



Article

# Detection of Multidrug Resistant Bacterial Infections Isolated from Patients with Diabetics Foot Ulcers in Iraq

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**Abstract:** Diabetes represent a chronic disorder that involve the decline in the levels of insulin produced by the pancreas due to many genetic and environmental factors. The disease involves the elevation of blood sugar in the circulation which may lead to vascular, neurological, ocular and renal complications. In addition, diabetic patients tend to develop ulceration due to wound infection swiftly in the lower limbs due to the reduce in the neurological sensation as well as to elevated sugar circulation which may greatly provoke wound infection and diabetic foot ulcerations. The current study aimed at isolation of the common causes of bacterial infection associated with diabetic foot ulcer, The present study was conducted in Salah Aldeen province during the period from 1<sup>st</sup> of November 2023 to 15<sup>th</sup> of May 2024 and included 150 patients with diabetic foot infection who attended Salah-Alden Hospitals: (Tikrit Teaching Hospital, Balad General Hospital and private surgery clinics). Swabs and whole blood from patients with diabetic foot ulcer were taken and submitted to routine culture and identification of the causative bacterial infection followed by biochemical identification and antibiotics susceptibility test for the isolated microbe. The current data revealed that most of the patients with diabetic foot ulcer were males 59.33% while females represented 40.67% with mean age 48 years for males and 53 years for females. Most of the patients with diabetic foot ulcer were residing urban areas 80.67% while the remaining 19.33% were living in rural areas. On the other hand, 18% of diabetic patients with foot ulceration were complaining of hypertension with majority having no other associated diseases 67.33%. Regarding bacterial isolates from diabetic foot ulceration, out of 150 clinical swabs only 47.33% were positive for bacterial isolates with predominant bacterial species species being *Pseudomonas aeruginosa* 38.03% followed by *Escherichia coli* 22.53%, *Klebsiella pneumoniae* 19.71%, *Staphylococcus aureus* 11.26% and *Streptococcus pyogenes* 8.45%. Antibiotics susceptibility test depicted that all isolates of *P. aeruginosa* 100% were resistant amoxicillin, ciprofloxacin and levofloxacin, while most of them were susceptible to imipenem 91%. whereas the vast isolates were resistant to amoxicillin group. Similarly, *E. coli* showed 93.7% resistance to amoxicillin group while 100% sensitivity to ciprofloxacin and levofloxacin antibiotics. Whereas, 92.9% of *K. pneumoniae* isolates were resistant to amoxicillin and azithromycin and 100% sensitive to imipenem. In the same way, 50% of *S. aureus* isolates were resistant to ceftazidime and 100% susceptible to imipenem. As for *S. pyogenes* 100% of the isolates were resistant to amoxicillin while 100% sensitive to cefixime, cefotaxime, amikacin, gentamycin and imipenem.

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**Keywords:** Diabetes, multidrug resistant, foot ulcer, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Streptococcus pyogenes*

## 1. Introduction

Diabetes poses a serious threat to human health and has the greatest incidence of any chronic illness in the world. Diabetes is classified into categories I and II clinically. In every

location and income bracket, type II diabetes mellitus (T2DM) is more common as people age. Nevertheless, because of bad lifestyle habits that have existed since childhood, the frequency is rising in younger age groups [1].

It was anticipated that the number of cases of diabetes in Europe would rise quickly, from 58 million in 2017 to 67 million cases by 2045, primarily in low- and middle-income nations. This would have a significant financial impact on all public health systems [2]. Higher prevalence of obesity and diabetes in many nations can be attributed to a variety of factors, including bad diets, urbanization, a more sedentary lifestyle, and, at the same time, a lack of funding for medical or preventive care for the populace. Diabetes foot ulcer (DFU) is defined by the World Health Organization as a foot ulcer accompanied by varying degrees of ischemia, infection, and neuropathy. It is a late consequence that is classified as a vascular issue in patients with diabetes mellitus [3]. T2DM is associated with insulin resistance (IR). Foot problems associated with diabetes are a major global source of disability.

Globally, diabetic foot ulcers (DFU) affect more than one-third of individuals with diabetes. DFU can lead to diabetic foot infections (DFI) and gangrene, which consume the majority of healthcare resources allocated to diabetic patients. Roughly 17% of DFI patients will need to have their feet amputated. According to studies, people with diabetes are more afraid of amputation than of dying [1]. Diabetic foot infections can take many different, intricate forms. In addition to the common occurrence of cellulitis, osteomyelitis and gangrene are additional potential outcomes. Because the increasingly severe form of prevention and treatment of diabetic foot ulcer infections was linked to a high rate of detection of these bacteria, it is imperative to focus on evaluating the risk factors of multi-drug resistant bacterial infections in order to find more effective treatment [4]. Clinical examples of common bacteria that are resistant to multiple drugs include enterobacteriaceae (including *Escherichia coli* and *Klebsiella pneumoniae*), vancomycin-resistant enterococcus (VRE), carbapenem-resistant enterobacteriaceae, multidrug-resistant *Pseudomonas aeruginosa* (MDR-PA), and multidrug-resistant *Acinetobacter baumannii* (MDR-AB) [4].

