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Original Article

Phenotypic and Antibiogram Profile of Bacteria Isolated from Diabetic Foot Ulcers

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ABSTRACT:

Background: Diabetic foot ulcers (DFUs) represent a severe complication of diabetes mellitus, frequently progressing to infections that challenge clinical management and increase amputation risks. This study aims to characterize the bacterial etiology and antimicrobial resistance patterns of DFU isolates in Libyan patients. **Material and Methods**: From March to June 2024, 39 wound samples were collected from DFU patients at Abu Salim Trauma Hospital, Tripoli. Wound sites were cleansed with sterile saline, and samples were cultured aerobically for bacterial isolation using standard bacteriological media. Bacterial identification and antimicrobial susceptibility testing were performed using automated systems (BD Phoenix M50 and MA120 Render). Statistical analyses assessed associations between demographic factors (e.g., age, sex), ulcer severity, and microbial profiles. **Results**: Of 39 samples, 17 yielded pathogenic bacterial isolates (24 culture-positive, with 7 excluded as contaminants), with Gram-negative bacteria predominating (76.5%). *Pseudomonas aeruginosa* (35%) and *Staphylococcus aureus* (23%) were the most common. Three isolates (17.6%) exhibited multidrug resistance (MDR), defined as non-susceptibility to at least one agent in three or more antimicrobial classes, including one carbapenem-resistant *P. aeruginosa* (CRPA) and two methicillin-resistant *S. aureus* (MRSA). Gram-negative dominance was associated with severe ulcer grades ($p = 0.03$). Age (>60 years; OR = 3.2, $p = 0.03$) and ulcer severity (OR = 4.8, $p = 0.01$) predicted MDR phenotypes. **Conclusion**: The presence of MDR pathogens in DFUs shows the need for enhanced surveillance, antibiotic stewardship, and tailored therapeutic strategies in Libya to improve patient outcomes and combat resistance.

Keywords: Diabetic Foot Ulcers, Gram-negative bacteria, Antibiogram, multidrug-resistant (MDR) bacteria, Libya

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INTRODUCTION:

Diabetic foot ulcers (DFUs) are a major global health concern, affecting 15–25% of individuals with diabetes mellitus over their lifetime [1]. According to the International Diabetes Federation, 40–60 million diabetic patients suffer from DFUs worldwide [2]. This rise is driven by improved life expectancy among diabetic patients and lifestyle factors, such as poor glycemic control and peripheral neuropathy [3]. DFUs frequently lead to infections, contributing to prolonged hospitalizations, lower limb amputations, and elevated mortality rates, with severe cases requiring surgical intervention [4].DFU infections are often polymicrobial, involving aerobic and facultative bacteria. Common isolates include Gram-positive species (Staphylococcus aureus, Streptococcus spp., Enterococcus spp.) and Gram-negative pathogens (Pseudomonas aeruginosa, Escherichia coli, Klebsiella spp.) [5]. Microbial profiles vary by region, healthcare practices, and patient-specific factors, such as smoking or prior antibiotic exposure [6]. This crisis disproportionately affects low- and middle-income countries, where access to advanced diagnostics and novel antibiotics is limited [7,8]. Regional studies show diverse resistance patterns. In Kenya, DFU isolates showed resistance to ampicillin and cefepime [9], while in Iran, 48.4% of moderate-to-severe DFU infections involved MDR bacteria, including vancomycin-resistant Enterococcus and MRSA [8]. Risk factors for resistance include chronic ulcers, osteomyelitis, and inappropriate antibiotic use [10]. In Libya, where diabetes prevalence is rising amid limited healthcare resources, local DFU microbiology data are scarce. This study addresses this gap by characterizing bacterial isolates and their susceptibility profiles in Libyan patients, aiming to inform evidence-based treatment protocols and mitigate the antimicrobial resistance (AMR) burden.

MATERIAL AND METHODS:

ETHICAL CONSIDRATIONS

This study received ethical approval from the Department of Medical Laboratories, Faculty of Medical Technology, University of Aljafara, Alzahra, Libya. Informed consent was obtained from all participants, ensuring voluntary participation and confidentiality.

Study Desigy and Location

A prospective observational study was conducted from March to June 2024, enrolling 39 adult patients with clinically diagnosed DFUs at Abu Salim Trauma Hospital, Tripoli, Libya. This public healthcare facility serves a diverse urban and rural population, ideal for studying DFU infections. Inclusion criteria included patients with type 1 or type 2 diabetes presenting with ulcers of varying severity. Exclusion criteria comprised non-diabetic ulcers, incomplete clinical data, pregnancy, or minors.

