**Journal of Neonatal Surgery**
***ISSN(Online): 2226-0439***
***Vol.14, Issue 33s (2025)***
*https://www.jneonatalsurg.com*

OPEN ACCESS

# Antimicrobial Resistance Patterns in Diabetic Foot Infections: A Microbiological Study in Pakistan

**Faiza Rehman[1], Tabbassum Imran[2], Sughra Kanwal[3], Mehwish Kharal[4], Naveen Farooq[5], Gotam Kumar[6], Nabiha Naeem[7]**

[1]Senior Lecturer, Shifa College of Medicine, Shifa Tameer e Millat University, H8/4 Islamabad, Pakistan

[2]Assistant Professor Microbiology, Pathology Department, Saidu Medical College Swat /Saidu Group of Teaching Hospital, Swat, Pakistan

[3]Department of Pharmacology, HITEC-IMS, HIT Taxila, Pakistan

[4]MD Internal Medicine Post Graduate Resident (PGR), Post Graduate Medical Institute (PGMI), Lahore, Pakistan

[5]Assistant Professor, Department of public Health and Healthcare Management, Tashkent State Medical University, Tashkent

[6]Associate Professor, Peoples Medical and Health Science University for Women (PUMHSW), Nawabshah, Pakistan.

[7]Department of Life Sciences, School of Science, University of Management and Technology (UMT), Lahore, Pakistan

**Corresponding Author:**

Nabiha Naeem,

Department of Life Sciences, School of Science, University of Management and Technology (UMT), Lahore, Pakistan. Email ID : nabiha.naeem@umt.edu.pk

Cite this paper as: Faiza Rehman, Tabbassum Imran, Sughra Kanwal, Mehwish Kharal, Naveen Farooq, Gotam Kumar, Nabiha Naeem, (2025) Antimicrobial Resistance Patterns in Diabetic Foot Infections: A Microbiological Study in Pakistan .Journal of Neonatal Surgery, 14, (33s) 489-494

**ABSTRACT**

**Background:** Diabetic foot infections (DFIs) are a major cause of morbidity and amputation worldwide, driven by polymicrobial infections and escalating antimicrobial resistance. Understanding local pathogen distribution and resistance trends is critical for guiding empirical therapy.

**Objective:** To identify bacterial isolates from DFIs, determine their antimicrobial resistance patterns, and assess the association of ulcer severity with multidrug resistance (MDR).

**Methodology:** This cross-sectional study included 150 patients with DFIs. Demographic and clinical data were collected, and specimens were processed for microbiological culture. Antimicrobial susceptibility was assessed using standard methods. Data were analyzed in SPSS v25, with descriptive statistics presented as mean ± SD, median (IQR), and n (%). Chi-square test assessed associations, with $p \leq 0.05$ considered significant.

**Results:** Of 172 isolates recovered, Staphylococcus aureus (31%) was most frequent, followed by *E. coli* (19%), Klebsiella pneumoniae (15%), and Pseudomonas aeruginosa (12%). MRSA accounted for 10% of S. aureus. Resistance was highest to ceftriaxone (60–81%) and ciprofloxacin (43–70%), whereas imipenem showed the lowest resistance (7–25%). MDR prevalence was 30% in S. aureus, 69% in *E. coli*, 73% in Klebsiella, and 60% in Pseudomonas. MDR was significantly more common in Wagner grade III–IV ulcers (54%) than in grade I–II (29%) ($p=0.012$).

**Conclusion:** DFIs in this cohort are characterized by a high burden of Gram-negative pathogens and MDR, especially in advanced ulcers. Early microbiological diagnosis, local antibiogram-guided empirical therapy, and antimicrobial stewardship are essential to improve outcomes and curb resistance.

**Keywords**: *Diabetic foot infections; antimicrobial resistance; multidrug resistance; Staphylococcus aureus; Enterobacterales*

## 1. INTRODUCTION

Diabetes mellitus is a major and growing public-health challenge worldwide, and its burden is particularly heavy in South Asia.