### **Aim of the study**

The goal of this research was to isolate and pinpoint the most prevalent bacteria that cause diabetic foot sores.

### **Objectives**

The objectives of the study are to:

1. The identification and isolation of the bacteria causing diabetic foot ulcers.
2. Research the resistance of isolated microorganisms to different types of antibiotics.

## **2. Materials and Methods**

### **Patients, Materials and Methods**

The current study involved collecting blood and swabbing diabetic patients with foot infection to detect the infection bacterial pathogens associated with foot ulcer. In addition to routine culture and identification antimicrobial susceptibility test was performed on all bacterial isolates.

### **Antimicrobial Susceptibility Testing**

The Kirby-Bauer disk diffusion method [5] was used to carry out the disc diffusion method. After choosing and inoculating pure bacterial isolates into a 0.9% saline solution,

the bacteria were allowed to grow until most of them required three to five hours of incubation. Next, Prior to suspension cultivation, the bacterial suspension's turbidity was measured and compared to the 0.5 McFarland standard, or  $1.5 \times 10^8$  CFU/ml. A Mueller-Hinton plate was used for the sensitivity test. A sterile cotton swab was dipped in the bacterial suspension and then wiped in three different directions to inoculate the plate's surface. After dispersing each antibiotic disc evenly over the plate's surface, the plate was incubated. To ensure that every antibiotic disc was fully positioned on the plate, seven of them were placed using sterile forceps and gently pressed to make sure they made contact with the medium's surface. Following an overnight incubation period at 35–37°C, the diameter of the inhibition zone was measured and compared to the standard inhibition zone.

### Patients and Sample Collection

Between November 1, 2023, and May 15, 2024, a cross-sectional study involving 130 diabetic patients with foot ulcers and 20 diabetic patients without a history of foot ulcers was conducted. Blood and swab specimens were collected from every case at the Salah-Alden Hospitals (Tikrit Teaching Hospital, Balad General Hospital, and private surgery clinic). The patients' informed consent was also acquired through the use of an investigator-designed questionnaire form. Every patient received an assurance that the privacy of their information would be maintained.

## 3. Results

### Socio Demographic Characteristics of study group

This study comprised 150 patients with diabetic foot ulcer infections; 71 (47.33%) of the sample tested positive for bacteria. Table 1 indicates that 61 (40.67%) of the participants were female and 89 (59.33%) were male. According to Table 1, the current study demonstrated that the age group of 41–50 years had the highest prevalence of diabetic patients, with 51 (34.0%), followed by age group of 31–40 years with 37 (24.67%), and the age group of less than 10 years old with 5 (3.33%).

**Table 1.** Sex distribution in patients' group

Sex distribution	Diabetic patients	
	No.	%
Males	89	59.33
Females	61	40.67
<b>Total</b>	150	100.00

**Table 2.** Distribution of patients according to age

Age group	Diabetic patients	
	No.	%
<10	5	3.33
11-20	13	8.67
21-30	17	11.33
31-40	37	24.67
41-50	51	34.00
>51	27	18.00

<b>Total</b>	150	100.00
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Regarding to the residence distribution the highest percentage of the study sample were residence at urban area 121 (80.67%) as seen in Table 3.

**Table 3.** Distribution of residence in study group

Residency distribution	Diabetic patients	
	No.	%
Urban	121	80.67
Rural	29	19.33
<b>Total</b>	150	100.00

Among of 150 patients, 49 had at least one risk factor for diabetes, with 27 (18%) having hypertension, 13 (8.67%) having visual impairment, and 9 (6.0%) having cardiovascular illnesses, according to the comorbidity criteria. as indicated by Table 4.

**Table 4.** Comorbidity factors in diabetic patients' group

Comorbidity factors	Diabetic patients	
	No.	%
Hypertension	27	18.00
visual impairment	13	8.67
Cardiovascular	9	6.00
Non	101	67.33
<b>Total</b>	150	100.00

#### Bacterial isolation and identification

The current investigation identified a number of bacterial species as the cause of diabetic foot ulcer infections. Based on biochemical tests, microscopy appearance, and culture properties. According to Table 5, the most frequently isolated bacteria were 27 (38.02%) for *P. aeruginosa*, 16 (22.53%) for *E. coli*, 14 (19.71%) for *Klebsiella pneumonia*, 8 (11.26%) for *S. aureus*, and 6 (8.45%) for *Strep. pyogenes*.

