



Cross-sectional Study



## Diabetic foot ulcer in Southern Jordan: A cross-sectional Study of Clinical and Microbiological Aspects

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### ABSTRACT

**Background:** Diabetes mellitus (DM) is a common metabolic disorder that significantly affects public health. Diabetic foot ulcer (DFU) is one of the serious complications of diabetes. DFU has a wide spectrum of bacterial isolates comprising Gram-positive, Gram-negative, aerobic bacteria and anaerobes. In the last two decades there has been an increase in the multidrug-resistant isolates (MDR).

**Materials and methods:** This cross-sectional prospective observational study was conducted in southern Jordan among patients with DFU. The included variables are sociodemographic and clinical information. Isolates from swab culture of ulcers and antimicrobial susceptibility pattern are also recorded.

**Results:** A total of 64 diabetic patients with DFU were included in this study. Most patients included in the study were males with male-to-female ratio of (2.2:1). The mean age was 54 years (SD ± 10.7). The mean duration of DM was 16.4 years (SD ± 7.5) and the mean HbA1c was 9.9% (SD ± 2.1). Neuropathy and anemia were noted in 72% and 44% of patients, respectively. The most frequent bacterial isolates were gram negative bacteria accounts for 29 isolates (45.3%). About 37.5% (24) of bacterial isolates showed MDR pattern. Previous antibiotic use in the last 30 days showed significant association with MDR bacteria (p-value <0.05). Previous history of amputations, presence of neuropathy, renal impairment, retinopathy, presence of anemia, limited joint mobility and presence of foot deformity were significantly associated with Wagner's grade ≥ three.

**Conclusion:** Many factors affect and increase the risk of having high grade diabetic foot ulcer. The most frequent bacterial isolates from diabetic foot ulcers were gram negative bacteria. High rates of MDR in this study reflect the loose implementation of regulations in Jordan regarding antibiotics dispensing.

### 1. Introduction

Diabetes mellitus (DM) is a common metabolic disorder that significantly affects public health [1]. Diabetic foot ulcer (DFU) is one of the serious complications of diabetes. DFU is a consequence of several pathological factors, such as neuropathy, peripheral vascular disease. Moreover, the impaired immune response and the decreased

microcirculation renders the ulcers more susceptible to infection [1,2]. DFU has high cost with disabling features due to its high morbidity and mortality [2].

The prevalence of DM among Jordanian increased dramatically in the last decade from 13% in 1994 to reach 23.7% in 2017 [3]. The estimated prevalence of DFU among Jordanian diabetic patients is 4.6–5.3% [4,5]. Due to diversity of DFU there are many classification

**Abbreviations:** DFU, Diabetic Foot Ulcer; MDR, multidrug-resistant isolates.

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systems to categorize this disease, one of the widely used clinical classifications of DFU is Wagner's classification system [6]. DFU has a wide spectrum of bacterial isolates comprising Gram-positive, Gram-negative, aerobic bacteria and anaerobes [6]. Treatment of diabetic foot infection requires the early surgical debridement, antimicrobial therapy, controlling blood sugar and improving of blood supply. Isolates from DFU have a wide susceptibility pattern, in the last two decades there has been an increase in multidrug-resistant isolates (MDR) [7]. We aimed to evaluate the clinical characteristics of DFU in southern Jordan and determine the microbiological aspects of DFU infection.

## 2. Materials and methods

### 2.1. Study design

This study was a cross-sectional prospective observational study that was conducted in southern Jordan among patients with DFU. Patients were evaluated at Alkarak governmental hospital, which is affiliated hospital to Mutah University, Alkarak, Jordan. Patients from Alkarak Italian hospital, Salam specialty hospital and Queen Rania hospital, Ma'an, Jordan were included in this study. Sample size was calculated using OpenEpi, Version 3.01. The calculated sample size according to the population in south of Jordan considering the local DM and DFU prevalence at 90% confidence level was 52 patients. The study is reported in line with the STROCSS criteria [8]. This study was registered in the research registry with unique identifying number (UIN): researchregistry 7620(<https://www.researchregistry.com/browse-the-registry#home/registrationdetails/6200578e7dd504001f7ec1e8/>).

