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## ORIGINAL ARTICLE

# Risk Factors for Multidrug-Resistant Bacterial Infection in Diabetic Foot Ulcers

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### SUMMARY

***Background*: This study aimed to analyze the distribution of multidrug-resistant (MDR) organisms (MDROs) in patients with diabetic foot ulcers (DFUs) and to identify risk factors for MDRO infections.**
***Methods*: Patients hospitalized with DFUs were enrolled, and ulcer swabs were cultured for bacterial identification and antibiotic susceptibility testing. Hematology and blood biochemistry were also assessed.**
***Results*: A total of 228 patients hospitalized with DFUs were enrolled. Out of 150 patients with positive cultures, 123 (82%) were infected with single strains, whereas 27 (18%) had mixed infections. Out of the 177 bacterial strains isolated, 78 (44%) were MDROs. Among the top 5 most common bacteria, coagulase-negative *Staphylococcus*, *Staphylococcus aureus*, and *Proteus* exhibited MDR rates of 92%, 56%, and 55%, respectively. *Pseudomonas aeruginosa* and *Enterobacter cloacae* had low MDR rates of 5% and 8%, respectively. Single variable logistic regression analysis showed that neutrophil percent (NEU%), creatinine, C-reactive protein, and fasting plasma glucose (FPG) were risk factors for MDRO infection, whereas hemoglobin and albumin levels were protective factors. Multivariable logistic regression analysis revealed that NEU% and FPG were independent risk factors for MDRO infection.**
***Conclusions*: A high percentage of the infections in patients with DFUs were caused by MDROs. To reduce MDRO infections in high-risk patients, it is important to use antibiotics rationally, improve patients' FPG levels and nutritional status, and strengthen hospital sterilization processes.**
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### KEYWORDS

diabetic foot infection, multidrug-resistant bacteria, risk factors

### INTRODUCTION

In Mainland China, an estimated 129.8 million people have diabetes [1]. Notably, 25% of the individuals with diabetes develop diabetic foot ulcers (DFUs) during their lifetime, which is one of the most serious complications of diabetes. In approximately 50 - 60% of the cases, DFUs become infected, and in about 20% of the cases, moderate-to-severe infections lead to lower extremity amputation. Patients with DFUs have a 5-year mortality rate of approximately 30%, whereas those with major amputations have a 5-year mortality rate of > 70% [2]. Diabetic foot infections (DFI) are risk fac-

tors for poor wound healing, amputation, and premature death [3].

Henig et al. [4] found that 56% of DFU infections are caused by multidrug-resistant (MDR) organisms **(**MDROs), and 30% of pathogenic bacteria are resistant to the recommended antibiotics. MDRO infection can lead to the deterioration of a condition, as characterized by poor wound healing, increased treatment failure, increased readmission rates, and increased mortality rates. MDROs, such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococci*, and extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae*, exhibit resistance to three or more classes of commonly used antibiotics to which they are usually sensitive [5].

Preventing and treating DFI is becoming more challenging as the proportion of infections caused by MDROs increases. Therefore, identifying the risk factors for MDRO infections is essential for clinicians to intervene promptly [6,7]. Studies have shown that the risk factors for MDRO infections include multiple hospitalizations, history of antibiotic use, type of diabetes, type of ulcer, ulcer size, osteomyelitis, and vasculopathy [8,9]. Few studies have assessed the risk of MDRO infections in patients with DFUs by monitoring the blood and biochemical indicators. This study analyzed the distribution of MDROs in patients with DFI and compared the levels of blood and biochemical indicators between the multidrug- and non-MDR bacterial infection groups. In addition, we aimed to identify risk and independent risk factors for MDRO infections in patients with DFI, using single variable and multivariable logistic regression analyses. Determining the risk factors for MDRO infections allows targeted interventions for patients susceptible to such infections. This can reduce the prevalence of MDRO infections, improve the cure rate of patients with DFUs and infections, and decrease the likelihood of foot or toe amputation. For patients with a history of DFUs and MDRO infections who have been successfully treated, detecting these indicators can serve as a timely reminder of their physical condition and help prevent the recurrence of MDRO infections.

## MATERIALS AND METHODS

### General information

Between October 2022 and April 2023, 228 patients with DFUs were admitted to the Diabetic Foot Center of the Air Force Hospital of the Eastern Theater Command, a 700-bed tertiary hospital in Nanjing, China. The inclusion criteria were the presence of diabetes and DFUs located below the ankle protrusion. The exclusion criteria were: 1) acute complications of diabetes other than DFUs; 2) other serious cardiovascular and cerebrovascular diseases and hepatic and renal insufficiency; and 3) other non-DFUs due to vascular insufficiency, cardiac diseases, neurological diseases, and malignant tumors. This was a monocentric study approved by the Ethics Committee of our hospital (DKKY no. 2022011), and written informed consent was obtained from all participants.