**Table 5.** Primary bacterial isolates in patients' group

Primary bacterial isolates	Gram stain	No.	%
<i>Pseudomonas aeruginosa</i>	Gram negative	<b>27</b>	38.02
<i>E. coli</i>	Gram negative	<b>16</b>	22.53
<i>Klebsiella pneumonia</i>	Gram negative	<b>14</b>	19.71
<i>Staph. aureus</i>	Gram positive	<b>8</b>	11.26
<i>Strep. pyogenes</i>	Gram positive	<b>6</b>	8.45
<b>Total</b>		71	100

### Biochemical tests result of isolated bacteria

The results of biochemical tests used for identification of isolated bacterial species in our study illustrated in Table 6.

**Table 6.** Biochemical tests used for identification of isolated bacterial species

Wound swab from diabetic foot	Citrate	Indole	Urease	Motility	K/G	Catalase	Oxidase
<i>Pseudomonas aeruginosa</i>	+	-	-	-	Alk/Alk	+	+
<i>E. coli</i>	-	+	-	+	Alk/Alk	+	-
<i>Klebsiella pneumonia</i>	+	-	+	-	Acid/Acid	+	-
<i>Staph. aureus</i>	+	-	+	-	Acid/Acid	+	-
<i>Proteus spp.</i>	+	-	+	+	H2S +	+	-
<i>Strep. pyogenes</i>	-	-	-	-	Acid/Acid	-	-

+: positive, - : negative, NT not tested.

### Antibiotic sensitivity of bacterial isolates

Table 7 shows the results of the antibiotic sensitivity tests conducted on the isolated bacteria in this investigation. Amoxicillin-clavulanic acid, Azithromycin, Cefixime, Cefotaxime, Cefepime, Gentamycin, Amikacin, Ceftazidime, Levofloxacin, Ciprofloxacin, Imipenem, and Clindamycin were the antibiotics utilized in this investigation. According to isolated *P. aeruginosa* in the current investigation, ceftazidime 26 (95%) was the most common sensitive antibiotic, while amoxicillin-clavulanic acid, levofloxacin, and ciprofloxacin 27 (100%) were the most common resistant antibiotics. On the other hand, Cefixime 14 (87.5%) was the most resistant antibiotic against isolated *E. coli*, while Levofloxacin (100%) and Ciprofloxacin (16%) were the most susceptible. Additionally, Imipenem 14 (100%) was the most sensitive antibiotic in an isolated *K. pneumonia* case, while Amoxicillin-clavulanic acid and Azithromycin 13 (92.9%) had the highest resistance. Imipenem 8(100%) was the antibiotic that *S. aureus* was most sensitive to, while Ceftazidime 4(50%) was the antibiotic that it was least resistant to. Lastly, in isolated *Strep. pyogenes*, the antibiotics with the highest resistance were amoxicillin-clavulanic acid (6(100%)), whereas cefixime, cefotaxime, gentamycin, amikacin, and imipenem had the lowest susceptibility (6(100%)).

**Table 7.** Antibiotic sensitivity of bacterial isolates

Antibiotics	<i>P. aeruginosa</i>		<i>E. coli</i>		<i>K. pneumonia</i>		<i>S. aureus</i>		<i>Strep. pyogenes</i>	
	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
<b>Amoxicillin-clavulanic acid</b>	0	27(100)	1(6.3)	15(93.7)	1(7.1)	13(92.9)	5(62.5)	3(37.5)	0	6(100)
<b>Azithromycin</b>	19(64)	8(36)	15(93.7)	1(6.3)	1(7.1)	13(92.9)	5(62.5)	3(37.5)	3(50)	3(50)
<b>Cefixime</b>	11(27)	16(73)	2(12.5)	14(87.5)	12(85.7)	2(14.3)	6(75)	2(25)	6(100)	0

<b>Cefotaxime</b>	10(45)	17(55)	13(81.25)	3(18.75)	2(14.3)	12(85.7)	5(62.5)	3(37.5)	6(100)	0
<b>Cefepime</b>	18(59)	9(41)	14(87.5)	2(12.5)	12(85.7)	2(14.3)	7(87.5)	1(12.5)	3(50)	3(50)
<b>Gentamycin</b>	21(73)	6(27)	14(87.5)	2(12.5)	13(92.9)	1(7.1)	7(87.5)	1(12.5)	6(100)	0
<b>Amikacin</b>	21(73)	6(27)	14(87.5)	2(12.5)	13(92.9)	1(7.1)	7(87.5)	1(12.5)	6(100)	0
<b>Ceftazidime</b>	26(95)	1(5)	13(81.25)	3(18.75)	13(92.9)	1(7.1)	4(50)	4(50)	3(50)	3(50)
<b>Levofloxacin</b>	0	27(100)	16(100)	0	13(92.9)	1(7.1)	6(75)	2(25)	3(50)	3(50)
<b>Ciprofloxacin</b>	0	27(100)	16(100)	0	13(92.9)	1(7.1)	6(75)	2(25)	3(50)	3(50)
<b>Imipenem</b>	25(91)	2(9)	15(93.7)	1(6.3)	14(100)	0	8(100)	0	6(100)	0
<b>Clindamycin</b>	22(77)	5(23)	14(87.5)	2(12.5)	12(85.7)	2(14.3)	7(87.5)	1(12.5)	3(50)	3(50)