### 2.2. Data collection

The data include sociodemographic, body mass index (BMI), type of diabetes mellitus, smoking history, clinical grade of DFU according to Wagner's classification system, bacterial isolates of DFU and their antibiotic susceptibility patterns. Wagner's classification of DFU is grade 0: no ulcers; grade 1: full-thickness skin ulcer; grade 2: ulcer penetrating to muscle, tendon or joint capsule; grade 3: deep ulcer reaching bone or joint with the evidence of deep-seated abscess or osteomyelitis; grade 4: limited gangrene in the forefoot; and grade 5: gangrene extending proximal to metatarsal head or whole foot gangrene [6]. Figs. 1 and 2 show different Wagner's grades of DFU among the study participants.

The medical records were reviewed for the presence of retinopathy, ischemic heart diseases, hyperlipidemia, use of antiplatelet, anticoagulant and lipid lowering agents. Moreover, the use of antibiotics in the last 30 days were documented. Absence of sensation at either foot using Semmes-Weinstein Monofilament (SWM) was considered as presence of neuropathy [9]. Renal function was assessed for each patient based on creatinine clearance with normal value more than 80 ml/min [10]. Assessment of skin changes as fissures, calluses, edema, tinea pedis, blisters and ingrown toenails were documented. Limited joint mobility, presence of foot deformity, previous amputations and poor footwear condition were examined for each patient. Joint mobility was evaluated by the range of motion at the ankle, subtalar joint, metatarsal joints, and interphalangeal joints to determine whether there is any pain or restricted motion [11]. Foot deformities include the presence of Charcot joint or hallux valgus or hammer/claw toes [12]. The results of hemoglobin (Hb), white blood cells (WBC's), c-reactive protein (CRP) and glycosylated hemoglobin (HbA1c) were measured. According to WHO classifications, for males, non-anemia is Hb more than or equal 13 g/dl, for nonpregnant female, non-anemia is Hb more than or equal 12 g/dl [13]. WBC's more than  $11.0 \times 10^9/L$  was considered leukocytosis [14]. CRP was reported qualitatively as positive or negative. HbA1c less than or equal 7% was considered controlled diabetes [15]. Patients with ankle brachial index less than 0.9 i.e., peripheral arterial disease were excluded from the study [16].

### 2.3. Swab collection and bacterial isolation

After thorough wound cleansing by normal saline, under aseptic technique swab was taken from the base of the wound. Specimens were immediately closed and transferred to the laboratory. Then, the specimens were inoculated on different culture media including MacConkey agar (MA; Merck, Germany), 5% (v/v) sheep blood agar (SBA) (Oxoid, USA), and sabouraud dextrose agar (Difco, BHIB) for fungi and then incubated at 37 °C up to 48 hr. If no microbial growth was detected at the end of 48 hours, the culture report was released as 'no growth'. The isolated colonies were identified by standard microbiological methods and the phenotypic and biochemical reactions using the specific cards of the automated VITEK 2 system (Bio-Merieux, France) as recommended by the Clinical Laboratory Standards Institute (CLSI) [17].



Fig. 1. Wagner's grade 1 and 2 among patients with diabetic foot ulcer participated in the study.



Fig. 2. Wagner's grade 3 and 4 among patients with diabetic foot ulcer participated in the study.

