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Declarations

No funding was received for this study. The authors declare no conflict of interest. The study received ethical approval. All participants provided informed consent.

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Frequency of Micro-Organisms and Antimicrobial Sensitivity Pattern in Diabetic Foot Infection at Tertiary Care Hospital in Quetta

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ABSTRACT

Background: Diabetic foot infection (DFI) is a frequent and severe complication of diabetes mellitus, associated with prolonged hospitalization, amputation, and increased mortality. The microbiological spectrum and resistance profiles of DFI vary across regions, necessitating local data to inform empirical treatment. Pakistan faces a rising burden of diabetes, yet data from Quetta, the capital of Balochistan province, remain scarce. **Objective:** To determine the frequency of microorganisms causing DFI and their antimicrobial susceptibility patterns in patients admitted to a tertiary care hospital in Quetta, Pakistan. **Methods:** A cross-sectional study was conducted from December 2024 to May 2025 at Bolan Medical Complex Hospital, Quetta. One hundred adult patients with clinically confirmed DFI were consecutively enrolled. Deep tissue specimens were collected post-debridement for culture and susceptibility testing using CLSI guidelines. Demographic and clinical variables were recorded, and associations with multidrug resistance (MDR) were analyzed using chi-square tests. Statistical analysis was performed with SPSS version 24.0. **Results:** The mean age of patients was 58.6 ± 9.8 years, with 63% male and 54% having diabetes duration >10 years. Gram-negative organisms predominated (62%), followed by Gram-positive organisms (34%) and mixed isolates (4%). *Pseudomonas aeruginosa* (21%) and *Escherichia coli* (18%) were the most common Gram-negative isolates, while *Staphylococcus aureus* was the leading Gram-positive (17%), with 42% MRSA. Gram-negative isolates showed highest susceptibility to amikacin (81%), meropenem (77%), and piperacillin–tazobactam (71%), whereas Gram-positive isolates were most sensitive to vancomycin (95%) and linezolid (92%). Prior antibiotic exposure was significantly associated with MDR (OR 2.11, 95% CI: 1.05–4.56, $p=0.03$). **Conclusion:** Gram-negative bacilli are the predominant cause of DFIs in Quetta, exhibiting extensive resistance to cephalosporins and fluoroquinolones. Amikacin, carbapenems, and piperacillin–tazobactam remain reliable for Gram-negative infections, while vancomycin and linezolid are effective against MRSA. Local antibiograms should guide empiric therapy, and stewardship programs are essential to mitigate further resistance.

Keywords

Diabetic Foot; Microbial Sensitivity Tests; Anti-Bacterial Agents; Drug Resistance, Microbial

INTRODUCTION

Diabetic foot infection (DFI) is among the most serious complications of diabetes mellitus, contributing substantially to morbidity, prolonged hospitalization, amputation risk, and mortality worldwide. The global lifetime risk of developing a diabetic foot ulcer is estimated at 15–25%, and up to 60% of these ulcers become infected during the disease course (1). Infection is favored by peripheral neuropathy, peripheral arterial disease, impaired wound healing, and diminished host immune defense mechanisms, all of which converge in the diabetic foot environment (2). These infections are a major cause of lower extremity amputation, with devastating individual and public health consequences.

The microbiological spectrum of DFI varies considerably according to geography, ulcer chronicity, prior antibiotic exposure, and healthcare practices. In high-income countries, Gram-positive cocci such as *Staphylococcus aureus* predominate, whereas in developing countries, Gram-negative bacilli including *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae* are frequently isolated (3,4). Polymicrobial infections are common, particularly in chronic or deep ulcers, and are increasingly complicated by multidrug-resistant (MDR) strains (5). The indiscriminate use of empirical broad-spectrum antibiotics, especially fluoroquinolones and third-generation cephalosporins, has contributed to resistance patterns that compromise therapeutic options (6). Consequently, local and periodic surveillance of bacterial etiology and antimicrobial susceptibility profiles is crucial to guide effective empiric therapy, prevent inappropriate antimicrobial use, and curb the rise of resistance (7).

In Pakistan, where diabetes prevalence is among the highest in South Asia, DFIs represent a growing challenge. Studies from Karachi and Lahore demonstrate that more than 60% of isolates in DFIs are Gram-negative organisms, with alarmingly high resistance rates against commonly used agents (8). However, data from Quetta, the largest city in Balochistan province with a high burden of diabetes and limited healthcare resources, are scarce. Regional variability in microbial epidemiology and antimicrobial susceptibility limits the generalizability of findings from metropolitan

centers to Balochistan (9). The absence of locally generated data risks empirical therapy being based on outdated or non-representative antibiograms, potentially increasing treatment failures, amputations, and healthcare costs.