### Analysis of blood and biochemical indicators

Fasting venous blood was drawn from patients on the morning of the day after admission, and blood and biochemical indices were measured. Routine blood tests were performed by using a Sysmex XN-1000 blood cell analyzer with the supporting reagents (Sysmex Corp., Kobe, Japan). Creatinine (Cr), C-reactive protein (CRP), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), albumin (Alb), and fasting plasma glucose (FPG) levels were measured, using a Hitachi 7600 automated biochemical analyzer with the reagents (Hitachi Ltd., Tokyo, Japan). Hemoglobin A1c (HbA1c) was measured, using a Bio-Rad glycated hemoglobin analyzer with the supporting reagents (Bio-Rad Laboratories Inc., Hercules, CA, USA). Fibrinogen (FIB) and D-dimer (D-D) levels were measured, using a Sysmex CS-5100 coagulation analyzer with the supporting reagents (Sysmex Corp., Kobe, Japan).

### Bacterial culture and drug sensitivity test

Wound specimen collection was aseptic. Superficial ulcer specimens were collected by dipping a sterile saline swab into the secretion or pus at the base of the ulcer, with care taken to avoid skin contamination around the wound. Secretions or pus were collected from deep ulcers, using sterile syringes after debridement. Huang et al. has reported that swab culturing may be reliable for identification of pathogens in superficial diabetic foot wounds, whereas in deep ulcers, swab culturing is associated with a high risk of missing pathogens [10]. Therefore, we selectively collected wound samples according to the assessment of the ulcers, which is the common practice adopted in similar studies. Samples were immediately sent to the microbiology laboratory for bacterial culture and drug sensitivity tests. The aerobic bacterial culture technique was used by inoculating the specimens into Columbia blood plates and incubating them under aerobic conditions at 35.5°C for 18 - 48 hours. Identification of the bacterial isolates was based on the DL-96A fully auto microbial ID/AST system (ZHUHAI DL BIOTECH Co., Ltd., Zhu Hai, Guangdong Province, China), which allows for the identification of microbial species by biochemical reactions and the determination of antimicrobial susceptibility patterns by specific panels. Anaerobic culturing was not performed in this study. The results of drug sensitivity tests were interpreted according to the standards of the American Committee for Clinical Laboratory Standardization (CLSI, 2020). Bacteria that were simultaneously resistant to three or more types of antibacterial drugs were recognized as MDR. Quality control strains obtained from Culti-Loops (Thermo Fisher Scientific Inc., Waltham, MA, USA) included *S. aureus* (ATCC25

923), *Klebsiella pneumoniae* (ATCC700603), *P. aeruginosa* (ATCC27853), *Enterococcus faecalis* (ATCC29 212), and *Escherichia coli* (ATCC25922). The first microbiological sample from all included patients during hospitalization was analyzed.

**Data collection**

The distribution of bacterial pathogens in patients with DFI and the multidrug-resistance rate of different strains were determined. Hematology and blood biochemistry measurements included white blood cell (WBC) count, NEU%, and Hb, FIB, D-D, Cr, Alb, CRP, TC, TG, HDL-C, LDL-C, FPG, and HbA1c levels.

**Statistical analysis**

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 27.0 (IBM Corp., Armonk, NY, USA). Continuous data were expressed as the mean and standard deviation, whereas categorical data were expressed as ratios or percentages. Independent samples *t*-tests were used to compare continuous variables between groups, and risk factors were analyzed by using single variable and multivariable logistic regression analyses. Statistical significance was set at $p < 0.05$ (two-tailed).

## RESULTS

**The distribution of MDROs in patients with DFUs**

This study enrolled 228 patients with DFUs, including 150 with positive bacterial cultures. Among these, 115 were men, whereas 35 were women, with an age range of 34 - 91 years. Single pathogen infections were observed in 123 cases (88.7%), whereas mixed pathogen infections were observed in 27 cases (11.3%). A total of 177 pathogenic bacterial strains were isolated. Out of the 177 bacterial strains analyzed, 87 (49.2%) were Gram-positive cocci, whereas 90 (50.8%) were Gram-negative bacilli. Among the 177 bacterial strains, 78 (44.1%) were MDR, whereas 99 (55.9%) were non-MDR. Out of the MDROs, 52 (66.7%) were Gram-positive, whereas 22 (33.3%) were Gram-negative. Out of the 150 patients, 66 (44%) were infected with MDROs, whereas 84 (56%) were infected with non-MDROs. The top 5 most prevalent pathogens were *S. aureus* (59 strains, 33.3%), *Proteus* spp. (22 strains, 12.4%), *P. aeruginosa* (20 strains, 11.3%), coagulase-negative *Staphylococcus* (12 strains, 6.8%), and *Enterobacter cloacae* (12 strains, 6.8%). Among them, 92% of the coagulase-negative *Staphylococcus* were resistant to methicillin, whereas *P. aeruginosa* and *E. cloacae* had lower MDR rates (5% and 8%, respectively). *S. aureus* exhibited a methicillin resistance rate of 56%, whereas *Proteus* spp. exhibited a rate of 55% (Table 1).