#### 2.4. Susceptibility testing

The antimicrobial susceptibility testing was done using standard disk diffusion method based on the Clinical and Laboratory Standards Institute (CLSI) guidelines [17]. Briefly, the turbidity of the bacterial cultures was standardized compared to the 0.5 McFarland standards in a sterile liquid PBS using a spectrophotometer. The antibiotic discs were applied on the surface of Mueller Hinton agar (MHA) plates after their inoculation by the isolated organisms. Different types of the antimicrobial discs and concentrations were used which includes ampicillin (Am: 30  $\mu$ g), gentamicin (Gm: 10  $\mu$ g), clindamycin (Cd: 2  $\mu$ g), streptomycin (S: 300  $\mu$ g), ceftriaxone (Cax: 30  $\mu$ g), amoxicillin/clavulanate (Amc: 30  $\mu$ g), amikacin (Ak: 30  $\mu$ g), aztreonam (Atm: 30  $\mu$ g), cefotaxime (Ct: 30ug), meropenem (Mem: 10ug), piperacillin/tazobactam (Pip/Tzp: 110  $\mu$ g), rifampicin (Rd: 30  $\mu$ g), trimethoprim-sulfamethoxazole (Tmp/Smz: 1.25/23.75), cefuroxime (Cxm: 30  $\mu$ g), cefoxitin (Fox: 30  $\mu$ g), ceftizoxime (Cz: 30  $\mu$ g), nitrofurantoin (Ft: 100  $\mu$ g), ceftazidime (Caz: 30  $\mu$ g), imipenem (Ipm: 10  $\mu$ g), erythromycin (E: 10  $\mu$ g), vancomycin (Va: 30  $\mu$ g), norfloxacin (Nor: 10  $\mu$ g), ciprofloxacin (Cp: 10  $\mu$ g), ofloxacin (Ofi: 10  $\mu$ g), levofloxacin (Le: 10  $\mu$ g), ceftazidime (Cz: 30  $\mu$ g), chloramphenicol (C: 30  $\mu$ g), doxycycline (Dxt: 30  $\mu$ g), oxacillin (Ox: 1  $\mu$ g), amoxicillin (Amr: 30  $\mu$ g), piperacillin (Pip: 100  $\mu$ g), tetracycline (Tet: 30  $\mu$ g), and metronidazole (Me: 5  $\mu$ g) (Becton Dickinson Microbiology Systems, BBL, MD, USA). The zone of inhibition was examined after 24–48 h incubation. To ensure the accurate performance of the assays, different quality control strains were used. Isolates resistance to at least one antibiotic in two or more major antibiotics groups considered as MDR in addition to Methicillin Resistant *Staphylococcus aureus* (MRSA) and Extended Spectrum Beta-Lactamase (ESBL) producing isolates [18].

#### 2.5. Data analysis

Data were analyzed using the statistical package for the social sciences version 23 (SPSS Inc., Chicago, IL) statistical software. Quantitative variables were expressed as mean  $\pm$  standard deviation. Chi-square test was used for testing the association between MDR and Wagner's grade with other variables. A p-value < 0.05 was considered statistically significant.

#### 2.6. Ethical consideration

The Institutional Review Board (IRB) at Mutah University has approved the research's protocol and granted its ethical approval; reference number: 19102021. In addition, written consent was obtained from all participants.

### 3. Results

The participants were 74 patients with DFU. Ten patients were excluded either due to incomplete data or ankle brachial index less than 0.9%. A total of 64 diabetic patients with DFU were included in this study between April 2021 and January 2022. Most patients included in the study were males 44 (68%) with male-to-female ratio of 2.2:1. The mean age was 54 years (SD  $\pm$  10.7) and ranged from 34 to 78 years. Table 1 shows the sociodemographic and clinical characteristics of patients with DFU. The mean Body Mass Index (BMI) was 28 kg/m<sup>2</sup> (SD  $\pm$  4.8) and about two-third of patients had BMI less than 30 kg/m<sup>2</sup>. Forty-seven patients (73%) had type 2 DM and seventeen patients (27%) had type 1 DM. The mean duration of DM was 16.4 years (SD  $\pm$  7.5) and the mean HbA1c was 9.9% (SD  $\pm$  2.1). Neuropathy was observed in 72% of patients. Anemia was noted in about 44% of patients. About 55% of participants declared a history of antibiotics use in the last 30 days. The most frequent bacterial isolates were *Escherichia coli* (17%), other isolates are shown in Fig. 3. Gram negative bacteria accounted for 29 isolates (45.3%) whereas gram positive isolates accounted for only 17.2%. About 37.5% (24) of bacterial isolates showed MDR for at least one antibiotic in two or more major antibiotics groups in addition to MRSA and ESBL producing organism. MRSA accounted for about 5% only and ESBL producing organism was noted in 3%. Among all selected clinical variables, presence of MDR bacteria showed statistically significant association (p value less than 0.05) with previous antibiotic use in the last 30 days. About 42% (27 cases) of DFU classified as Wagner's grade three or more. Previous history of amputations, presence of neuropathy, renal impairment, retinopathy, presence of anemia, limited joint mobility and presence of foot deformity were associated significantly with Wagner's grade  $\geq$  three (Table 2).