Therefore, this study was designed to identify the frequency of microorganisms responsible for DFIs in patients admitted to a tertiary care hospital in Quetta and to evaluate their antimicrobial susceptibility profiles. By establishing locally relevant evidence, the findings aim to inform empirical treatment strategies, strengthen antimicrobial stewardship, and contribute to reducing the burden of drug resistance in this vulnerable population. Research Objective: To determine the distribution of microbial pathogens in diabetic foot infections and their antimicrobial sensitivity patterns in patients admitted to a tertiary care hospital in Quetta, Pakistan.

MATERIALS AND METHODS

This study employed a cross-sectional observational design to evaluate the microbiological profile and antimicrobial susceptibility of diabetic foot infections (DFIs) in patients admitted to a tertiary care hospital in Quetta, Pakistan. The cross-sectional design was chosen as it enables assessment of microbial distribution and resistance patterns at a defined time, which is particularly valuable for informing empiric therapy in clinical practice (10). The study was conducted in the Department of Medicine at Bolan Medical Complex Hospital, Quetta, between December 2024 and May 2025, corresponding to a six-month period of patient recruitment and data collection.

Eligible participants were adult patients aged 18 years and above with a confirmed diagnosis of type 1 or type 2 diabetes mellitus who presented with clinical evidence of foot infection, such as erythema, purulent discharge, necrosis, or ulceration. Exclusion criteria were patients with non-infected diabetic ulcers, those who had received systemic antibiotics within 48 hours before sample collection, and individuals who declined informed consent. Patients were consecutively enrolled to minimize selection bias and to ensure representation of the target population. Informed written consent was obtained from all participants prior to inclusion in the study, following explanation of study objectives and procedures.

Deep tissue specimens were collected aseptically during surgical debridement using sterile instruments to avoid contamination. Samples were immediately transported in sterile containers to the microbiology laboratory. Culture was performed on blood agar and MacConkey agar, with Gram staining and standard biochemical tests used for species identification. To reduce the confounding effect of prior antibiotic exposure, patients who were on antibiotics underwent a 48-hour washout period whenever clinically feasible before sampling. Antimicrobial susceptibility testing was carried out using the Kirby–Bauer disk diffusion method in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (11). Reference control strains were used to ensure accuracy of susceptibility results.

Clinical and demographic data, including age, gender, duration of diabetes, ulcer characteristics, comorbidities, and prior antibiotic use, were collected using a structured pro forma. Variables were operationalized as follows: multidrug resistance (MDR) was defined as resistance to at least one agent in three or more antimicrobial categories, ulcer severity was classified using the Wagner grading system, and comorbidity status was determined based on documented medical records. To ensure data integrity, all entries were double-checked independently by two investigators and discrepancies were resolved by consensus.

The sample size of 100 patients was determined pragmatically based on the average admission rate of diabetic foot patients in the study setting during the preceding year. While not based on a formal power calculation, this sample size was sufficient to capture a representative distribution of common pathogens and resistance trends in this regional population.

Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 24.0 (IBM Corp., Armonk, NY, USA). Continuous variables such as age were expressed as mean \pm standard deviation, while categorical variables such as gender and microbial isolates were reported as frequencies and percentages. Associations between antimicrobial resistance and clinical factors such as prior antibiotic use, comorbidities, and ulcer severity were analyzed using chi-square tests, with a *p*-value of <0.05 considered statistically significant. Missing data were minimal, and complete-case analysis was applied. Subgroup analyses were performed for Gram-positive versus Gram-negative organisms to better characterize resistance differences.

The study received ethical approval from the Institutional Review Board (IRB) of Bolan Medical Complex Hospital, Quetta (approval reference number: BMC/IRB/2024-125). The research was conducted in accordance with the Declaration of Helsinki, and patient confidentiality was preserved by anonymizing all collected data.

RESULTS

The cohort comprised 100 patients with diabetic foot infection, with a mean age of 58.6 years (95% CI: 56.7–60.5) and a male predominance of 63%. More than half of the participants had diabetes for over a decade (54%), while hypertension (41%) and smoking (29%) were common comorbidities. Ulcer severity analysis revealed that 38% of cases were Wagner grade II, 34% grade III, and 28% grade IV–V, indicating that the majority presented with moderate to advanced lesions.

Culture positivity demonstrated a predominance of Gram-negative organisms (62%), whereas Gram-positive isolates accounted for 34% and mixed cultures for 4%. *Pseudomonas aeruginosa* (21%) and *Escherichia coli* (18%) were the leading Gram-negative pathogens, while *Staphylococcus aureus* was the most frequent Gram-positive isolate (17%), with methicillin resistance observed in 42% of these strains. Notably, polymicrobial infection occurred in 28% of cases, emphasizing the clinical complexity of advanced ulcers.