**Blood and biochemical indices in the MDR- and non-MDR bacterial infection groups**

Hb and Alb levels were significantly lower in the MDR bacterial infection group than in the non-MDR bacterial infection group. The NEU% and Cr, CRP, and FPG levels were significantly higher in the MDRO infection group than in the non-MDRO infection group (Table 2).

Univariate logistic regression analysis of risk factors for MDRO infections in patients with DFUs

Univariate logistic regression analysis was performed to explore the risk factors for patients with MDRO infections. Independent variables were selected based on significant differences between the two groups. As presented in Table 3, NEU% and Cr, CRP, and FPG levels were identified as risk factors for multidrug-resistant bacterial infections in patients with DFUs, whereas Hb and Alb levels were identified as protective factors. Notably, these findings are based on objective evaluations and exclude subjective assessments.

**Multivariable logistic regression analysis of independent risk factors for MDR bacterial infection in patients with DFUs**

Multivariable logistic regression analysis indicated that NEU% and FPG level were independent risk factors for MDRO infections in patients with DFUs (Table 4).

## DISCUSSION

DFUs can significantly affect patients' daily lives and even pose a threat to their overall health. Patients with DFUs may be infected with MDROs due to various factors, including wound healing delay, inappropriate antibiotic therapy, frequent hospitalizations, neuropathy, nephropathy, and peripheral vascular disease [11]. DFUs are caused by repetitive trauma resulting from a combination of factors, such as loss of protective sensation, peripheral vasculopathy, and impaired immunity. Microorganisms can colonize and proliferate in ulcers, worsening tissue damage and causing infections. This makes it challenging for antimicrobial drugs to reach the infection site and have a sufficient antimicrobial effect, leading to rapid bacterial resistance [12]. The prevalence, severity, and pathophysiology of DFUs are influenced by pathogen- and host-related factors, such as immune status, nerve damage, microbial virulence, and degree of antibiotic resistance. According to Dai et al. [9], MDRO infections are associated with neuropathy, osteomyelitis, and ulcer size. The risk of MDRO infections is independently associated with poor glycemic control, infection duration, and ulcer size. In particular, several studies have reported that MDROs significantly increase the recurrence and amputation rates in patients with DFUs [13-16], which adversely affects the quality of life and life expectancy of patients with diabetes. Effective control of DFI is a pressing concern for clinicians because it is a significant factor in the deterioration of the condition. Therefore, antibiotic treatment is

**Table 1. Distribution characteristics of multidrug-resistant pathogenic bacteria in patients with diabetic foot ulcers.**

| Bacteria | MDR [a] strains n (row %) | NMDR strains n (row %) | Total strains n |
|---|---|---|---|
| **Gram-positive bacteria** | **52 (60)** | **35 (40)** | **87** |
| ***Staphylococcus aureus*** | **33 (56)** | **26 (44)** | **59** |
| **Coagulase-negative *Staphylococcus*** | **11 (92)** | **1 (8)** | **12** |
| ***Streptococcus*** | **1 (17)** | **5 (83)** | **6** |
| ***Staphylococcus intermedius*** | **4 (80)** | **1 (20)** | **5** |
| ***Enterococcus faecalis*** | **2 (50)** | **2 (50)** | **4** |
| ***Staphylococcus hominis*** | **1 (100)** | **0 (0)** | **1** |
| **Gram-negative bacteria** | **26 (29)** | **64 (71)** | **90** |
| ***Proteus*** | **12 (55)** | **10 (45)** | **22** |
| ***Pseudomonas aeruginosa*** | **1 (5)** | **19 (95)** | **20** |
| ***Enterobacter cloacae*** | **1 (8)** | **11 (92)** | **12** |
| ***Escherichia coli*** | **5 (56)** | **4 (44)** | **9** |
| ***Serratia*** | **2 (29)** | **5 (71)** | **7** |
| ***Klebsiella pneumoniae*** | **1 (17)** | **5 (83)** | **6** |
| ***Acinetobacter*** | **1 (17)** | **5 (83)** | **6** |
| ***Morganella morganii*** | **3 (60)** | **2 (40)** | **5** |
| ***Pantoea agglomerans*** | **0 (0)** | **3 (100)** | **3** |