### 4. Discussion

Our study presents the first study in southern part of Jordan about

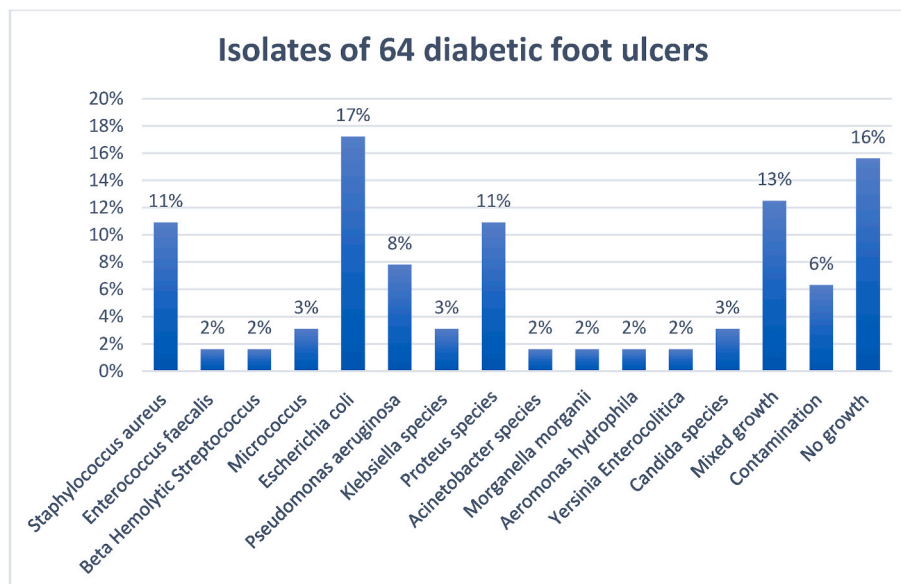


**Table 1**  
The sociodemographic and clinical characteristics of 64 patients with diabetic foot ulcer.

Characteristics	Numbers	Percentage %
<b>Gender</b>		
Male	44	68.8%
Female	20	31.2%
<b>Age (Mean ± SD)</b>	(54.2 ± 10.7)	
30–49 years	22	34.4%
50–65 years	31	48.4%
More than 65 years	11	17.2%
<b>BMI (Mean ± SD)</b>	(28 ± 4.8)	
Less than 30	46	71.9%
More 30	18	28.1%
<b>DM duration (Mean ± SD)</b>	(16.4 ± 7.5)	
Less than 20 years	50	78.1%
More than 20 years	14	21.9%
<b>DM</b>		
Type 1	17	26.6%
Type 2	47	73.4%
<b>Associated comorbidities</b>		
Hypertension	23	35.9%
Ischemic heart disease	8	12.5%
Hyperlipidemia	31	48.4%
<b>DM complications</b>		
Neuropathy	46	71.9%
Retinopathy	33	51.6%
Renal impairment	5	7.8%
<b>Smoking</b>		
Current smoker	25	39.1%
Non-smoker	31	48.4%
Ex-smoker <sup>1</sup>	8	12.5%
<b>HbA1c (Mean ± SD)</b>	(9.9 ± 2.1)	
Less than 6.5%	3	4.7%
6.6–8%	12	18.8%
8.1–10%	20	31.2%
More than 10.1%	29	45.3%

SD: standard deviation, BMI: body mass index kg/m<sup>2</sup>, DM: diabetes mellitus, HbA1c: glycosylated hemoglobin. 1: Ex-smoking<sup>1</sup> refers to someone who has smoked more than 100 cigarettes in their lifetime but has not smoked in the last 28 days.

