

Microbial Identification And Antibiotic Resistance Patterns In Diabetic Foot Ulcers From Patients In Erbil, Kurdistan Region, Iraq

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Abstract

Diabetic foot ulcer (DFU) is a complication of diabetes and is associated with microbial infections. Early identification of causative microorganisms and their resistance patterns is critical for effective management. This study aimed to identify bacterial profiles in DFU patients and sought to characterize the isolated bacteria. A cross-sectional study was involving 110 DFU patients. Wound specimens were aseptically collected and cultured. Microbial identification and antibacterial susceptibility were performed. Extended-spectrum β -lactamase (ESBL)-producing strains and Methicillin-resistant *Staphylococcus aureus* (MRSA) were identified. Results showed the majority of DFU patients were males aged 51-60 years, with most residing in urban areas. Bacterial infections were predominant, with Gram-negative bacteria accounting for 66.67% of isolates. The most common pathogens were *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, while *Staphylococcus aureus* was the most frequent Gram-positive isolate. Biochemical tests supported differentiation of isolates. Antibiotic resistance was common, particularly among Gram-negative isolates, with *Klebsiella pneumoniae* and *Acinetobacter baumannii* showing multidrug resistance. Among Gram-positive bacteria, all isolates were resistant to erythromycin but remained sensitive to vancomycin, teicoplanin, and linezolid. ESBL production was highest in *Klebsiella pneumoniae*, and all *Staphylococcus aureus* and *Staphylococcus haemolyticus* isolates showed ceftioxin resistance. The study highlights a high prevalence of multidrug-resistant bacterial infections in DFUs, particularly among Gram-negative isolates. The frequent detection of ESBL-producing strains and MRSA underscores the urgent need for routine microbial identification and antibiotic susceptibility testing to guide effective treatment. These findings support the importance of targeted antibacterial treatment and continuous surveillance to reduce complications and improve clinical outcomes in DFU patients.

Keywords: DFU, biochemical tests, Antibiotic resistance, Gram-negative bacteria, Gram-positive bacteria, ESBL, MRSA.

1. Introduction

Diabetic foot ulcer (DFU) is a severe chronic diabetic complication, which affected 15–25% of diabetic patients in their lifetime [1]. Approximately 18.6 million people worldwide are affected by a DFU each year [2]. It is associated with significant morbidity and mortality and can subsequently lead to hospitalization and lower limb amputation if not recognized and treated in a timely manner [3]. DFUs are open sores or wounds that typically occur on the lower extremities of individuals with diabetes [4] and primarily resulting from a combination of many risk factors [5, 6]. Therefore, the development of DFUs is often multifactorial, involving trauma, poor circulation, and impaired immune response, which can lead to severe bacterial colonization and infection [7]. One of the most challenging aspects of managing DFUs is the high rate of infection, primarily due to microbial colonization.

Microorganisms would colonize and proliferate in the ulcer, including foot ulcer of diabetic patients, accentuating tissue damage and resulting in infection [8] which can be polymicrobial [9, 10]. Bacteria including Gram-negative and Gram-positive and fungi are considered potential causes of infection. Microorganisms have been isolated from DFUs involving gram-positive bacteria, especially *Staphylococcus aureus* [11]. However, deep and chronic wounds have often yielded aerobic gram-negative or obligate anaerobic bacteria [12]. The early diagnosis of DFU infection and appropriate treatment based on the identification of the pathogens and their antimicrobial susceptibility pattern is important for good prognosis [13]. In addition, the complexity of treating DFUs is compounded by the emergence of antibiotic resistance and extended-spectrum β -lactamase (ESBL)-producing bacteria [14]. Such resistance limits the efficacy of conventional antibiotics, presenting substantial challenges to patient management and healthcare systems.