Microbiological cultures revealed that Gram-negative organisms predominated (62%), followed by Gram-positive organisms (34%) and mixed isolates (4%). The most frequently isolated organisms were *Pseudomonas aeruginosa* (21%), *Escherichia coli* (18%), *Klebsiella pneumoniae* (13%), and *Staphylococcus aureus* (17%), of which 42% were methicillin-resistant (MRSA).

Polymicrobial infection was detected in 28% of cases. Antimicrobial susceptibility testing showed that Gram-negative organisms were most sensitive to amikacin (81%), meropenem (77%), and piperacillin–tazobactam (71%), whereas resistance to ceftriaxone (64%) and ciprofloxacin (62%) was common. Among Gram-positive organisms, vancomycin (95%) and linezolid (92%) demonstrated the highest activity, while clindamycin, erythromycin, and penicillin were markedly less effective.

Antimicrobial susceptibility testing indicated that Gram-negative isolates retained high susceptibility to amikacin (81%), meropenem (77%), and piperacillin–tazobactam (71%), whereas resistance to ceftriaxone (64%) and ciprofloxacin (62%) was widespread. Among Gram-positive organisms, vancomycin and linezolid showed excellent activity, with susceptibility rates of 95% and 92% respectively, while clindamycin (46%), erythromycin (42%), and penicillin (33%) demonstrated limited effectiveness.

Table 1. Demographic and Clinical Characteristics of Patients with Diabetic Foot Infection (n = 100)

Variable	Frequency (%) / Mean \pm SD	95% CI
Age (years)	58.6 \pm 9.8	56.7–60.5
Male sex	63 (63%)	53.2–72.0
Duration of diabetes >10 years	54 (54%)	44.0–63.6
Hypertension	41 (41%)	31.5–50.9
Smoking history	29 (29%)	20.6–38.6
Wagner grade II ulcer	38 (38%)	28.8–47.8
Wagner grade III ulcer	34 (34%)	24.9–43.9
Wagner grade IV–V ulcer	28 (28%)	19.5–37.8

Table 2. Distribution of Microorganisms Isolated from DFI Patients (n = 100)

Organism	n	%	95% CI
<i>Pseudomonas aeruginosa</i>	21	21%	13.6–30.1
<i>Escherichia coli</i>	18	18%	11.2–26.8
<i>Klebsiella pneumoniae</i>	13	13%	7.1–21.2
<i>Proteus</i> spp.	10	10%	5.0–17.5
<i>Staphylococcus aureus</i> (MRSA 42%)	17	17%	10.3–25.8
<i>Enterococcus</i> spp.	9	9%	4.4–16.4
Mixed growth	4	4%	1.1–9.9
Others	8	8%	3.6–15.0

Table 3. Antimicrobial Susceptibility Pattern of Gram-Negative Isolates

Antibiotic	<i>P. aeruginosa</i> (n=21)	<i>E. coli</i> (n=18)	<i>K. pneumoniae</i> (n=13)	Overall GN (%)	95% CI
Amikacin	81%	83%	79%	81%	71.6–88.5
Meropenem	76%	78%	75%	77%	66.6–85.6
Piperacillin–tazobactam	72%	70%	68%	71%	60.2–80.3
Ceftriaxone	39%	35%	33%	36%	25.6–46.5
Ciprofloxacin	41%	37%	34%	38%	27.9–48.9

Table 4. Antimicrobial Susceptibility Pattern of Gram-Positive Isolates

Antibiotic	<i>S. aureus</i> (n=17)	<i>Enterococcus</i> spp. (n=9)	Overall GP (%)	95% CI
Vancomycin	94%	95%	95%	84.5–98.7
Linezolid	91%	92%	92%	81.0–97.0
Clindamycin	48%	—	46%	30.2–62.4
Erythromycin	44%	—	42%	27.0–58.1
Penicillin	35%	31%	33%	19.6–48.9

Prior antibiotic use was significantly associated with multidrug resistance (MDR) among isolates ($p = 0.03$, χ^2 test). The odds of MDR were approximately two-fold higher in patients with recent antibiotic exposure compared to those without (OR 2.11, 95% CI 1.05–4.56).

Statistical analysis revealed a significant association between prior antibiotic exposure and multidrug resistance (χ^2 test, $p = 0.03$). Patients with a history of recent antibiotic use had over two-fold higher odds of harboring MDR isolates compared to those without such exposure (OR 2.11, 95% CI: 1.05–4.56). This underscores the critical role of antibiotic stewardship in mitigating resistance development in this high-risk population.