DFU with its clinical and microbiological data. Most patients included in this study with DFU were males which comes in concordance with national and global studies [5,18]. This male predominance may be attributed to fact that males have higher physical activity than females [19]. In contrary to Jordanian reports, Bakri et al. stated that obesity present in 50% of diabetic patients, this study showed that most DFU patients have BMI less than 30 kg/m<sup>2</sup>. This finding comes in consistence with recent studies [18,19]. In contrary to a review study by Ambrosch et al. where *Staphylococcus aureus* and other gram-positive bacteria were the most common isolates, *Escherichia coli* and other gram-negative isolates were the most common isolated organism in our study and in another study from Iran [20,21]. The higher rate of gram-negative isolates as *Escherichia coli* from DFU in eastern countries compared to western one, might be attributed to hands contamination by fecal flora during sanitary habits and abluition that may contaminate the DFU [22]. The rates of MDR bacteria in DFU patients vary from one study to another according to the national antibiotics prescription policy [18]. Our study showed that 37.5% of isolates from DFU were MDR which is consistent with other studies from China, France and Oman [7,17,22]. High rates of MDR in this study reflect the loose implementation of regulations in Jordan regarding antibiotics dispensing. Inappropriate previous use of antibiotics was the main determinant of MDR isolates in consistence with Wu et al. [23]. In concordance with Jordanian study by Bakri et al. the peripheral neuropathy increases the risk of DFU, our study showed that 72% of patients with DFU have peripheral neuropathy [5]. Moreover, our study showed that peripheral neuropathy associated with high Wagner’s grade (three or more). We also showed an association between renal impairment and high Wagner’s grade (three or more) in consistence with a study from United Kingdom [24]. Although, this association was not observed in other studies [5,18]. Previous history of amputation and retinopathy increase the risk of having high Wagner’s grade (three or more) as observed in our study and other studies [24–26]. Current smoking among DFU patients was not a risk factor for high Wagner’s grade (three or more) in this study as observed by Merza et al. but not in other studies [19,26,27]. This may be attributed to the exclusion of patients with peripheral vascular disease in which smoking is the main contributor. Most of our patients have uncontrolled diabetes with average HbA1c of 9.9% but it fails to show significant association with high Wagner’s grade (three or more) as Shatnawi et al. found [25]. Anemia is a common finding in diabetic patients and especially those with DFU ranging from 50% to 90% [27,



**Fig. 3.** Isolates among 64 patients with diabetic foot ulcer with percentage of each one.

**Table 2**  
Association between variables and high Wagner's grade among 64 diabetic foot ulcer patients.

variable	Wagner's grade 1 or 2 Number (percentage)	Wagner's grade 3 or more Number(percentage)	p-value
Gender			0.1
Male	22(34.4%)	22(34.4%)	
Female	15(23.4%)	5(7.8%)	
Age			0.2
less 50 years	19(29.7%)	9(14.1%)	
more than 50 years	18(28.1%)	18(28.1%)	
Obesity according to BMI			0.17
Non obese	24(37.5%)	22(34.4%)	
obese	13(20.3%)	5(7.8%)	
DM duration			0.55
Less than 20 years	30(46.9%)	20(31.3%)	
More than 20 years	7(10.9%)	7(10.9%)	
Retinopathy			0.047
No	22(34.4%)	9(14.1%)	
YES	15(23.4%)	18(28.1%)	
Nephropathy			0.011
NO	37(57.8%)	22(34.4%)	
YES	0(0.0%)	5(7.8%)	
Neuropathy			0.002
NO	16(25.0%)	2(3.1%)	
YES	21(32.8%)	25(39.1%)	
Smoking			0.3
Current smoker	12(18.8%)	13(20.3%)	
Non- or Ex-smoker <sup>a</sup>	25(39.1%)	14(21.9%)	
DM controlled according HbA1c <sup>2</sup>			0.99
Controlled	4(6.3%)	3(4.7%)	
Uncontrolled	33(51.6%)	24(37.5%)	
Hb level <sup>3</sup>			0.022
Normal	25(39.1%)	10(15.6%)	
Anemia	12(18.8%)	17(26.6%)	
Amputation			0.044
NO	31(48.4%)	16(25%)	
YES	6(9.4%)	11(17.2%)	
Limited Joint Mobility			0.003
NO	33(51.6%)	15(23.4%)	
YES	4(6.3%)	12(18.8%)	
Foot deformity			0.003
NO	30(46.9%)	12(18.8%)	
YES	7(10.9%)	15(23.4%)	

