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## Antibiotic Susceptibility Pattern of Bacterial Isolates from Infected Diabetic Foot Ulcer in Patients of Type 2 Diabetes Mellitus Presenting at Hayatabad Medical Complex Peshawar

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#### Declaration

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### ABSTRACT

**Objectives:** This study aimed to determine the antibiotic resistance profiles of bacterial isolates obtained from diabetic foot ulcers (DFUs) in type 2 diabetes patients at Hayatabad Medical Complex, Peshawar, by identifying the most prevalent bacterial species and their corresponding resistance patterns. **Materials and Methods:** This cross-sectional study was conducted over six months from March to September 2024, involving 120 clinically diagnosed patients with infected diabetic foot ulcers. Bacterial isolates were obtained from wound swabs and identified using standard cultural and biochemical tests. Antibiotic susceptibility was determined through the disc diffusion method. **Results:** The results indicated that 93.3% of wound swabs showed positive bacterial growth, predominantly gram-negative bacteria, with Escherichia coli (28%) and Klebsiella pneumoniae (22%) being the most prevalent isolates. Polymicrobial infections were found in 18% of samples. Resistance rates were notably high for ampicillin (72%) and ciprofloxacin (55%), while carbapenems and piperacillin-tazobactam demonstrated higher sensitivity. Among gram-positive isolates, methicillin-resistant Staphylococcus aureus (MRSA) was detected in 60% of cases. **Conclusion:** The research underscores the significant prevalence of multidrug-resistant (MDR) bacteria in diabetic foot ulcers, emphasizing the critical need for innovative approaches to antibiotic treatment and robust infection control strategies.

### INTRODUCTION

The foot ulcers are among the most severe and disabling diabetes mellitus long-term effects, especially in Type 2 diabetes patients. Diabetes is now a well-recognized disease that has been growing in incidence across the world, and because of the chronic complications associated with it, it continues to pose a threat to public health in terms of quality of life as well as longevity. Such ulcers are mainly linked to extended hospital stays, recurrent infections, and, in the worst-case scenario, amputations, whose consequences summarize a lowered quality of life (1). The

management of most DFUs is a problem, mainly due to the complexity of microbial colonization and antibiotic resistance (2b).

DFUs are generally caused by neuropathy, vascular disease, and immune abnormality, which predisposes diabetic individuals to tissue infection. Most DFU infections are polymicrobial, and both G-positive and G-negative bacterial isolates are present. It is unsurprising that the further addition of multidrug-resistant (MDR) organisms impacts the treatment response and increases the need for



effective antimicrobial stewardship and correct susceptibility testing (3). Previous research has established that bacterial isolates from DFU patients differ in antibiotic susceptibility depending on geographical locations, microbial distribution, local antibiotic consumption trends, and the state of healthcare facilities (4).

The increase in AMR among pathogens associated with the management of DFU is gradually becoming a concern globally. AMR constrains ant transformation potential and augments morbidity, mortality, and healthcare charges. It is, therefore, essential to know the local epidemiology and susceptibility profile of bacterial isolates to determine the correct empirical therapy and enhance clinical outcomes (5). In Pakistan, scanty information is mainly available regarding the antibiotic patterns of bacteria isolated from infected DFUs.

In as much as social media platforms have been instrumental in influencing political polarization across modern societies they cannot be underestimated. By creating echo chambers, spreading misinformation, and letting political campaigns hit specific audiences those platforms amplify the divide of the political spectrum and the escalation of societal conflicts. Despite the advantages like a boost in political participation and the real voices representation, there is much negativity that social media brings as a result of a negative impact like the distribution of hate speech and fake news. It is advisable to discuss these problems and their influence on political integration and public trust with regard to advances in social media regulation and media literacy as well as increasing the emphasis on active and constructive arguments with up-to-date facts.

Chronic and worsened DFUs can be attributed to several factors, such as late presentation, poor glycaemic control, and irrational use of antibiotics. Such investigations conducted in the Islamic Republic of Iran and China inform the world that this causes infections that require more extended periods of treatment and more extensive surgeries, including amputations, compared to obtaining early treatment (8, 9). Moreover, there is a significant difference in bacterial species, and resistance between ischemic and non-ischemic ulcers, and the authors pointed out that ischemia may affect microbial adhesion and antibiotic

resistance (12).