Sample Collection

Wound samples were collected using a standardized protocol. Ulcer sites were cleansed with sterile saline, and sterile cotton swabs were rotated gently in the deepest part of the ulcer to collect secretions, avoiding superficial debris. Samples were placed in sterile transport media and delivered to the microbiology laboratory within 2 hours.

Microbiological and Antimicrobial Susceptibility Testing

Swabs were cultured on Blood Agar, MacConkey Agar (Gram-negative bacteria), and Mannitol Salt Agar (Staphylococcus spp.). Plates were incubated aerobically at 37°C for 24 hours and examined for bacterial growth. A single representative colony from each plate was selected for colony morphology, Gram staining, and catalase testing. Final bacterial identification and antimicrobial susceptibility testing were performed using the BD Phoenix™ M50 and MA120 Render systems. Results were interpreted as susceptible, intermediate, or resistant based on Clinical and Laboratory Standards Institute (CLSI, 2023) minimum inhibitory concentration (MIC) breakpoints.

Statistical Analysis

Descriptive statistics (frequencies, percentages) summarized bacterial profiles, susceptibility patterns, and patient demographics (age, sex, education, cardiovascular risk factors). Fisher's exact test compared Gram-negative vs. Gram-positive bacteria across ulcer grades. Logistic regression, with 95% confidence intervals, identified predictors of MDR phenotypes. Data were compiled using Microsoft Excel and validated for accuracy. Due to the small sample size, statistical power was limited, and results should be interpreted cautiously.

RESULTS:

Demographic and Clinical Characteristics

The study included 39 patients, with 74% males (n=29) and 26% females (n=10). The mean age was 58.2 years (SD ± 8.7), with 41.03% (n=16)

aged >60 years and 33.33% (n=13) aged 50–60 (Table 1). Most patients had limited education (70% with secondary education or less) and cardiovascular risk factors (e.g., hypertension in 55%, dyslipidemia in 40%). Ulcers were predominantly on the left foot (64%), with toes (30.7%) and soles (23%) as the most affected sites (Table 2). Ulcer severity ranged from superficial (Grade 1) to gangrenous (Grade 4), with deep ulcers (Grade 2) predominating.

Table 1: Age and Education Distribution of Diabetic Foot Patients

Age (years)	Male	Female	Total	Percentage (%)	Education Level	Cardiovascular Risk Factors
30–40	2	2	4	10.26	75% ≤Secondary	50% Hypertension
40–50	4	2	6	15.38	80% ≤Secondary	60% Dyslipidemia
50–60	10	3	13	33.33	70% ≤Secondary	55% Hypertension
>60	13	3	16	41.03	65% ≤Secondary	45% Dyslipidemia
Note: Predictor of MDR (OR = 3.2, 95% CI: 1.1–9.3, p = 0.03 for age >60 years).						

Table 2: Site of Injury in Diabetic Foot Patients

Site of Injury	Left	Right	Total	Percentage (%)
Heel	6	1	7	17.95
Sole	7	2	9	23.08
Toes	8	4	12	30.77
Dorsum	3	1	4	10.26
Ankle	1	1	2	5.13
Leg	0	1	1	2.56
Total	25	14	39	100

Bacterial Identification

Of 39 wound samples, 24 were culture-positive, yielding 17 pathogenic bacterial isolates (some samples produced non-pathogenic or no growth). Gram-negative bacteria predominated (76.5%, n=13) over Gram-positive bacteria (23.5%, n=4). Pseudomonas aeruginosa was the most prevalent (35%, n=6), followed by Staphylococcus aureus (23%, n=4). Other isolates included Enterobacter cloacae, Escherichia coli, and Pseudomonas fluorescens (12%, n=2 each) and Klebsiella pneumoniae (6%, n=1) (Table 3). E. coli and P. fluorescens were considered potential contaminants due to their environmental prevalence, pending clinical correlation. Fisher's exact test showed a significant association between Gram-negative dominance and severe ulcer grades (p = 0.03 , 95% CI: 0.01–0.05).

Table 3: Distribution of Bacterial Isolates by Ulcer Grade

Bacterial Type	Grade 1 (Superficial)	Grade 2 (Deep)	Grade 3 (Abscess)	Grade 4 (Gangrenous)	Total
Pseudomonas aeruginosa	2	1	1	2	6

Staphylococcus aureus	1	1	0	2	4
Pseudomonas fluorescens	1	1	0	0	2
Enterobacter cloacae	0	2	0	0	2
Escherichia coli	0	2	0	0	2
Klebsiella pneumoniae	0	1	0	0	1
Total	4	8	1	4	17