Pakistan ranks among the countries with the highest adult prevalence of diabetes: recent estimates from the International Diabetes Federation and the IDF Diabetes Atlas report that Pakistan has tens of millions of adults living with diabetes and one of the highest national prevalence rates globally.(1, 2)

Diabetic foot ulcers (DFUs) are one of the most important and costly complications of diabetes. DFUs affect a substantial proportion of persons with diabetes and frequently progress to infection; infected diabetic foot ulcers are the leading cause of non-traumatic lower-limb amputation and are associated with prolonged hospital stays, high healthcare costs, and increased mortality. In Pakistan the published prevalence of DFUs shows wide variation between studies, but pooled estimates indicate a considerable burden (pooled prevalence ≈12.2% in a recent meta-analysis), reflecting both the magnitude of the problem and between-study heterogeneity due to differences in population and care access.(3)

The microbiology of diabetic foot infections (DFIs) is complex and often polymicrobial. Gram-positive organisms such as Staphylococcus aureus, including MRSA, gram-negative bacilli including Escherichia coli, Klebsiella *spp.,* Pseudomonas *spp*., and anaerobes are commonly recovered from infected wounds; the relative frequency of these pathogens depends on ulcer severity, prior antibiotic exposure, and local epidemiology. Importantly, recent systematic reviews and regional studies describe high and rising rates of antimicrobial resistance among isolates from DFIs, including methicillin resistance in S. aureus, extended-spectrum β-lactamase (ESBL) production and other multidrug resistance mechanisms among Enterobacterales, and notable resistance among non-fermenters, all of which complicate empirical therapy and are associated with poorer outcomes.(4, 5)

Antimicrobial resistance (AMR) is a recognized public-health threat in Pakistan. Surveillance and point-prevalence surveys from tertiary hospitals document frequent and sometimes inappropriate antibiotic prescribing and a heavy burden of resistant organisms in clinical isolates, a context that fosters selection and spread of resistant pathogens in community and hospital settings alike. The combination of a very large diabetic population, variable access to foot-care services, and substantial AMR pressures makes the management of DFIs in Pakistan particularly challenging.(6, 7)

Given Pakistan's high diabetes prevalence, the substantial proportion of patients who develop foot ulcers, and accumulating evidence of resistance among common wound pathogens, locally generated microbiological and susceptibility data are essential to guide empiric antibiotic choices, inform antimicrobial stewardship, and help reduce limb-threatening complications. Yet published studies from Pakistan are limited by small samples, single-center designs, or outdated susceptibility profiles. This study characterizes the bacterial spectrum and antimicrobial resistance patterns of isolates from diabetic foot infections seen at our tertiary care centre in Pakistan, providing current, actionable data to improve empirical treatment protocols and stewardship efforts. The present study aimed to determine the microbiological profile and antimicrobial susceptibility patterns of bacterial isolates in patients with diabetic foot infections.

## 2. METHODOLOGY

The present study was designed as a cross-sectional descriptive study conducted over a period of one year. A total sample size of 150 patients was calculated using OpenEpi, considering a prevalence of diabetic foot infections to be 6.3%, with a 95% confidence interval and a 5% margin of error.(8) Patients were recruited consecutively using a non-probability consecutive sampling technique until the required sample size was achieved.

All adult patients with a confirmed diagnosis of diabetes mellitus presenting with clinically infected diabetic foot ulcers, as defined by the presence of local signs of infection (erythema, warmth, tenderness, purulent discharge), were included. Patients who had received systemic antibiotic therapy within 72 hours before sample collection, those with severe comorbidities such as terminal malignancy, and patients unwilling to provide consent were excluded from the study.

