Klebsiella	23 (21.70)	3 (8.82)	0.093
	Staphylococcus aureus	19 (17.92)	11 (32.35)	0.074
	Streptococcus species	7 (6.60)	2 (5.88)	0.881
Vancomycin	Escherichia coli	56 (56.00)	19 (47.50)	0.362
	Klebsiella	17 (17.00)	9 (22.50)	0.449
	Staphylococcus aureus	21 (21.00)	9 (22.50)	0.845
	Streptococcus species	6 (6.00)	3 (7.50)	0.743
Tazocin	Escherichia coli	59 (57.28)	16 (43.24)	0.141
	Klebsiella	16 (15.53)	10 (27.03)	0.123
	Staphylococcus aureus	23 (22.33)	7 (18.92)	0.664

	Streptococcus species	5 (4.85)	4 (10.81)	0.202
Gentamicin	Escherichia coli	50 (52.08)	25 (56.82)	0.602
	Klebsiella	17 (17.71)	9 (20.45)	0.698
	Staphylococcus aureus	23 (23.96)	7 (15.91)	0.281
	Streptococcus species	6 (6.25)	3 (6.82)	0.898
Cefotaxime	Escherichia coli	59 (55.66)	16 (47.06)	0.381
	Klebsiella	20 (18.87)	6 (17.65)	0.873
	Staphylococcus aureus	20 (18.87)	10 (29.41)	0.192
	Streptococcus species	7 (6.60)	2 (5.88)	0.881
Ceftazidime	Escherichia coli	50 (51.02)	25 (59.52)	0.355
	Klebsiella	20 (20.41)	6 (14.29)	0.393
	Staphylococcus aureus	20 (20.41)	10 (23.81)	0.653
	Streptococcus species	8 (8.16)	1 (2.38)	0.201
Imipenem	Escherichia coli	60 (55.56)	15 (46.88)	0.387
	Klebsiella	20 (18.52)	6 (18.75)	0.976
	Staphylococcus aureus	22 (20.37)	8 (25.00)	0.575
	Streptococcus species	6 (5.56)	3 (9.38)	0.439
Meropenem	Escherichia coli	53 (51.96)	22 (57.89)	0.531
	Klebsiella	19 (18.63)	7 (18.42)	0.977
	Staphylococcus aureus	23 (22.55)	7 (18.42)	0.596
	Streptococcus species	7 (6.86)	2 (5.26)	0.731

4. DISCUSSION

In this study, we found that *Escherichia coli* was the most frequently isolated pathogen in diabetic foot infections (DFIs), accounting for 53.57% of cases, followed by *Staphylococcus aureus* **(**21.43%**)**, *Klebsiella* species **(**18.57%**),** and *Streptococcus* species (6.43%)**.** The predominance of *E. coli* in our cohort is consistent with previous reports, where Gram-negative bacteria have increasingly been implicated as primary agents in DFIs [12]. In contrast, studies from Western countries frequently identify *S. aureus*, particularly methicillin-resistant *S. aureus* (MRSA), as the leading pathogen [13]. This regional variation may be explained by differences in hygiene practices, antibiotic usage, and healthcare infrastructure.

Age-stratified analysis revealed that *Klebsiella* was significantly more prevalent in older patients (23.53% in ≥55 years vs. 5.26% in <55 years, p=0.013), while *E. coli* remained common across all ages (49.02% vs. 65.79%**,** p=0.076). Previous studies have similarly reported a higher prevalence of *Klebsiella* in elderly diabetic populations, possibly due to frequent hospital exposure and prior antibiotic use [14]. The distribution of *S. aureus* and *Streptococcus* species did not vary significantly by age, in agreement with earlier findings that Gram-positive isolates tend to remain stable across age groups [15].

When stratified by ulcer severity, *E. coli* was consistently the predominant isolate across all Wagner grades, ranging from 50.00% in grade 1 to 71.43% in grade 5 cases (p=0.828). Notably, *Streptococcus* species were more frequent in grade 3 ulcers (11.63%) compared to other grades (p=0.000), suggesting that deeper soft tissue involvement may favor their growth. Similar trends have been reported in previous studies, where *Streptococcus* species were commonly associated with higher-grade ulcers [16]. These findings underscore the need for broad empirical coverage in severe DFIs. Clinical characteristics also influenced microbial patterns. *Klebsiella* was significantly more common in patients with a history of toe or foot amputation (22.77% vs. 7.69%, p=0.039), consistent with studies suggesting that repeated surgical interventions and prolonged hospital stays increase colonization with resistant Gram-negative organisms [17].

Antimicrobial susceptibility testing revealed widespread resistance. *E. coli* demonstrated sensitivity rates between 51.02%

with ceftazidime and 57.28% with piperacillin–tazobactam, while *Klebsiella* showed higher resistance, with the lowest sensitivity to Tazocin (15.53%). *S. aureus* remained relatively sensitive, with 21.19–23.96% distribution across antibiotics. Previous regional studies have also highlighted alarmingly high resistance among Gram-negative isolates, particularly to third-generation cephalosporins, while carbapenems such as imipenem and meropenem maintained comparatively better efficacy [18,19]. The observed resistance patterns highlight the urgent need for antimicrobial stewardship to prevent further escalation of multidrug resistance in DFI pathogens.

Strengths and Limitations

The main strength of this study is its focus on local microbiological profiles and antimicrobial resistance patterns in diabetic foot infections, providing region-specific evidence that can guide empirical therapy. The study also employed standardized laboratory methods (CLSI guidelines) and stratified analysis across demographic and clinical variables, enhancing the validity of the findings. However, the study was limited by its single-center design, relatively small sample size (n=140), and cross-sectional nature, which restricts causal inference. Additionally, fungal and anaerobic organisms were not evaluated, and molecular characterization of resistance mechanisms was not performed, which could have provided deeper insights into resistance trends.

5. CONCLUSION

This study demonstrated that *Escherichia coli* was the most prevalent pathogen in diabetic foot infections, followed by *Staphylococcus aureus* and *Klebsiella* species, with notable resistance to commonly used antibiotics, particularly cephalosporins. Resistance patterns underscore the growing challenge of multidrug-resistant Gram-negative organisms, while factors such as age, ulcer severity, and prior amputation history influenced pathogen distribution. These findings highlight the importance of continuous local surveillance and rational antibiotic use to optimize treatment strategies and improve patient outcomes in diabetic foot infections.

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