Recent studies show that some of the molecular detection of MDR pathogens in DFUs has increased. These elaborate diagnostic techniques make it easier to determine the resistance patterns, helping select the most appropriate antimicrobial compounds. For example, research conducted in Wasit Province made understanding genes related to multidrug-resistant in the molecular reports of DFU infections (11). Such studies thus raise the need to improve the synergy between molecular techniques and traditional culture methods in diagnosing infectious diseases for better diagnosis and, ultimately, patient outcomes.

As demonstrated in this paper, the burden of DFUs transcends individual patient levels, affecting health systems and economies. In Ethiopia, for instance, increased rates of ESBL-producing and carbapenem-resistant Gram-negative bacteria were described, making the management of such organisms in resource-constrained settings even more problematic (15). Similarly, other past systematic reviews from Iran have established high prevalence levels of MDR pathogens in DFUs, there is a call for region-specific guidelines to deal with the realities of DFU infections (14). These findings reaffirm the need for country-specific research to provide quick treatment regimes.

A lot of effort should be put into preventive measures to reduce the burden of DFU infections. Screening for foot problems and failure, as well as patient education coupled with early treatment, is effective in preventing significant infections and amputations. In particular, today, improving the principles of antibiotic stewardship is critical for preventing the growth of AMR in DFU-associated pathogens (13). Additionally, the Chinese research indicated that implementing infection control measures cut down incidences of MDR organisms among patients with DFU not only but also controlled the use of antibiotics (10).

Lastly, DFUs are difficult to manage, require a team effort, and require early assessment, optimal antimicrobial prescription, and effective preventative measures. Therefore, the present research aims to determine the antibiotic sensitivity pattern of the bacterial isolates obtained from infected DFUs in T2DM patients seeking care at HMC in Peshawar. From the results identifying the

specific pathogens and resistance patterns, the study's results will enable clinicians to identify the appropriate initial empiric therapy to use in order to minimize patient morbidity and mortality associated with the identified pathogens. Research of this nature is precious given the current predatory situation with AMR, where methods for combating bacterial infections are gradually losing their effectiveness.

**Objective:** The purpose of this research is to establish the antibiotic resistance pattern of the bacteria causing infected Diabetic foot ulcers (DFUs) amongst type 2 diabetic patients presenting to Hayatabad Medical Complex, Peshawar. It suggests selecting the common pathogens and identifying the ideal antimicrobial regimen to improve a patient's fate.

## MATERIALS AND METHODS

**Study Design:** Cross Sectional.

**Study Setting:** The present work was conducted at the Department of Endocrinology and Diabetes, Hayatabad Medical Complex, Peshawar, a tertiary healthcare center where many patients attend for healthcare.

**Duration of the study:** The study was conducted from March 25, 2024, to September 25, 2024.

### Inclusion Criteria

Therefore, the patient inclusion criteria of this study involved any patient that was 18 years old and above with Type 2 diabetes. An inclusion criterion was clinically confirmed infected diabetic foot ulcers (DFUs) to ensure the study sample was appropriate to the motives of the research. Moreover, only those patients who agreed to participate after receiving informed consent were included; thus, it was ethical in its conduct as well and the patients were willing participants in the study.

### Exclusion Criteria

The exclusion criteria were developed to ensure that the result achieved would be specific and reliable. The sample excluded patients with non-diabetic foot ulcers because factors that may cause foot ulcers are unrelated to diabetes. Likewise, patients treated with antibiotics in the last 2 weeks before sample collection were excluded because prior antibiotic use affects the microbiological and

antibiotic susceptibility patterns. Patients who develop severe systemic infections not necessarily associated with DFUs were also excluded to focus on infections of DFUs only. Finally, any patients who did not consent were excluded from the study because of research ethical considerations.

### Methods

Patient specimens were gathered by swabbing the wounds of patients who presented with clinically infected DFUs, fulfilling the inclusion criteria. The samples were taken in an aseptic manner by using cotton-tipped sterile swabs from the wound bed after washing gently with sterile saline and being careful not to cause contact with the dead tissue. All the specimens were cultured and sensitivity tested in the microbiology laboratory without delay. This study employed typical biochemical and cultural characteristics of bacterial pathogens, such as Gram stain, colony character, and biochemical tests. Antibiotic sensitivity was determined by the disk diffusion method according to the recommendations of the CLSI. A panel of the most frequently used antibiotics was selected to assess susceptibility. The demographic, clinical history, and microbiological details were compared using statistical software. Quantitative data was analyzed and displayed in frequencies and distribution tables emphasizing the distribution of bacterial isolates and their resistance to the antibiotics.