The challenges faced by the clinicians in treating DFU are the antibiotic resistance, which restricts the choice of antibiotics for the treatment process. So the treatment of DFU requires appropriate antibacterial selection, continuous updates of the microorganisms responsible of infection and their resistance pattern remain a keystone in the management process, since infection with multidrug resistant strains is increasing and poses additional morbidity and mortality [15]. In addition, the role of polymicrobial infections remains insufficiently understood. This incomplete understanding may contribute to delayed healing, treatment failure, and recurrent infections, thereby exacerbating the burden of the disease. Therefore, the current study aimed to study the prevalence of microbial infection in DFU patients and more focusing on gender differences. Moreover, investigating types of bacteria and their characteristics, including biochemical tests and antibiotic susceptibility patterns in DFU patients were the purpose of the present study.

2. Materials and methods

2.1. Study and setting

The current research study was conducted in Erbil city, Kurdistan region, Iraq, between 1st June 2024 to 1st August 2025. Participants are involving diabetic patients diagnosed with foot ulcers at Galiawa diabetes and endocrinology teaching center in Erbil. The research was conducted in collaboration with Erbil teaching hospital-endocrine department. From DFU patients, demographic and clinical data will be collected, ensuring adherence to ethical protocols. In addition, samples were collected under sterile conditions and transported immediately to the laboratory for microbial analysis. The microbiological analysis was performed following standard biosafety and quality control protocols. The informed consent was obtained from all participants.

2.2. Patients' selection

Patients aged 30 years and above with DFUs were recruited. Then, only patients which were clinically diagnosed diabetes mellitus (HbA1c = 9-15%) and the presence of at least one-foot ulcer, classified according to the Wagner grading system (Grade 1–5) are involved in current study. Therefore, a total of 110 patients who met the eligibility criteria were enrolled in the study.

2.3. Sample collection and handling

Wound specimens were collected from DFUs after gently cleaning the wound area with sterile normal saline to remove surface contaminants, necrotic tissue and debris. All specimens were immediately placed into sterile containers and transported to the microbiology laboratory immediately. Samples were processed without delay. Each sample was labeled with a unique patient code and accompanying demographic and clinical data were documented in a secure case record form.

2.4. Microbial inoculation and incubation

Samples were inoculated onto a variety of culture media including blood, MacConkey and mannitol salt agars. The culture plates were incubated at 37°C for 24 hours to facilitate bacterial growth. For the Anaerobic bacteria, the specimen will be inoculated in Thioglycolate broth incubated anaerobically at 37°C overnight for primary isolation. After that specimen inoculated on Fastidious Anaerobic Agar media (FFA). For the identification of fungi, samples will be inoculated onto Sabouraud's dextrose agar and incubated at 25°C for 4 days and at 35 °C for 2 days. After the incubation periods, bacterial and fungal growth will be observed and analyzed.

2.5. Microbial identification

2.5.1. Gram's staining

Gram's staining was performed as a preliminary step in the identification of bacterial isolates according to standard protocol.

2.5.2. VITEK automated bacterial identification system

Bacterial identification was performed using the VITEK automated system (bioMérieux, France). Pure colonies were first isolated by appropriate culture media, and a standardized bacterial suspension equivalent to a 0.5 McFarland turbidity standard was prepared in sterile saline. The suspension was then inoculated into VITEK identification cards specific to either Gram-positive, Gram-negative, or yeast isolates. Cards were automatically sealed and loaded into the VITEK instrument, where they were incubated and monitored for biochemical reactions through optical systems. Identification was achieved by comparing reaction profiles to a proprietary database.

2.5.3. Biochemical tests

Biochemical testing was performed to further identify and differentiate bacterial isolates based on their metabolic properties. Tests were selected based on the Gram reaction and observed morphological characteristics.

2.6. Antimicrobial Susceptibility Test

Antibiotic susceptibility will be assessed using the Kirby-Bauer disc diffusion method. This will involve testing antibiotics according to the isolated bacteria. The resistance patterns will be documented to determine the susceptibility profiles of the isolated microorganisms.