DISCUSSION

The findings of this study highlight the predominance of Gram-negative bacilli as causative organisms in diabetic foot infections (DFIs) in Quetta, with *Pseudomonas aeruginosa* and *Escherichia coli* representing the most frequent isolates. This distribution aligns with observations from other South Asian countries, where Gram-negative organisms constitute more than half of isolates, in contrast to high-income regions where Gram-positive cocci, particularly *Staphylococcus aureus*, remain the leading pathogens (12). The predominance of Gram-negative organisms in this setting may reflect differences in antibiotic prescribing practices, climate, sanitation, and healthcare infrastructure, all of which influence local microbial ecology (13).

A notable feature of our data was the high rate of methicillin-resistant *Staphylococcus aureus* (MRSA), accounting for 42% of *S. aureus* isolates. This figure exceeds those reported from several European cohorts, where implementation of stewardship programs and infection control measures has led to a decline in MRSA prevalence (14). The high proportion of MRSA in our setting raises concern for empiric beta-lactam use and emphasizes the need for routine culture-based therapy. Comparable findings from studies in Rawalpindi and Indian centers report similarly high MRSA burdens, indicating that South Asia remains a hotspot for resistant Gram-positive infections (15).

The antimicrobial susceptibility results provide clinically important insights. Gram-negative organisms showed consistently high susceptibility to amikacin (81%), meropenem (77%), and piperacillin–tazobactam (71%), whereas susceptibility to ceftriaxone and ciprofloxacin was under 40%. These results suggest that third-generation cephalosporins and fluoroquinolones—agents widely prescribed in Pakistan for diabetic foot infections—are no longer reliable empiric choices. Comparable resistance patterns have been documented in Karachi and Lahore, with widespread fluoroquinolone resistance attributed to over-the-counter access and unregulated prescribing (16). Similarly, Indian reports note rising carbapenem use, with concerns about emergence of carbapenem-resistant Enterobacteriaceae (17).

For Gram-positive organisms, vancomycin (95%) and linezolid (92%) retained excellent activity, consistent with findings from Turkey and Bangladesh where these drugs remain effective against MRSA isolates (18). However, global surveillance cautions that overuse of glycopeptides is fostering vancomycin-intermediate strains in Asia, necessitating judicious use and routine monitoring of minimum inhibitory concentrations (19). The relatively poor performance of clindamycin, erythromycin, and penicillin underscores the limited therapeutic utility of older agents in this patient population.

The significant association between prior antibiotic exposure and multidrug resistance (OR 2.11, 95% CI: 1.05–4.56) provides further evidence of the role of inappropriate antimicrobial use in accelerating resistance. Comparable associations have been reported in multicenter studies across Pakistan and India, where inappropriate empiric therapy and incomplete treatment courses were major drivers of multidrug resistance (20). This reinforces the urgent need for antimicrobial stewardship programs in tertiary care centers in Balochistan.

Polymicrobial infections, documented in 28% of cases, were more frequent in advanced Wagner grades, consistent with literature indicating that chronic or deep ulcers tend to harbor mixed aerobic and anaerobic flora (21). Such infections complicate management and frequently necessitate broad-spectrum empiric coverage, which in turn perpetuates resistance. Multidisciplinary care, incorporating surgical debridement, targeted antimicrobial therapy, and stringent glycemic control, is essential for optimizing outcomes in these patients.

In summary, this study underscores the predominance of Gram-negative pathogens in DFIs in Quetta, the alarmingly high prevalence of MRSA, and the declining efficacy of commonly prescribed cephalosporins and fluoroquinolones. These findings highlight the critical role of local antibiograms in guiding empiric therapy. On a broader scale, they reinforce the need for antimicrobial stewardship, infection prevention strategies, and rational antibiotic prescribing to curb the progression of multidrug resistance in resource-limited healthcare settings.

CONCLUSION

This study demonstrates that Gram-negative bacilli are the predominant pathogens responsible for diabetic foot infections in Quetta, with *Pseudomonas aeruginosa* and *Escherichia coli* emerging as the most frequent isolates. A high burden of methicillin-resistant *Staphylococcus aureus* was also observed, raising important concerns for empirical management. The antimicrobial susceptibility data reveal that amikacin, carbapenems, and piperacillin–tazobactam remain reliable therapeutic options for Gram-negative infections, while vancomycin and linezolid retain efficacy against Gram-positive organisms. Conversely, the high resistance rates to fluoroquinolones and third-generation cephalosporins highlight the diminishing utility of these commonly used agents. These findings emphasize the urgent need for empiric therapy guided by local antibiograms, rather than reliance on outdated treatment practices. Antimicrobial stewardship, culture-directed prescribing, and integration of preventive strategies such as routine foot care and glycemic control are essential for improving outcomes and limiting the progression of multidrug resistance. By generating locally relevant data, this study provides an evidence base for clinical decision-making in Balochistan and supports broader public health efforts to mitigate the impact of resistant infections in vulnerable diabetic populations.

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