**MDR [a] - non-susceptible to ≥ 1 agent in ≥ 3 antimicrobial categories.**
**MDR - multidrug-resistant, NMDR - non-multidrug-resistant.**

**Table 2. Comparison of blood and biochemical indices between multidrug-resistant (MDR) and non-MDR bacterial infection groups.**

| Demographic | MDR group (n = 66) | NMDR strains (n = 84) | p |
|---|---|---|---|
| **Age (years)** | **64.12 ± 11.39** | **65.98 ± 13.59** | **0.375** |
| **Duration of disease (years)** | **16.03 ± 6.38** | **15.42 ± 7.24** | **0.591** |
| **WBC ($\times 10^9$/L)** | **10.00 ± 4.42** | **8.82 ± 3.68** | **0.083** |
| **NEU (%)** | **77.15 ± 8.71 [a]** | **70.67 ± 10.22** | **< 0.001** |
| **Hb (g/L)** | **109.03 ± 21.06 [a]** | **121.24 ± 20.61** | **< 0.001** |
| **FIB (g/L)** | **4.95 ± 1.48** | **4.56 ± 1.43** | **0.103** |
| **D-D (mg/L)** | **1.20 ± 1.32** | **0.87 ± 0.93** | **0.091** |
| **Cr (µmol/L)** | **148.54 ± 196.93 [a]** | **94.45 ± 88.55** | **0.026** |
| **Alb (g/L)** | **31.88 ± 5.93 [a]** | **35.17 ± 5.56** | **0.001** |
| **CRP (mg/L)** | **52.97 ± 54.37 [a]** | **25.83 ± 27.34** | **< 0.001** |
| **TC (mmol/L)** | **3.82 ± 1.28** | **3.83 ± 1.04** | **0.952** |
| **TG (mmol/L)** | **1.37 ± 0.66** | **1.25 ± 0.62** | **0.235** |
| **HDL-C (mmol/L)** | **1.13 ± 0.36** | **1.08 ± 0.26** | **0.355** |
| **LDL-C (mmol/L)** | **2.13 ± 0.98** | **2.23 ± 0.83** | **0.503** |
| **FPG (mmol/L)** | **11.19 ± 4.39 [a]** | **9.39 ± 3.27** | **0.005** |
| **HbA1c (%)** | **8.90 ± 2.62** | **8.48 ± 1.99** | **0.260** |

**Alb - albumin, Cr - creatinine, CRP - C-reactive protein, D-D - D-dimer, FIB - fibrinogen, FPG - fasting plasma glucose, Hb - hemoglobin, HbA1c - hemoglobin A1c, HDL-C - high-density lipoprotein cholesterol, LDL-C - low-density lipoprotein cholesterol, NEU - neutrophils, TC - total cholesterol, TG - triglycerides, WBC - white blood cell.**

**Table 3. Unadjusted logistic regression analysis of risk factors for MDR infection.**

| Risk factor | p-value | OR | 95% CI |
|---|---|---|---|
| Hb (g/L) | < 0.05 | 0.972 | 0.956 - 0.988 |
| Alb (g/L) | < 0.05 | 0.904 | 0.851 - 0.961 |
| FPG (mmol/L) | < 0.05 | 1.146 | 1.037 - 1.266 |
| Cr (μmol/L) | < 0.05 | 1.003 | 1.000 - 1.006 |
| NEU (%) | < 0.05 | 1.077 | 1.036 - 1.120 |
| CRP (mg/L) | < 0.05 | 1.018 | 1.008 - 1.027 |

**Alb - albumin, CI - confidence interval, Cr - creatinine, CRP - C-reactive protein, FPG - fasting plasma glucose, Hb - hemoglobin, MDR - multidrug-resistant, NEU - neutrophil, OR - odds ratio.**

**Table 4. Multivariable logistic regression analysis of risk factors for MDR infection.**

| Risk factor | p-value | OR | 95% CI |
|---|---|---|---|
| NEU (%) | < 0.05 | 1.051 | 1.006 - 1.099 |
| Cr (μmol/L) | 0.374 | 1.001 | 0.998 - 1.004 |
| CRP (mg/L) | 0.074 | 1.010 | 0.999 - 1.021 |
| FPG (mmol/L) | < 0.05 | 1.119 | 1.003 - 1.247 |

**CI - confidence interval, Cr - creatinine, CRP - C-reactive protein, FPG - fasting plasma glucose, MDR - multidrug-resistant, NEU - neutrophil, OR - odds ratio.**

necessary to improve the prognosis of DFI. The initial treatment of DFI is typically empirical and varies based on the severity of infection and local microbiological data. MDRO infections increase the difficulty of treating DFI and can lead to failure of antibiotic therapy. It is essential to promptly perform bacterial cultures, characterization, and drug sensitivity testing of diabetic ulcer wound secretions to select appropriate antimicrobial agents to control the infection as soon as possible.