3.7. Detection of ESBL-Producing strains and MRSA

For gram-negative bacteria, ESBL production was screened using the double-disk synergy test. In brief, isolates were inoculated on Mueller-Hinton agar, and disks of ceftazidime and ceftazidime-clavulanic acid were placed 20 mm apart (center to center). After incubation at 37°C for 18–24 hours, an enhanced zone of inhibition toward the clavulanic acid-containing disk indicated ESBL production. For MRSA detection, a 30 ug ceftazidime disc diffusion susceptibility tests will be performed, Muller Hinton Agar plates will be inoculated with a suspension (equivalent to a 0.5 McFarland standard) of each MRSA considered at 37°C, and zone diameters will be read after 24 hrs. The following breakpoint will be considered: resistant ≤ 21 mm, susceptible > 22 mm.

2.8. Statistical analysis

Statistical analysis was achieved by GraphPad Prism version 9.0. Categorical variables were compared using chi-square or Fisher's exact test. Continuous variables were presented as medians with minimum to maximum. In addition, the frequency and proportion of most findings were shown. Heatmap created by GraphPad Prism. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Demographic and clinicopathologic characteristics of DFU patients

A total of 110 DFU patients were included in the current study. Their HbA1C was between 9-15% and the majority of patients were male. The most common age group was 51–60 years, comprising 43.64% of the patients, followed by 61–70 years (23.64%). Regarding residence, the majority lived in urban areas (70.91%), while the remaining 29.09% resided in rural areas. A significant proportion of participants had hypertension (65.45%), and more than nine out of ten patients were found to have peripheral neuropathy (Table 1).

Table 1. Demographic and clinical characteristics of DFU patients

Variable	Category	Frequency (n)	Percentage (%)
Gender	Male	68	61.81
	Female	42	38.19
Age	31-40 years	2	1.82
	41-50 years	20	18.18
	51-60 years	48	43.64
	61-70 years	26	23.64
	71-80 years	14	12.73
	Urban	78	70.91
Residence	Rural	32	29.9
	Yes	72	65.45
Hypertension	No	38	34.55
	Yes	102	92.73
Peripheral neuropathy	No	8	7.27
	Yes	102	92.73

3.2. Isolated microorganisms in DFU patients

Out of all the clinical samples analyzed, bacteria were the most isolated microorganisms, accounting for more than half of DFU patients. No microbial growth was detected in 18% of patients (Fig.1 and Table 2). The distribution of isolated microorganisms was compared between male and female patients. Bacteria were isolated more frequently in males, while fungi were isolated more often in female patients. However, statistical analysis revealed no significant association between gender and type of microorganism isolated (Table 3). Concerning the kinds of isolated bacteria, 28% of patients were infected with multiple different kinds of bacteria (Fig 2).

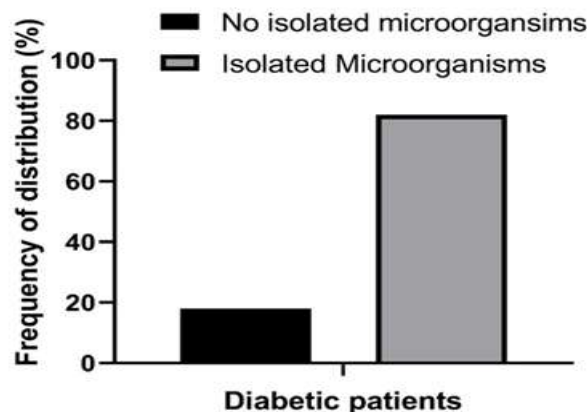


Figure 1: Percentage of microbial infected diabetic patients with foot ulcers

Table 2. Distribution of isolated microorganisms among clinical samples

	Category	Percentage (%)
Isolated microorganisms	Bacteria	66
	Fungi	8
	Bacteria & fungi	8
	Not isolated	18

Table 3. Comparison of isolated microorganisms between male and female patients

Isolated Microorganism	Male (%)	Female (%)	χ^2 / Fisher's Exact	p-value
Bacteria	70.83	52.38	2.207	0.53
Fungi	4.17	14.29		
Bacteria & Fungi	8.33	9.52		
Not isolated	16.67	23.81		