Data collection was performed following informed consent and ethical approval from the Institutional Review Board. After detailed clinical examination and documentation of demographic and clinical variables, wound samples were collected aseptically using sterile cotton swabs or tissue specimens, depending on the wound depth. Samples were immediately transported to the microbiology laboratory and processed according to standard protocols. Bacterial isolates were identified by conventional microbiological methods, including Gram staining, culture on selective media, and biochemical tests. Antimicrobial susceptibility testing was carried out using the Kirby-Bauer disc diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI).(9) Results were recorded systematically in a structured proforma.

Data were entered, cleaned, and analyzed using the Statistical Package for Social Sciences (SPSS) version 25.0. Descriptive statistics were applied to summarize the study variables. Categorical variables such as gender, comorbidities, bacterial isolates, and antimicrobial resistance profiles were expressed as frequencies and percentages. Continuous variables, including age and duration of diabetes, were first tested for distribution using the Shapiro–Wilk test. Normally distributed variables were presented as means with standard deviations, while non-normally distributed variables were summarized as medians with interquartile ranges. The distribution of pathogens and resistance rates across different subgroups, such as age, gender, and ulcer severity, was assessed. For categorical variables, the Chi-square test was employed to determine associations, and Fisher's exact test was applied where expected cell counts were <5. A p-value ≤0.05 was considered statistically significant.

## 3. RESULTS

A total of 150 patients with diabetic foot infections were included in the study. The mean age of the patients was 56.8 ± 10.7 years, and the majority were male (61%). The median duration of diabetes was 12 years (IQR: 8–16). More than half of the patients were hypertensive (56%), and most presented with advanced ulcers (Wagner grade III–IV, 68%) (Table 1).

**Table 1. Demographic and Clinical Characteristics of Patients (n = 150)**

| Variable | Category | n (%)/ Mean ± SD / Median (IQR) |
|---|---|---|
| Gender | Male | 92 (61%) |
| | Female | 58 (39%) |
| Age (years) | | 56.8 ± 10.7 |
| Duration of diabetes (years) | | 12 (8–16) |
| Hypertension | Yes | 84 (56%) |
| | No | 66 (44%) |
| Ulcer severity (Wagner grade) | Grade I–II | 48 (32%) |
| | Grade III–IV | 102 (68%) |

Microbiological culture yielded 172 isolates, with polymicrobial growth observed in several cases. The predominant pathogen was Staphylococcus aureus (31%), including 10% methicillin-resistant strains (MRSA). Among Gram-negative bacteria, Escherichia coli (19%) and Klebsiella pneumoniae (15%) were the most common, followed by Pseudomonas aeruginosa (12%) (Table 2).

**Table 2. Bacterial Isolates from Diabetic Foot Infections (n = 172 isolates)**

| Isolate | n (%) |
|---|---|
| *Staphylococcus aureus* | 54 (31%) |
| MRSA (subset of S. aureus) | 18 (10%) |
| *Escherichia coli* | 32 (19%) |
| *Klebsiella pneumoniae* | 26 (15%) |
| *Pseudomonas aeruginosa* | 20 (12%) |
| *Proteus* spp. | 12 (7%) |
| *Enterococcus* spp. | 10 (6%) |
| Anaerobes | 8 (5%) |
| Others | 6 (4%) |

Antimicrobial resistance patterns revealed high levels of resistance to third-generation cephalosporins and fluoroquinolones across both Gram-positive and Gram-negative isolates. Notably, 61% of S. aureus and nearly 80% of Enterobacterales (*E. coli* and *Klebsiella*) were resistant to ceftriaxone. In contrast, imipenem retained good activity, with resistance ranging from 7% in *S.* aureus to 25% in Pseudomonas (Table 3).