This study included 228 patients with DFUs, including 150 with positive bacterial cultures, resulting in a positivity rate of 65.8%, which was lower than the rate (81.1% [146/180]) reported by Yan et al. [17] but comparable to that (68.2% [178/289]) reported by Saseedharan et al. [18]. In the early stages, DFIs are typically caused by Gram-positive cocci and are usually single bacterial infections. During the chronic phase, DFIs are caused mainly by a mixture of Gram-negative aerobic and anaerobic bacteria, and fungi are rarely present [19].

In this study, 44% (66/150) of the patients with positive bacterial cultures had MDRO infections. This proportion was lower than that reported by Henig et al. (56.2% [364/648]) [13] and Yan et al. (56.8% [84/148]) [17] and similar to that reported by Liu et al. (41.3% [271/656]) [20]. The most common MDRO in this study was MRSA, which accounted for 56% (33/59) of the *S. aureus* strains that were isolated, similar to the reports by Pontes et al. (63%) [21] and Xie et al. (53.7%) [22]. Notably, the MDR rate of coagulase-negative *Staphylococcus* was 92%, which is similar to the rate (91%) reported by Liu et al. [6]. DFUs caused by MDROs, particularly MRSA, are associated with more severe infections, resulting in longer ulcer duration, longer hospital stays, and higher treatment costs than DFUs infected with the methicillin-susceptible *S. aureus* [23]. This finding suggests that early identification of individuals at risk of MDRO infection is crucial for reducing the overuse of broad-spectrum antibiotics. Systemic antibiotic therapy is typically required for moderate-to-severe DFIs. The initial selection of drugs is usually empirical and based on locally available clinical and epidemiological data [24].

The diversity of MDROs in inflamed wounds is associated with demographics, age, gender, length of hospitalization, and history of antibiotic therapy [25]. Therefore, identifying the risk of MDRO infection in patients with DFUs as early as possible is crucial [26]. Notably, many studies have explored the risk factors for the emergence of MDROs; however, few have reported the risk factors identified in studies involving routine blood and biochemical markers. This study showed that Hb and Alb levels were lower in the MDRO infection group than in the non-MDRO infection group, and the

NEU% and Cr, CRP, and FPG levels were higher in the MDRO infection group. Among patients with DFI and prolonged hyperglycemia, the MDRO infection group exhibited significantly lower Hb and Alb levels than the non-MDRO infection group. This suggests a possible association between poor nutritional status and immunocompromise, which in turn increases the risk of MDRO infections. Single variable logistic regression analysis indicated that Hb and Alb were protective factors in patients with MDRO-induced DFI. To the best of our knowledge, this is a novel finding. Previous studies have noted that anemia is prevalent in diabetic patients, especially those with DFUs [27,28], and that it is significantly associated with larger, deeper ulcers, more severe infections, higher risk of amputation, and higher mortality [29]. Anemia is a risk factor for impaired wound healing, and addressing anemia is essential to promote ulcer healing in patients with DFUs [30]. Recurrent ulcers and delayed healing are known to be important risk factors for the development of MDROs [31], and in conjunction with the finding of this study, it is reasonable to infer that correcting anemia in patients with DFIs can help reduce the incidence of MDRO. With regard to albumin, it is established that it enhances microcirculatory blood flow and reduces inflammation and oxidative damage [32]. Correction of albumin levels and nutrient supply is of great importance in facilitating DFU healing. Therefore, clinicians should focus on improving the nutritional status of these patients. If necessary, blood transfusions can be used to increase the Hb levels.

Bharathi et al. [33] indicated poor glycemic control in patients with MDRO infections of the diabetic foot, which was confirmed in this study. Hyperglycemia activates polyol channels, increases end-product glycosylation, and ultimately promotes the release of reactive oxygen species and nitric oxide, leading to oxidative stress and inflammation [34]. This study showed that high FPG level is an independent risk factor for MDRO infections in patients with DFUs. Therefore, patients should regularly monitor their FPG levels and control excessive levels in a timely manner to reduce the risk of such infections.