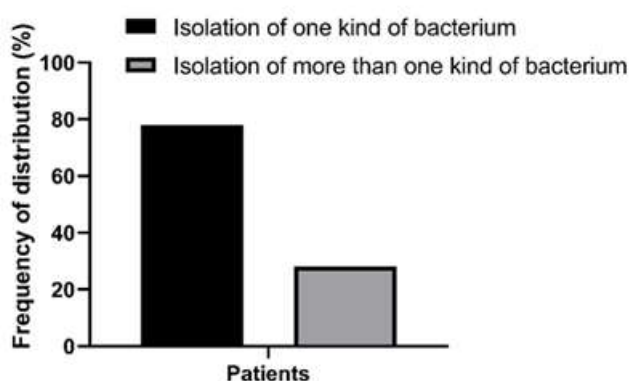


Figure 2: Multiple infection in diabetic patients with foot ulcers

3.3. Distribution and biochemical characterization of Gram-negative and Gram-positive bacteria isolated from DFUs.

Among infected patients with bacteria, 66.67% and 33.33 were infected with Gram negative and positive bacteria respectively. Isolated Gram-negative bacteria represent nine different species. The most frequently isolated organism was *Pseudomonas aeruginosa* (21.43%), followed by *Klebsiella pneumoniae* (19.05%). Less frequently isolated bacteria include *Klebsiella oxytoca*, *Morganella morganii*, *Acinetobacter baumannii*, and *Stenotrophomonas maltophilia*, each accounting for 2.38% of the isolates (Fig 3A). Regarding biochemical profiles of the isolated Gram-negative bacteria, the Simmons citrate test was positive in the majority of isolated bacteria in DFU patients. *Pseudomonas aeruginosa* showed positive oxidase activity, distinguishing it from most other isolates. Concerning the triple sugar iron (TSI) test components, more importantly, hydrogen sulfide (H₂S) production was primarily associated with *Proteus mirabilis*. Lactose fermentation varies across species (Fig 4A).

Regarding isolated Gram-positive bacteria in DFU patients, five different species were isolated and the most frequently isolated organism was *Staphylococcus aureus* (47.62%), followed by *Enterococcus faecalis* (19.04%) (Fig 3B). In addition, *Staphylococcus aureus* demonstrated positive results for all biochemical tests and bile-esculin and 6.5% NaCl tolerance tests were not applicable. *Staphylococcus haemolyticus* was catalase-positive

and beta hemolytic producer. *Corynebacterium* sp. showed only catalase positivity. *Streptococcus viridans* was alpha positive for hemolysis but negative for all other biochemical tests. *Enterococcus faecalis* was positive for bile-esculin hydrolysis and 6.5% NaCl tolerance, distinguishing it from other isolates. Moreover, coagulase, Dnase and Mannitol fermentation were not applicable for *Enterococcus faecalis* (Fig 4B).

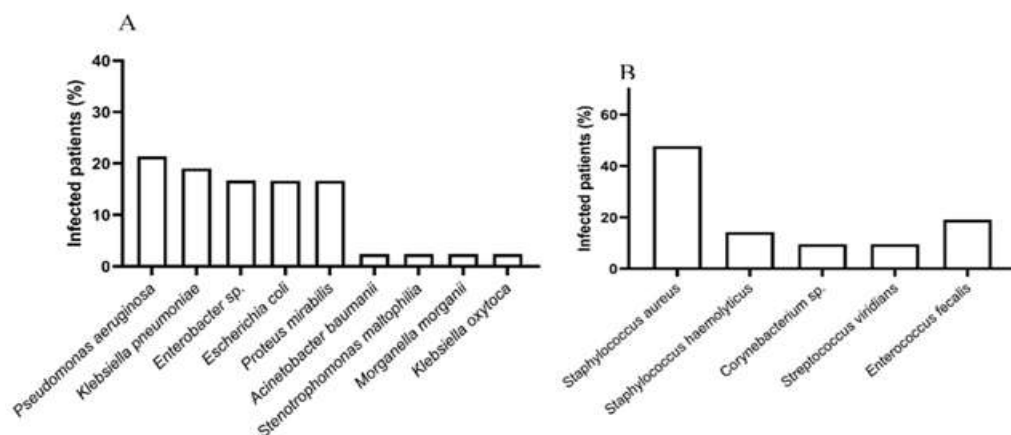


Figure 3: Distribution of Gram (A) negative (B) positive bacterial pathogens isolated from infected DFU patients