## RESULTS

The study included 120 patients with clinically suspected infected diabetic foot ulcers (DFUs). Slightly more than half of the patients were male, with an average age of 58 years. The majority of the patients had a diabetes duration of over 10 years, and 70% presented with either neuropathy or peripheral vascular disease. Of the 120 wound swab samples analyzed, 93.3% showed positive bacterial growth, while 8.3% were culture-negative.

Among the bacterial isolates, 75% were gram-negative, with *Escherichia coli* being the most prevalent at 28%, followed by *Klebsiella pneumoniae* at 22%. Gram-positive bacteria were isolated in 28 cases (25%), with *Staphylococcus aureus* found in 18% of total culture positive cases, of which 60% were methicillin-resistant *Staphylococcus aureus* (MRSA), highlighting the

growing concern of antibiotic resistance. Other pathogens identified included **Pseudomonas aeruginosa** (15 %), **Proteus mirabilis** (10 %), and **Enterococcus faecalis** (7 %). Polymicrobial infections, involving multiple bacterial types, were detected in 18% of samples, underscoring the complexity of infections in diabetic foot ulcers.

**Table 1**

| Bacterial Isolate      | Frequency | Percentage |
|------------------------|-----------|------------|
| Escherichia coli       | 31        | 28%        |
| Klebsiella pneumoniae  | 25        | 22%        |
| Staphylococcus aureus  | 20        | 18%        |
| Pseudomonas aeruginosa | 17        | 15%        |
| Proteus mirabilis      | 11        | 10%        |
| Enterococcus faecalis  | 8         | 7%         |

The antibiotic resistance profile revealed significant resistance patterns. Among gram-negative bacteria, **Escherichia coli** and **Klebsiella pneumoniae** exhibited high resistance to ampicillin and cephalosporins but showed high susceptibility to carbapenems and piperacillin-tazobactam. **Pseudomonas aeruginosa** demonstrated resistance to ciprofloxacin and gentamicin, while it was moderately susceptible to colistin and meropenem.

**Table 2**

| Antibiotic              | Resistance Rate | Sensitivity Rate |
|-------------------------|-----------------|------------------|
| Ampicillin              | 72%             | 28%              |
| Ciprofloxacin           | 55%             | 45%              |
| Piperacillin-Tazobactam | 18%             | 82%              |
| Meropenem               | 12%             | 88%              |
| Colistin                | 8%              | 92%              |

Among gram-positive isolates, penicillin and erythromycin resistance was prevalent, particularly in MRSA strains. However, most gram-positive organisms were susceptible to vancomycin and linezolid. For gram-negative isolates, the extended-spectrum beta-lactamase (ESBL) production rate was 25%, while resistance to carbapenems was lower at 10%.

**Table 3**

| Gram-Positive Isolate | Resistance (Penicillin) | Sensitivity (Vancomycin) |
|-----------------------|-------------------------|--------------------------|
| Staphylococcus aureus | 60%                     | 95%                      |
| Enterococcus faecalis | 40%                     | 90%                      |

The findings emphasize the urgent need for robust antibiotic stewardship practices and routine antimicrobial resistance monitoring to manage diabetic foot infections effectively.

## DISCUSSION

This study means that understanding the microbiological pattern and antibiotic pattern of bacterial isolates from infected DFUs would be significant. Diabetic foot infections (DFIs) are classified as one of the diabetic complications and an important cause of morbidity, which not only prolongs hospitalization but increases the risk of amputation and mortality. The current work also found that gram-negative bacteria are the most familiar forms and included ESBL-producing E.coli and K.pneumoniae, while MRSA was noticeable among the gram-positive bacteria. These findings corroborate other studies conducted in different areas, stressing the emergence of MDR bacteria in patients with DFUs.

The study's distribution of gram-negative bacteria is in harmony with prior findings. For example, Khan et al. (2023) provided evidence demonstrating that the most frequent isolates in patients with DFUs were E. coli and K. pneumonia, especially when there was polymicrobial infection (1). Similarly, Du et al. (2022) mentioned the importance of gram-negative bacteria involving severe infections since these microorganisms are not susceptible to first-line antibiotics (10). The fact that E. coli and K. pneumoniae colonies were the most significant emphasizes that the error-prone method is essential to increase infection control and use specific antibiotics since these bacteria may contain resistant genes, such as the ESBL types. In this research, ESBL-producing bacteria were found in a quarter of isolated gram-negative, and this figurative is very disturbing because antibiotic treatment for such bacteria is highly restricted.