CRP has traditionally been used as a marker of infection and cardiovascular events. However, growing evidence suggests that it plays a crucial role in the inflammatory process of and host response to infection, including involvement in the complement pathway, apoptosis, phagocytosis, nitric oxide release, and production of cytokines, particularly interleukin-6 and tumor necrosis factor-α. Elevated CRP levels have been observed in patients with bacterial infections [35]. Our analysis showed that the CRP level is an independent risk factor for MDRO infection. The risk of MDRO infection increases with higher CRP levels. NEU% is also a common indicator of inflammation, and its level directly reflects the severity of inflammation. Al-Shammaree et al. [36] found that the NEU% in patients with DFI was 71.97%, which was significantly higher than that in patients with non-DFI (57.47%). In this study, NEU% was elevated in both the MDRO and non-MDRO infection groups, but was significantly higher in the MDRO infection group, which may be indicative of the greater severity of MDRO infections. Multivariable logistic regression analysis indicated that NEU% was an independent risk factor for MDRO infection. This finding suggests that clinicians should monitor the NEU% and take timely and effective measures to prevent and treat MDRO infections.

Diabetic nephropathy (DN) affects approximately 20 - 40% of patients with diabetes. In addition, approximately 39.3% of patients with DFUs have chronic kidney disease [37]. DN is characterized by increased urinary protein excretion and a decreased glomerular filtration rate (GFR). Patients with moderately reduced estimated GFR (eGFR) are at a higher risk of treatment failure than those with normal eGFR [38]. A study by Ardelean et al. [39] confirmed a strong association between impaired renal function and the progression of DFI. Akbari et al. [40] reported a decline in renal function after antibiotic treatment, and a higher prevalence of renal function decline was observed among patients receiving antibiotics who had higher rates of nephrotoxicity. In this study, we found that patients with MDRO infections had significantly higher Cr levels than those with non-MDRO infections. This finding suggests that renal function is severely impaired in patients with MDRO infections. We hypothesized that higher Cr levels may be due to DFU progression and the increased use of broad-spectrum antibiotics, which can impair renal function in patients.

This study has several limitations that must be acknowledged. Firstly, it lacks data on anaerobic bacteria in patients with diabetic foot ulcer infections due to the lack of facilities within the microbiology laboratory to isolate and culture anaerobic bacteria. Secondly, two alternative methods of specimen collection were employed in this study. While the surface of the infected area was thoroughly rinsed to avoid contamination with skin colonizing bacteria prior to sampling, it is indubitable that tissue biopsy represents the most standard method. Thirdly, although our hospital is a provincial center for the diagnosis and treatment of diabetic foot disease, with radiating capacity to serve patients from Jiangsu and Anhui provinces, this is still a monocentric study. Consequently, the results may not be generalizable to populations with different demographic characteristics.

## CONCLUSION

In conclusion, this study revealed that a high percentage of infections were caused by MDROs in patients with DFUs. It is recommended that microbiology laboratories regularly, typically on a quarterly basis, report on the distribution of local MDROs and their resistance profiles. This would assist clinicians in rationalizing the use of antibiotics in the early empirical phase of dosing,

thereby reducing and preventing such infections. The risk of MDRO infections can be clinically assessed by regularly monitoring NEU% and Hb, Alb, CRP, FPG, and Cr levels. To reduce the likelihood of MDRO infections in high-risk patients, nutritional status should be improved to enhance immunity, FPG levels should be effectively controlled, and hospital sterilization and strict aseptic procedures should be strengthened.

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**Ethical Approval Statement:**
The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Air Force Hospital of Eastern Theater Command (DKKY no. 2022011). Informed consent was obtained from all subjects involved in the study. Written informed consent was obtained from the patients to publish this paper.

**Data Availability Statement:**
The data used in this study are available from the corresponding author upon reasonable request.

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**Declaration of Interest:**
The authors declare that they have no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**References:**

1. Li Y, Teng D, Shi X, et al. Prevalence of diabetes recorded in mainland China using 2018 diagnostic criteria from the American Diabetes Association: national cross sectional study. BMJ 2020; 369:m997. (PMID: 32345662)

2. Armstrong DG, Tan T-W, Boulton AJM, Bus SA. Diabetic foot ulcers: A review. JAMA 2023;330(1):62-75. (PMID: 37395769)

3. Du F, Ma J, Gong H, et al. Microbial infection and antibiotic susceptibility of diabetic foot ulcer in China: Literature review. Front Endocrinol (Lausanne) 2022;13:881659. (PMID: 35663325)