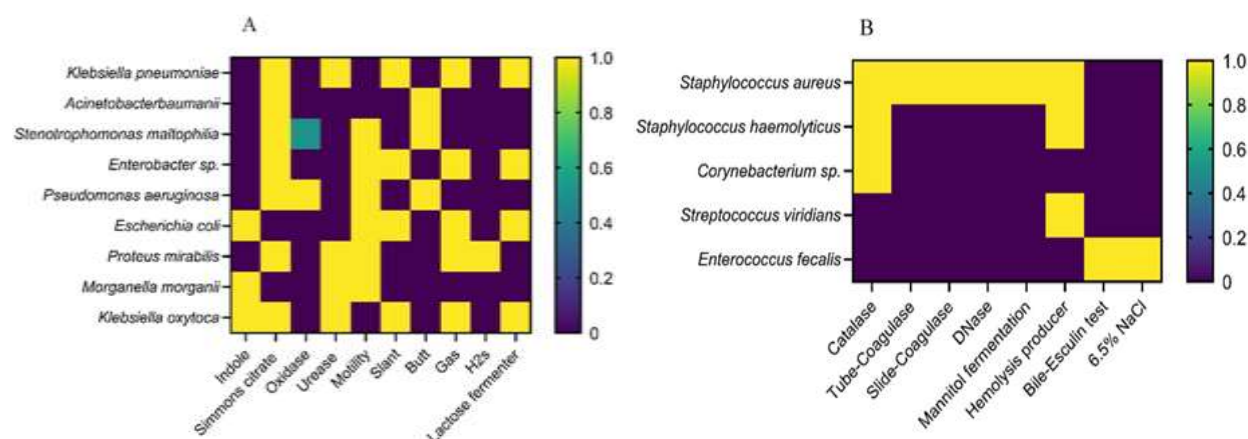


Figure 4: (A) A heatmap to visualize the biochemical profiles, TSI reactions, and lactose fermentation capabilities of nine species Gram positive bacteria. Each characteristic was coded numerically (positive = 1, negative = 0, partial/weak = 0.5) to standardize interpretation. Also, TSI slant and butt encoded numerically (Yellow = 1, Red = 0). (B) A heatmap to visualize the biochemical profiles capabilities of five species Gram negative bacteria. Each characteristic was coded numerically (positive = 1, negative = 0) to standardize interpretation. Bile-esculin and 6.5% NaCl tolerance tests were not applicable for *Staphylococcus aureus* and *Staphylococcus haemolyticus*. coagulase, Dnase and Mannitol fermentation were not applicable for *Enterococcus faecalis*.

3.4. Antibiotic resistance patterns of Gram negative and Gram-positive bacteria isolated from DFUs

In patients of current study, *Klebsiella pneumoniae* exhibited high resistance to amoxicillin, ampicillin and aztreonam with better sensitivity observed toward imipenem, meropenem, tigecycline and colistin. *Escherichia coli* and *Enterobacter* sp. showed moderate susceptibility to third-generation cephalosporins and fluoroquinolones, while *Proteus mirabilis* showed higher resistance to fluoroquinolones and moderate sensitivity to gentamicin and amikacin. *Acinetobacter baumannii*, *Morganella morganii* and *Klebsiella oxytoca* were largely resistant to most antibiotics tested (Fig 5A).