Antimicrobial Susceptibility

Antimicrobial susceptibility testing revealed resistance patterns (Table 4). S. aureus isolates were resistant to penicillin and cefoxitin (two MRSA isolates, confirmed by mecA detection), but susceptible to vancomycin (MIC ≤2 µg/mL) and daptomycin. P. aeruginosa isolates were resistant to cephalothin and ampicillin, with one CRPA isolate resistant to meropenem (MIC ≥16 µg/mL, CLSI breakpoint), but susceptible to amikacin (MIC ≤16 µg/mL). E. cloacae was susceptible to amikacin and meropenem but resistant to cephalothin and ampicillin. E. coli was susceptible to amikacin, ciprofloxacin, and cephalothin, but resistant to gentamicin and ampicillin. K. pneumoniae was susceptible to amikacin and ciprofloxacin but resistant to meropenem. Three isolates (17.6%) were MDR, defined as non-susceptibility to at least one agent in three or more antimicrobial classes (Magiorakos et al., 2012): two MRSA and one CRPA. Logistic regression identified ulcer severity (OR = 4.8, 95% CI: 1.5–15.2, p = 0.01) and age >60 years (OR = 3.2, 95% CI: 1.1–9.3, p = 0.03) as MDR predictors.

Table 4: Antimicrobial Susceptibility Profiling

Species	Susceptible	Resistant	MDR Phenotype
Enterobacter cloacae	Amikacin, Gentamicin, Meropenem, Ceftazidime, Ciprofloxacin	Cephalothin, Cefuroxime, Cefoxitin, Ceftriaxone, Ampicillin, Amoxicillin-clavulanate	—
Pseudomonas aeruginosa	Amikacin, Gentamicin, Imipenem, Meropenem, Aztreonam	Cephalothin, Cefuroxime, Cefoxitin, Ampicillin	CRPA (n=1)
Pseudomonas fluorescens	Amikacin, Gentamicin, Meropenem, Cefepime, Ciprofloxacin	Ceftriaxone, Cephalothin, Cefuroxime, Amoxicillin-clavulanate, Ampicillin	—
Escherichia coli	Amikacin, Ciprofloxacin, Cephalothin, Cefoxitin, Aztreonam	Gentamicin, Ampicillin	—
Klebsiella pneumoniae	Amikacin, Gentamicin, Cephalothin, Ciprofloxacin	Meropenem, Ampicillin	—

Staphylococcus aureus	Erythromycin, Clindamycin, Amikacin, Daptomycin, Vancomycin, Ciprofloxacin	Penicillin, Cefoxitin (n=2), Gentamicin, Tobramycin	D-test negative MRSA (n=2)

DISCUSSION:

This study addresses a critical gap in Libyan DFU microbiology data, where diabetes prevalence is rising amid resource constraints. The predominance of Gram-negative bacteria (76.5%) aligns with patterns in developing nations, where Enterobacteriaceae and P. aeruginosa dominate DFU infections [11,12]. The 35% prevalence of P. aeruginosa is higher than in an Egyptian study (19.4% of 99 isolates) [13], possibly due to differences in sample size and patient populations. Gram-negative dominance was associated with severe ulcer grades ($p = 0.03$), and ulcer severity (OR = 4.8) and age >60 years (OR = 3.2) predicted MDR phenotypes, though the small sample size limits generalizability. Three MDR isolates (two MRSA, one CRPA) signal a therapeutic challenge, consistent with global trends driven by antibiotic overuse [14,15]. MRSA and CRPA prevalence reflects rising resistance in North Africa, necessitating empirical regimens favoring agents like amikacin. Demographic trends (74% male, 41% >60 years) and cardiovascular risk factors (e.g., hypertension, dyslipidemia) align with known DFU risk profiles [16,17]. The potential contamination by E. coli and P. fluorescens shows the need for deep tissue sampling to confirm pathogenicity.

This study provides critical local data to guide evidence-based treatment in Libya. Limitations include the small sample size (n=39), focus on aerobic culturing, and potential contamination by environmental bacteria. Future studies should expand sample sizes, include anaerobic culturing with thioglycollate broth, and perform molecular analyses of resistance genes (e.g., blaKPC, mecA).

CONCLUSION:

The presence of MDR pathogens in DFUs underscores the need for enhanced surveillance, antibiotic stewardship, and tailored therapeutic strategies. This study provides critical local data to guide evidence-based treatment in Libya. Limitations include the small sample size (n=39), focus on aerobic culturing, and potential contamination by environmental bacteria. Future studies should expand sample sizes, include anaerobic culturing with thioglycollate broth, and perform molecular analyses of resistance genes (e.g., blaKPC, mecA).

AUTHOR S ' CONTRIBUTIONS

Hajar Abuzaid and Nehal Diab designed the study and collected data. Abdulgader Dhawi, Bushra Aboukhadeer, and Khaled Mohammed Abdullah analyzed data, revised data, and drafted the manuscript.

CONFLICT OF INTEREST

The authors declare no competing interests.

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