4. Henig O, Pogue JM, Cha R, et al. Epidemiology of diabetic foot infection in the Metro-Detroit area with a focus on independent predictors for pathogens resistant to recommended empiric antimicrobial therapy. Open Forum Infect Dis 2018;5(11):ofy245. (PMID: 30402532)

5. Magiorakos A-P, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012;18(3):268-81. (PMID: 21793988)

6. Liu X, Ren Q, Zhai Y, Kong Y, Chen D, Chang B. Risk factors of multidrug-resistant organism infection in diabetic foot ulcer. Infect Drug Resist 2022;15:1627-35. (PMID: 35418765)

7. Ismail AA, Meheissen MA, Elaaty TAA, Abd-Allatif NE, Kassab HS. Microbial profile, antimicrobial resistance, and molecular characterization of diabetic foot infections in a university hospital. Germs 2021;11(1):39-51. (PMID: 33898340)

8. Xia W, He W, Luo T, Tang N. Risk factors for multidrug-resistant bacterial infections in patients with diabetic foot ulcers: a systematic review and meta-analysis. Ann Palliat Med 2021; 10(12):12618-30. (PMID: 35016446)

9. Dai J, Jiang C, Chen H, Chai Y. Assessment of the risk factors of multidrug-resistant organism infection in adults with type 1 or type 2 diabetes and diabetic foot ulcer. Can J Diabetes 2020; 44(4):342-9. (PMID: 32005564)

10. Huang Y, Cao Y, Zou M, et al. A comparison of tissue versus swab culturing of infected diabetic foot wound. Int J Endocrinol 2016;2016:8198714. (PMID: 27123004)

11. Datta P, Chander J, Gupta V, Mohi GK, Attri AK. Evaluation of various risk factors associated with multidrug-resistant organisms isolated from diabetic foot ulcer patients. J Lab Physicians 2019; 11(1):58-62. (PMID: 30983804)

12. Li X, Du Z, Tang Z, Wen Q, Cheng Q, Cui Y. Distribution and drug sensitivity of pathogenic bacteria in diabetic foot ulcer patients with necrotizing fasciitis at a diabetic foot center in China. BMC Infect Dis 2022;22(1):396. (PMID: 35459117)

13. Henig O, Pogue JM, Martin E, et al. The impact of multidrug-resistant organisms on outcomes in patients with diabetic foot infections. Open Forum Infect Dis 2020;7(5):ofaa161. (PMID: 32500092)

14. Saltoglu N, Surme S, Ezirmik E, et al.; KLİMİK Society, Diabetic Foot Study Group. The effects of antimicrobial resistance and the compatibility of initial antibiotic treatment on clinical outcomes in patients with diabetic foot infection. Int J Low Extrem Wounds 2023;22(2):283-90. (PMID: 33856261)

15. Matta-Gutiérrez G, García-Morales E, García-Álvarez Y, Álvaro-Afonso FJ, Molines-Barroso RJ, Lázaro-Martínez JL. The influence of multidrug-resistant bacteria on clinical outcomes of diabetic foot ulcers: A systematic review. J Clin Med 2021;10(9): 1948. (PMID: 34062775)

16. Chang M, Nguyen TT. Strategy for treatment of infected diabetic foot ulcers. Acc Chem Res 2021;54(5):1080-93. (PMID: 33596041)

17. Yan X, Song J-F, Zhang L, Li X. Analysis of risk factors for multidrug-resistant organisms in diabetic foot infection. BMC Endocr Disord 2022;22(1):46. (PMID: 35189877)

18. Saseedharan S, Sahu M, Chaddha R, et al. Epidemiology of diabetic foot infections in a reference tertiary hospital in India. Braz J Microbiol 2018;49(2):401-6. (PMID: 29157899)

19. Pitocco D, Spanu T, Di Leo M, et al. Diabetic foot infections: a comprehensive overview. Eur Rev Med Pharmacol Sci 2019;23(2 Suppl):26-37. (PMID: 30977868)

20. Liu W, Song L, Sun W, Fang W, Wang C. Distribution of microbes and antimicrobial susceptibility in patients with diabetic foot infections in South China. Front Endocrinol (Lausanne) 2023;14:1113622. (PMID: 36761201)

21. Pontes DG, Silva ITDCE, Fernandes JJ, et al. Microbiologic characteristics and antibiotic resistance rates of diabetic foot infections. Rev Col Bras Cir 2020;47:e20202471. (PMID: 32667581)

22. Xie X, Bao Y, Ni L, et al. Bacterial profile and antibiotic resistance in patients with diabetic foot ulcer in Guangzhou, Southern China: Focus on the differences among different Wagner's grades, IDSA/IWGDF grades, and ulcer types. Int J Endocrinol 2017; 2017:8694903. (PMID: 29075293)