Regarding Gram positive bacteria, *Staphylococcus aureus* and *Staphylococcus haemolyticus* showed high resistance to erythromycin and β -lactams (penicillin, oxacillin). *Corynebacterium* sp. displayed resistance to multiple agents, mainly including ciprofloxacin, clindamycin, erythromycin and levofloxacin. *Streptococcus viridans* showed complete resistance to ciprofloxacin, clindamycin, erythromycin and gentamicin. *Enterococcus faecalis* demonstrated strong resistance to erythromycin and gentamicin. Notably, all current study Gram positive bacteria were resistance to erythromycin and sensitive to teicoplanin, vancomycin and linezolid (Fig 5B).

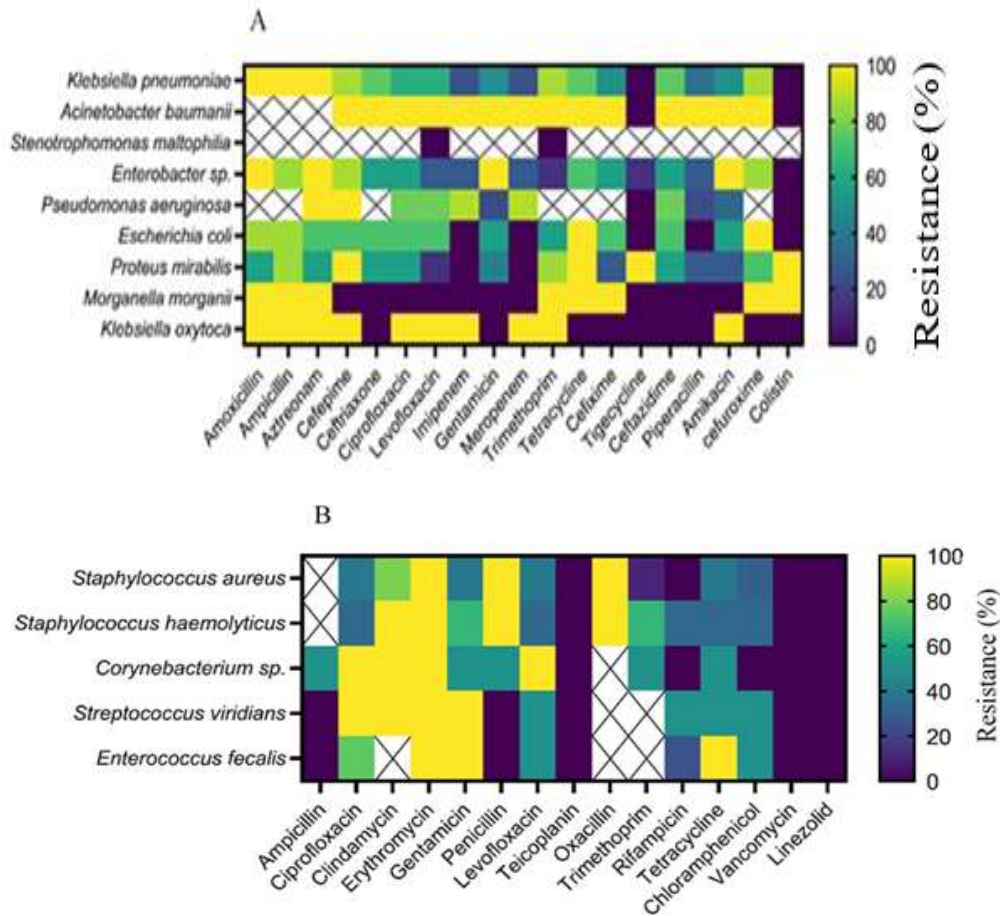


Figure 5: Heatmap of antibiotic susceptibility patterns among isolated (A) Gram negative bacteria (B) Gram positive bacteria in DFU patients. cross-hatched squares are indicators as not detected.