**Table 3. Antimicrobial Resistance Patterns of Major Isolates**

| Antibiotic | S. aureus (n=54) | E. coli (n=32) | Klebsiella (n=26) | Pseudomonas (n=20) |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| **Amoxicillin–clavulanate** | 30 (56%) | 23 (72%) | 20 (77%) | 13 (65%) |
| **Ciprofloxacin** | 23 (43%) | 20 (63%) | 15 (58%) | 14 (70%) |
| **Gentamicin** | 16 (30%) | 15 (47%) | 14 (54%) | 11 (55%) |
| **Ceftriaxone** | 33 (61%) | 25 (78%) | 21 (81%) | 12 (60%) |
| **Imipenem** | 4 (7%) | 5 (16%) | 5 (19%) | 5 (25%) |
| **Linezolid (Gram+ only)** | 3 (6%) | | | |
| **Vancomycin (Gram+ only)** | 2 (4%) | | | |

Multidrug resistance (MDR) was particularly concerning among Gram-negative organisms, observed in 69% of E. coli, 73% of Klebsiella, and 60% of Pseudomonas isolates, compared with 30% of S. aureus (Table 4).

**Table 4. Multidrug Resistance (MDR) among Common Pathogens**

| **Pathogen** | **MDR isolates n (%)** |
|---|---|
| Staphylococcus aureus (n=54) | 16 (30%) |
| Escherichia coli (n=32) | 22 (69%) |
| Klebsiella pneumoniae (n=26) | 19 (73%) |
| Pseudomonas aeruginosa (n=20) | 12 (60%) |

Furthermore, MDR isolates were significantly more common in patients with severe ulcers (Wagner grade III–IV) compared to those with mild ulcers (53.9% vs. 29.2%, p = 0.012), underscoring the link between ulcer severity and resistance burden (Table 5).

**Table 5. Association of Ulcer Severity with Multidrug Resistance (n = 150)**

| **Ulcer severity** | **MDR isolates n (%)** | **Non-MDR isolates n (%)** | **p-value** |
|---|---|---|---|
| Grade I–II (n=48) | 14 (29%) | 34 (71%) | 0.012* |
| Grade III–IV (n=102) | 55 (54%) | 47 (46%) | – |
| *Statistically significant ($p \leq 0.05$) | | | |

## 4. DISCUSSION

In this study of 150 patients with diabetic foot infections we observed a mixed microbiological picture with Staphylococcus aureus as the single most frequent isolate (31%) but a high burden of Gram-negative pathogens and a worrying level of multidrug resistance (MDR) among Enterobacterales (69% E. coli, 73% Klebsiella) and Pseudomonas (60%). High resistance to third-generation cephalosporins and fluoroquinolones, with relatively preserved activity of carbapenems (imipenem resistance 7–25% across species), and a stronger MDR signal in advanced (Wagner III–IV) ulcers were the study's principal findings.

Several recent reports broadly similar patterns. A large cross-sectional studies also documented predominance of S. aureus with frequent polymicrobial infections and an increasing proportion of Gram-negative organisms in higher-grade ulcers, findings that mirror our observation of polymicrobial growth and the link between ulcer severity and MDR. The authors specifically reported high MRSA incidence in advanced grades and an increasing gram-negative share with higher ulcer grade.(10-12)

Other contemporary Pakistani reports emphasize Gram-negative dominance or a high Pseudomonas burden in some settings: a CMH Lahore study found Gram-negative organisms notably P. aeruginosa, to be the most common isolates and reported meropenem/ carbapenem retained activity against many Gram-negatives, a pattern similar to our finding of lower carbapenem resistance compared with cephalosporins and fluoroquinolones.(13) A single-centre Peshawar study likewise

highlighted a very high proportion of P. aeruginosa among DFU isolates and substantial resistance to commonly used agents, stressing local variation in dominant pathogens across Pakistan.(14)

Comparisons with regional and international work showed concordant themes. A systematic review of DFI microbiology and susceptibility found widespread heterogeneity between centres but consistent rises in resistance to cephalosporins and fluoroquinolones, and noted that Gram-negative organisms are an increasingly important component of DFIs in many LMIC settings, findings that align with our high Enterobacterales MDR rates and broad cephalosporin resistance.(4) Studies from South America and beyond similarly report high MDR prevalence among Gram-negatives and frequent resistance to oral and injectable agents commonly used empirically, while preserving relative sensitivity to carbapenems and select aminoglycosides, again echoing our susceptibility profile.(15, 16)