BMI: body mass index kg/m<sup>2</sup>, DM: diabetes mellitus, HbA1c: glycosylated hemoglobin.

<sup>a</sup> Ex-smoking refers to someone who has smoked more than 100 cigarettes in their lifetime but has not smoked in the last 28 days. 2: HbA1c less or equal 7% considered controlled DM, uncontrolled when HbA1c more than 7%. 3: For males, non-anemia is Hb more than or equal 13 g/dl, for nonpregnant female, non-anemia is Hb more than or equal 12 g/dl.

[28]. Our study reported 44% prevalence of anemia among DFU that was associated with high Wagner's grade (three or more). Anemia leads to poor wound healing and higher amputation rate [28]. Previous history of amputation and foot deformities increase the risk of DFU [26,29]. Our findings suggest that history of amputation and foot deformities were associated with high Wagner's grade (three or more). Limited joint mobility increases the risk to have DFU [30]. In this study, limited joint mobility increased the risk to have high Wagner's grade (three or more).

#### 4.1. Limitations

Our study suffers from several limitations. First, the small sample size would limit the generalizability of the results to a larger population.

While a larger sample size will add more value to our results, our targeted population covered a certain geographical under-studied region in Jordan. Then, the end-result of DFU such as completely healed, chronic wound, amputation or mortality were not addressed in this study. However, the strength of our study lies in its prospective nature of data collection and strict follow-up. In fact, this study is first of its kind in southern Jordan that combined clinical and microbiological aspects of DFU.

## 5. Conclusion

Many factors affect and increase the risk of having high grade diabetic foot ulcer. Neuropathy, retinopathy, anemia, renal impairment and previous history of amputation were associated with high Wagner's grade (three or more). This highlights the needs for preventive strategy in high-risk patients. The most frequent bacterial isolates from diabetic foot ulcers were gram negative bacteria. High rates of MDR in this study reflect the loose implementation of regulations in Jordan regarding antibiotics dispensing.

## Ethical approval

The Institutional Review Board (IRB) at Mutah University has approved the research's protocol and granted its ethical approval; reference number: 19102021.

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## Author contribution

Aborajooch A. Emad: study concept or design, data collection, data analysis or interpretation, writing the paper, Talal M. Alqaisi: study concept or design, data collection, data analysis or interpretation, writing the paper, Muhammad Yassin: study concept or design, data analysis or interpretation, writing the paper, Eyad Alqpelat: data analysis or interpretation, writing the paper, Alaa Abofaraj: study concept or design, data analysis or interpretation, writing the paper, Tariq Alrawajih: data analysis or interpretation, writing the paper, Hamed Alzoubi: study concept or design, data analysis or interpretation, writing the paper, Mohammad Abu lubad: study concept or design, data analysis or interpretation, writing the paper.

## Trail registry number

1. Name of the registry: Diabetic Foot Ulcer in South of Jordan: A Cross-sectional Study of Clinical and Microbiological Aspects
2. Unique Identifying number or registration ID: researchregistry7620
3. Hyperlink to your specific registration (must be publicly accessible and will be checked): <https://www.researchregistry.com/browse-the-registry/#home/registrationdetails/6200578e7dd504001f7ec1e8/>

## Guarantor

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.amsu.2022.103552>.

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