3.5. Detection of ESBL and MRSA markers in isolated bacteria

Among the isolated Gram-negative bacteria, *Pseudomonas aeruginosa* (21.42%) and *Klebsiella pneumoniae* (19.04%) were the most frequently detected species. ESBL production was most prevalent in *Klebsiella pneumoniae* (75%), followed by *Escherichia coli* (71.4%), *Proteus mirabilis* (57.5%) and *Enterobacter* sp. (57.1%). In contrast, ESBL production was not detected in *Acinetobacter baumannii*, *Stenotrophomonas maltophilia*, and *Pseudomonas aeruginosa*. In addition, *Morganella morganii* and *Klebsiella oxytoca* showed no ESBL activity despite their low isolation rates (2.38% each) (Table 4).

Among Gram-positive isolates, *Staphylococcus aureus* was the most frequently detected species (47.62%), followed by *Staphylococcus haemolyticus* (14.28%). Notably, cefoxitin resistance was observed in 100% of both *Staphylococcus aureus* and *Staphylococcus haemolyticus* isolates in DFU patients (Table 5).

Table 4. Prevalence of ESBL production among Gram-negative bacterial isolated from DFU patients

Bacteria	Total isolates (%)	ESBL positive (%)
<i>Klebsiella pneumoniae</i>	19.04	75
<i>Acinetobacter baumannii</i>	2.38	Not detected
<i>Stenotrophomonas maltophilia</i>	2.38	Not detected
<i>Enterobacter</i> Sp.	16.66	57.1
<i>Pseudomonas aeruginosa</i>	21.42	Not detected
<i>Escherichia coli</i>	16.66	71.4
<i>Proteus mirabilis</i>	16.66	57.5
<i>Morganella morganii</i>	2.38	0
<i>Klebsiella oxytoca</i>	2.38	0

Table 5. Cefoxitin resistance among *Staphylococcus* isolated in DFU patients

Bacteria	Total isolates (%)	Cefoxitin resistance (%)
<i>Staphylococcus aureus</i>	47.62	100
<i>Staphylococcus haemolyticus</i>	14.28	100

4. Discussion

Diabetic foot ulcers (DFUs) persist as a major public health concern, especially in low- and middle-income countries, due to their association with infection and poor glycemic control. The current research which was conducted in Erbil, Kurdistan region-Iraq, investigated the microbiological profile and antibiotic resistance patterns among 110 DFU patients, providing important insights for guiding local clinical management and antimicrobial stewardship. Most of current research DFU patients were male and aged 51-60 years, aligning with global epidemiological trends that showed a higher incidence of DFUs among older males with long-standing diabetes mellitus [16]. Poor glycemic control and comorbidities like hypertension and peripheral neuropathy were prevalent and consistent with findings of international study [17].

Microbiological analysis discovered that bacteria were the predominant pathogens, with polymicrobial infections observed in 28% of cases. Gram-negative bacteria, particularly *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, were more frequently isolated than Gram-positive bacteria in DFU patients. These results agree with other studies which showed Gram negative bacteria were more prevalent than gram positive bacteria and the most common isolated bacteria in DFU patients were *Pseudomonas* sp. followed by *Escherichia coli* and *Staphylococcus aureus* [18, 19]. This predominance of Gram-negative pathogens, especially *Pseudomonas* sp. has important implications for treatment, as these organisms often exhibit multidrug resistance mechanisms [20]. In addition, these results might be related to the niche of bacteria as the pathogen distribution in chronic wounds in rural Ghana observed with a predominance of *Pseudomonas aeruginosa* and *Staphylococcus aureus* [21] and other study revealed that resistant Gram-negative bacteria play a dominant role in military wound infections [22].

Moreover, biochemical characterization helped distinguish between species and revealed specific resistance traits. For instance, *Proteus mirabilis* uniquely produced hydrogen sulfide on TSI, while *Pseudomonas aeruginosa* was oxidase-positive, aiding their rapid identification. Therefore, these biochemical insights remain valuable in laboratories with limited access to molecular tools. Higher breath hydrogen sulfide levels correlated with greater duodenal prevalences of hydrogen sulfide producers, including *Proteus mirabilis* [23]. Regarding *Pseudomonas aeruginosa*, it is oxidase-positive, motile gram-negative bacillus widespread in nature. The virulence factors of *Pseudomonas aeruginosa* including the ability to grow under minimal growth conditions, the widespread presence

in nature, and the ability to form biofilms make *Pseudomonas aeruginosa* a highly important bacterium along with its resistance mechanisms against many antibiotics [24].