Where our results differ from some published reports is in the magnitude of MRSA. While we found MRSA in 10% of isolates, some Pakistani series reported markedly higher MRSA proportions (including reports of MRSA exceeding 60% in subsets of ulcers), highlighting sizable local and methodological differences (sample type, inclusion of deep tissue vs. swabs, prior antibiotic exposure) that influence observed MRSA rates. This underlines the point that direct comparisons must account for sampling approach, patient case-mix, and prior antibiotic use.(10)

Two consistent messages emerge from the cross-study comparison. First, the predominance between Gram-positive and Gram-negative organisms varies by centre, patient population (community v. hospital acquired, prior antibiotics), and ulcer severity, but the trend toward rising Gram-negative and polymicrobial infections in many recent series is clear and reflected in our data. Second, high rates of resistance to commonly used oral and parenteral agents (third-generation cephalosporins, fluoroquinolones) plus a high prevalence of MDR among Enterobacterales is a recurring finding, reinforcing the limited utility of empirical therapy without local susceptibility data and the need to reserve carbapenems and other last-line drugs for confirmed or strongly suspected resistant infections.(4)

Strengths and implications. Concordance with multiple reports supports the external validity of our key observations: that DFIs in Pakistan frequently involve Gram-negative MDR pathogens and that MDR correlates with ulcer severity, an important operational point for clinicians managing advanced DFUs. Our data support early culture and sensitivity testing, stewardship to reduce inappropriate broad-spectrum antibiotic use, and local antibiogram-guided empirical protocols (especially for severe ulcers).(11)

Limitations in light of other studies. Differences between our results and some reports (notably MRSA frequency and the relative proportion of *Pseudomonas*) probably reflect sampling methods (swab versus deep tissue), prior outpatient antibiotic exposure, single-centre design, and modest sample size; these same limitations are highlighted by other authors and systematic reviews as key causes of inter-study heterogeneity. Future multicentre prospective work with standardized sampling (deep tissue or bone where appropriate) would help produce more generalizable national antibiograms.

In summary, our findings are broadly consistent with recent Pakistani and international literature showing high MDR among Gram-negative DFU pathogens and frequent resistance to cephalosporins and fluoroquinolones, with carbapenems retaining the best activity in most centres. This convergence of evidence argues strongly for routine culture before escalation of therapy, local antibiogram development, and strengthened antimicrobial stewardship in diabetic foot services.

## 5. CONCLUSION

This study demonstrates that diabetic foot infections in our setting are predominantly caused by Staphylococcus aureus and Gram-negative organisms, with a high prevalence of multidrug resistance, particularly among Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa. The strong association between advanced ulcer severity and MDR underscores the clinical importance of early diagnosis, prompt culture and sensitivity testing, and tailored antimicrobial therapy. Preservation of carbapenem sensitivity, despite widespread resistance to cephalosporins and fluoroquinolones, highlights the urgent need for antimicrobial stewardship to avoid further erosion of last-line agents. These findings call for the development of local antibiograms and evidence-based empirical treatment guidelines to optimize outcomes in patients with diabetic foot infections.

## REFERENCES

1. Hossain MJ, Al-Mamun M, Islam MR. Diabetes mellitus, the fastest growing global public health concern: Early detection should be focused. Health Science Reports. 2024;7(3):e2004.
2. Jokhio AH, Talpur KI, Shujaat S, Talpur BR, Memon S. Prevalence of diabetic retinopathy in rural Pakistan: A population based cross-sectional study. Indian Journal of Ophthalmology. 2022;70(12):4364-9.
3. Akhtar S, Ali A, Ahmad S, Khan MI, Shah S, Hassan F. The prevalence of foot ulcers in diabetic patients in Pakistan: A systematic review and meta-analysis. Frontiers in Public Health. 2022;10:1017201.
4. Maity S, Leton N, Nayak N, Jha A, Anand N, Thompson K, et al. A systematic review of diabetic foot infections: pathogenesis, diagnosis, and management strategies. Frontiers in Clinical Diabetes and Healthcare. 2024;5:1393309.
5. Boschetti G, Sgarabotto D, Meloni M, Bruseghin M, Whisstock C, Marin M, et al. Antimicrobial resistance patterns