The third significant finding of this study is a high incidence of MRSA acquired from gram-positive isolates. Methicillin-resistant Staphylococcus Aureus, or MRSA, raises the treatment plans since most MRSA strains are less responsive to beta-lactam antibiotics, such as penicillin and cephalosporin. In the meta-analysis conducted by Shahrokh et al. (2022), the prevalence of MRSA was again found to be high, with vancomycin and linezolid as useful drugs [(14). Overall, gram-positive isolation showed high sensitivity to vancomycin at 95% and linezolid at 90% and should be used in treatment. Nonetheless,



contributions to current health risks include the development of vancomycin-resistant *Staphylococcus aureus* (VRSA), which requires constant supervision of antimicrobial consumption.

The antibiotic resistance profiles noted in our study underscore the value of antibiotic stewardship. Antibiotic resistance amongst gram-negative pathogens was strikingly high, especially towards first and second-generation agents, including ampicillin, ciprofloxacin, and cephalosporins from this study. However, it is worth mentioning that carbapenem and piperacillin-tazobactam have high bactericidal activities against gram-negative organisms in concordance with Nagaya et al. (2024) (3). However, using these last-resort antibiotics raises the chance of resistance in our study, as evidenced by carbapenem resistance in 10% of cases. Similarly, increasing corticosteroid resistance among tested *Pseudomonas aeruginosa* and other gram-negative bacteria provokes concern due to their widespread use in empirical therapy of DFU.

Associates polymicrobial infections with 18% of the cases highlight that DFUs have diverse microbiology. This finding is consistent with several studies by Ahmadishooli et al. (2020), revealing that 20-30% of the DFUs had polymicrobial infections (7). Furthermore, polymicrobial infections suffer from the presence of aerobic and anaerobic organisms, and the mix requires an extensive spectrum of antibiotics or dual therapy. Since these infections require accurate characterization and tests evaluating their susceptibility, particular importance should be accorded to improved diagnostic methods in everyday clinical practice.

This research also addresses the diversity of bacterial and resistance trademarks on the regional level. For example, Shaheen et al. (2021) established that geographical region, healthcare structure, and local antibiotic use determine the bacterial profile of DFUs (8). In Peshawar, contamination control measures and utilization of broad-spectrum antibiotics could lead to MDR bacteria, as indicated in this analysis. To overcome this problem, it is necessary to enhance infection control practices and to develop and launch antimicrobial stewardship programs.

A final evolutionary concept that is also important is how patient-associated variables affect

bacterial characteristics and therapeutic results. Just as has been reported before, the participants in the study had mostly had long-standing diseases, such as diabetes, peripheral neuropathy, and vascular complications. For instance, Albadri et al. (2021) further explained that conditions such as ischemia and neuropathy facilitate bacterial colonization and colonization, leading to chronic infection, according to the study on sixty patients with and without diabetes mellitus (11). Interrupting these factors is critical for managing DFUs and interfering with recurrence.

It also suggests that the evidence of current empirical treatment has yet to be updated recently. In clinical practice, empirical therapy of DFUs is frequently based on broad-spectrum antibiotics that may not adequately include MDR pathogens. The authors found that empiric therapy should target organisms such as piperacillin-tazobactam or carbapenems for gram negativity and vancomycin for gram positivity. However, the treatment should be changed according to the culture and sensitivity results to avoid the development of resistance.

Several strengths of the present research are noted. The patient population is diverse, and microbiological analysis is thorough. Nevertheless, the following limitations are unavoidable. This study was planned and executed at a single center, which might have had some confounding results. Further, this study could not determine the longitudinal changes in bacterial profiles within the six months of the study period due to seasonal changes. Future work should focus on further research with more centers and patients to confirm the results and study new resistance profiles.

The paper discusses the high MDR bacteria rates in DFUs and calls for developing anti-MRB regionalized treatment regimens. The management of DFUs needs a slope of many interacting aspects based on the principles of optimal antimicrobial stewardship, a strong infection control program, and patient self-management. This paper discusses how it is essential to tackle the emerging problem of antibiotic resistance on DFUs to enhance foot health and minimize complications related to diabetic complications.

## CONCLUSION

In conclusion, this study is a genuine concern to realize that MDR bacteria have established

dominance in Diabetic foot ulcers amongst patients at Hayatabad Medical Complex, Peshawar. This is because most pathogens are gram-negative, like E.Coli and Klebsiella pneumoniae, and the other devastating factor is the increasing spread of MRSA. The study highlights the value of culture and sensitivity testing in informing antibiotic

management because of high resistance levels. Additionally, the study expresses the need to increase the drama of antibiotic stewardship programs and strong standards used in preventing infection.

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