Antibiotic resistance patterns showed alarming trends. Gram-negative isolates exhibited resistance to penicillin and third-generation cephalosporins. Particularly, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Escherichia coli* demonstrated high rates of ESBL production. *Klebsiella pneumoniae*, a gram-negative bacterium, has emerged as a significant causative agent in DFU infections, raising concerns due to increasing antibiotic resistance, particularly in ESBL [25]. Carbapenems (imipenem and meropenem) and last-line antibiotics (tigecycline and colistin) retained high efficacy against most Gram-negative isolates. On the other hand, Gram-positive bacteria were uniformly resistant to erythromycin and β -lactams but remained susceptible to linezolid, vancomycin, and teicoplanin. These results of current research match with the outcomes of other study which showed Gram-positive bacteria were sensitive to linezolid, vancomycin, and teicoplanin. More than 50% of Gram-negative bacteria were resistant to third-generation cephalosporins, while the resistance rates of piperacillin/tazobactam, amikacin, meropenem, and imipenem were relatively low [26]. To combat Gram-Positive bacteria, vancomycin, linezolid, and rifampicin are considered excellent antimicrobial agents [27] which supports the current research results.

Furthermore, the high prevalence of ESBL and MRSA strains in current research may be attributed to uncontrolled antibiotic use, poor infection control practices, and inadequate diabetic foot care which is very clear in Erbil, Kurdistan region-Iraq. Regarding ESBL production, results of current research are in parallel with a study which tested 76 isolates, 53.9% were phenotypically ESBL producers and *Klebsiella pneumoniae* 75% (6/8) had the highest frequency of ESBL production [28]. Concerning the MRSA, the ulcer of diabetic foot became infected by MRSA [29, 30] which supports the results of current study in which 100% of *Staphylococcus aureus* and *Staphylococcus haemolyticus* isolates exhibited resistance to cefoxitin, suggesting widespread MRSA infections. Therefore, most *Staphylococcus aureus* isolates exhibit methicillin resistance, so treatment is recommended with antimicrobials active against MRSA in patients who have risk factors associated with MRSA infections [31].

5. Conclusion

In current study, the majority of DFU patients were middle-aged to elderly males with poor glycemic control, hypertension and peripheral neuropathy. Bacterial infections predominated over fungal infections. Gram-negative bacteria, notably *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were the most frequently isolated pathogens, while *Staphylococcus aureus* was the most common Gram-positive isolate. Biochemical profiling discovered distinctive species-specific features aiding in identification. In addition, high levels of antibiotic resistance were observed, especially among Gram-negative isolates, with notable ESBL production in *Klebsiella pneumoniae* and *Escherichia coli*. Furthermore, 100% cefoxitin resistance among *Staphylococcus aureus* and *Staphylococcus haemolyticus* isolates indicates a high prevalence of MRSA. These findings emphasize the need for robust infection control strategies, antimicrobial stewardship, and routine microbial surveillance to improve outcomes in DFU patients.

Declarations

Ethical approval

The current research project (No: 4, May 21, 2024) has been approved by the Ethics and Scientific committee of the Kurdistan Higher Council of Medical Specialties for Scientific and ethical approval.

Informed consent

Written informed consent was obtained from all participants prior to their inclusion in the study

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Author contributions

Sarheed jabar Muhammed was responsible for methodology, data collection, data analysis, and manuscript writing. Mazyar Jabbar Ahmed designed the study, supervised the overall project implementation, and contributed to the critical revision of the manuscript. Azhin Dlshad Aziz was responsible for recruiting participants, conducting clinical assessments, and interpreting the results. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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Conflict of interest

The authors declare no conflicts of interest.

Availability of data

The corresponding author can provide the datasets used and/or analyzed during the current study upon reasonable request.

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