23. Chen Y, Yang J, Wang Y, et al. Community-associated methicillin-resistant Staphylococcus aureus infection of diabetic foot ulcers in an eastern diabetic foot center in a tertiary hospital in China: a retrospective study. BMC Infect Dis 2023;23(1):652. (PMID: 37789270)

24. Pessoa e Costa T, Duarte B, João AL, et al. Multidrug-resistant bacteria in diabetic foot infections: Experience from a Portuguese tertiary centre. Int Wound J 2020;17(6):1835-9. (PMID: 32820614)

25. Hassan MA, Abd El-Aziz S, Elbadry HM, El-Aassar SA, Tamer TM. Prevalence, antimicrobial resistance profile, and characterization of multi-drug resistant bacteria from various infected wounds in North Egypt. Saudi J Biol Sci 2022;29(4):2978-88. (PMID: 35531185)

26. Silvia N S, Velrajan M. Deciphering diabetic foot wounds: A comprehensive review on classification, multidrug resistance, microbial insights, management & treatment strategies, and advanced diagnostic tools. Curr Diabetes Rev 2024. (PMID:38798205)

27. Olgun ME, Altuntaş SÇ, Sert M, Tetiker T. Anemia in patients with diabetic foot ulcer: effects on diabetic microvascular complications and related conditions. Endocr Metab Immune Disord Drug Targets 2019;19(7):985-90. (PMID: 30636618)

28. Jiang F, Liu Q, Wang Q. Association between hemoglobin levels and diabetic foot ulcer in patients with type 2 diabetes: a cross-sectional study. Wounds 2024;36(3):73-9. (PMID:38684121)

29. Kumar R, Singh SK, Agrawal NK, et al. The prevalence of anemia in hospitalized patients with diabetic foot ulcer (DFU) and the relationship between the severity of anemia and the severity of DFU. Cureus 2023;15(7):e41922. (PMID: 37583722)

30. Lee SH, Kim SH, Kim KB, Kim HS, Lee YK. Factors influencing wound healing in diabetic foot patients. Medicina (Kaunas) 2024;60(5):723. (PMID: 38792906)

31. Răducu L, Moraru OE, Gheoca-Mutu D-E, et al. Confronting a new challenge in plastic surgery: MDR infections in patients with chronic wounds. Life (Basel) 2024;14(4):444. (PMID: 38672715)

32. Cheng P, Dong Y, Hu Z, et al. Biomarker prediction of postoperative healing of diabetic foot ulcers: a retrospective observational study of serum albumin. J Wound Ostomy Continence Nurs 2021;48(4):339-44. (PMID: 34186553)

33. Bharathi SP, Sukumaran SK. A review on the current principles of antibiotic therapy for diabetic foot infection. Infect Disord Drug Targets 2021;21(5):e270421188440. (PMID: 33243133)

34. Yang T, Qi F, Guo F, et al. An update on chronic complications of diabetes mellitus: from molecular mechanisms to therapeutic strategies with a focus on metabolic memory. Mol Med 2024; 30(1):71. (PMID: 38797859)

35. Sun H, Ma Y, Heng H, Liu X, Liang J, Geng H. Microbiological distribution, antimicrobial susceptibility and risk factors of polymicrobial infections in diabetic foot. Clin Lab 2024;70(4). (PMID:38623675)

36. Al-Shammaree SAW, Abu-AlKaseem BA, Salman IN. Procalcitonin levels and other biochemical parameters in patients with or without diabetic foot complications. J Res Med Sci 2017;22:95. (PMID: 28900451)

37. Zhang L, Fu G, Deng Y, et al. Risk factors for foot ulcer recurrence in patients with comorbid diabetic foot osteomyelitis and diabetic nephropathy: A 3-year follow-up study. Int Wound J 2023;20(1):173-82. (PMID: 35673930)

38. He Y, Qian H, Xu L, et al. Association between estimated glomerular filtration rate and outcomes in patients with diabetic foot ulcers: a 3-year follow-up study. Eur J Endocrinol 2017;177(1): 41-50. (PMID: 28424173)

39. Ardelean A, Neamtu A-A, Balta D-F, et al. Lipid profile paradox: investigating improved lipid levels in diabetic mellitus patients with foot ulcer infections - a prospective descriptive study. Diagnostics (Basel) 2023;13(23):3531. (PMID: 38066772)

40. Akbari R, Javaniyan M, Fahimi A, Sadeghi M. Renal function in patients with diabetic foot infection; does antibiotherapy affect it? J Renal Inj Prev 2016;6(2):117-21. (PMID: 28497087)