in diabetic foot infections, an epidemiological study in northeastern Italy. Antibiotics. 2021;10(10):1241.
6. Saleem Z, Haseeb A, Godman B, Batool N, Altaf U, Ahsan U, et al. Point prevalence survey of antimicrobial use during the COVID-19 pandemic among different hospitals in Pakistan: Findings and implications. Antibiotics. 2022;12(1):70.
7. Bashir N, Dablool AS, Khan MI, Almalki MG, Ahmed A, Mir MA, et al. Antibiotics resistance as a major public health concern: A pharmaco-epidemiological study to evaluate prevalence and antibiotics susceptibility-resistance pattern of bacterial isolates from multiple teaching hospitals. Journal of Infection and Public Health. 2023;16:61-8.
8. Zhang P, Lu J, Jing Y, Tang S, Zhu D, Bi Y. Global epidemiology of diabetic foot ulceration: a systematic review and meta-analysis. Annals of medicine. 2017;49(2):106-16.
9. Yin D, Guo Y, Han R, Yang Y, Zhu D, Hu F. A modified Kirby-Bauer disc diffusion (mKB) method for accurately testing tigecycline susceptibility: a nation-wide multicenter comparative study. Journal of Medical Microbiology. 2023;72(8):001671.
10. Naeem H, Khan S, Siddiqui MA, Ali MM, Siddiqui SN, Ghafoor A, et al. Microbial Profile and Antimicrobial Resistance among the Most Common Grades of Diabetic Foot Ulcers (DFUs) at Mayo Hospital, Lahore, Pakistan. BioScientific Review. 2023;5(3):15-25.
11. Khan MS, Azam M, Khan MN, Syed F, Ali SHB, Malik TA, et al. Identification of contributing factors, microorganisms and antimicrobial resistance involved in the complication of diabetic foot ulcer treatment. Microbial Pathogenesis. 2023;184:106363.
12. Kibunto P, Peter B, Munisi Y, Byabato S, Jaka H, Sam S, et al. Patterns of Pathogens and Antimicrobial Susceptibility Profiles in Diabetic Foot Ulcers' Infections among Patients at Bugando Medical Centre in Mwanza, Tanzania. 2023.
13. Hussain C, Farooque MA, Khan SH, Younas A. Bacterial Diversity and Their Antimicrobial Susceptibility Patterns in Diabetic Foot Infections, a Tertiary Care Hospital Study. Life and Science. 2024;5(4):06-.
14. Qasim M, Haroon A, Ullah M, Amir N, Imtiaz H, Tara T, et al. Prevalence and Antibiotic Susceptibility Pattern of Pseudomonas Aeruginosa from the Foot Ulcer of Diabetic Patients in Hayatabad Medical Complex Peshawar, Pakistan. Journal of Health and Rehabilitation Research. 2024;4(2):414-20.
15. Moya-Salazar J, Chamana JM, Porras-Rivera D, Goicochea-Palomino EA, Salazar CR, Contreras-Pulache H. Increase in antibiotic resistance in diabetic foot infections among peruvian patients: a single-center cross-sectional study. Frontiers in Endocrinology. 2023;14:1267699.
16. Yang S, Hu L, Zhao Y, Meng G, Xu S, Han R. Prevalence of multidrug-resistant bacterial infections in diabetic foot ulcers: A meta-analysis. International Wound Journal. 2024;21(4):e14864