#### 4. Discussion

##### Socio Demographic Characteristics of study group

In the present study the number of infected males 89 (59.33%) has shown a greater increase compared to females 61 (40.67%), that agreed with the study conducted by Al-Allak *et al* [7]. Other study by Almuhana *et al.* who collected 30 specimens from a total of 22 male and 8 female patients, whose ages varied between 45 and 85 years [8]. The observation was made that a higher percentage of males (61.7%) were affected compared to females (38.3%) among a total of 120 diabetic individuals suffering from foot infections. According to Shekhar *et al.*, the male-to-female ratio in the age group of 36 to 75 years was 2:1, with men accounting for 72.2% of the population [9]. While peritoneal closure and wound irrigation with normal saline greatly reduce the incidence of wound infection in both sexes, Abdul-Razzak *et al.* [10] found no significant variations in the incidence of wound infection between males and females. This suggests that differences in sex do not increase the likelihood of wound infection, and wounds can occur in both sexes equally [11].

The current discovery of a higher infection rate in male patients relative to female patients can be explained by the fact that men in Iraq are more exposed to outside environmental factors. Higher levels of outdoor employment, sex-related differences in lifestyles and professional activities that put more strain on the foot from work, and inadequate adherence to foot care practices compared to females may all be contributing factors to the male population in the DFU [11].

In terms of residential distribution, the majority of the study sample (66.67%) resided in urban areas, as seen In Table 1. This study contradicts a prior study that indicated that the majority of the study group resided in rural areas (58.2%) [11]. The majority of research, including those undertaken by Abdulhameed *et al.* in Iraq [12] have shown similar findings with a large number of urban residents (70.7%) exhibiting the same outcomes, which aligns well with our own findings. The variations in outcomes were ascribed to the geographic dispersion of the populace, which differs across various nations.

Only 49 out of 150 patients had one or more risk factors for diabetes, according to the comorbidity factors in the current results. Of these patients, 27 (18%) had hypertension, 13 (8.67%) had visual impairment, and 9 (6.0%) had cardiovascular disorders. These findings are consistent with a study by Abdulghani *et al.* that found the patients had comorbid complications like hypertension (61.4%), dyslipidemia (58.6%), retinopathy (23.3%), heart disease (14.4%), and severe foot complications (3.9%) with diabetes [13]. In another study conducted by Gershater *et al.*, Comorbidity diseases associated with DM patients were conflicting with our data in which neuropathies were the highest compliant 93%, followed by visual impairment 73%, cardiovascular 60% and cerebral damage comes the least (34%) [14].

### Antibiotic sensitivity of bacterial isolates

The study of isolated *P. aeruginosa* produced the following results: imipenem 25(91%) was the most commonly used sensitive antibiotic, and amoxicillin-clavulanic acid, levofloxacin, and ciprofloxacin 27 (100%) were the most commonly used resistant antibiotics. These results are consistent with those of Mahmoudi *et al.* [15], who showed that *P. aeruginosa* exhibited complete resistance to all antibiotics except imipenem, meropenem, gentamycin, and azithromycin. On the other hand, *P. aeruginosa* isolates shown intermediate sensitivity to imipenem and amikacin and substantial resistance to ceftazidime, piperacillin, gentamycin, levofloxacin, and tobramycin, according to a research by Yayan *et al.* [16]. Numerous resistance mechanisms are known to exist. These include inherent mechanisms like lower outer membrane permeability and improved efflux pump production, as well as acquired mechanisms like gene mutations or the acquisition of genes encoding for porins and other proteins. *Pseudomonas aeruginosa* develops resistance to penicillin, carbapenems, and cephalosporins due to  $\beta$ -lactamases, which are enzymes that sever the amide link in the  $\beta$ -lactam ring, making the antibiotics ineffective. Furthermore, aminoglycoside resistance is mediated by transferring enzymes that change aminoglycosides [17].

According to *E. Coli*, the antibiotics levofloxacin and Ciprofloxacin 16 (100%) were the most susceptible, while Cefixime 14 (87.5%) was the most resistant. A recent study, however, discovered that *E. Coli* had the highest levels of resistance to Amoxicillin-clavulanic acid (100%), cephalexin (100%), cefotaxime (90%), and cefepime (90%), while its highest levels of sensitivity were to Ciprofloxacin (90%), amikacin (80%), and imipenem (70%). It is evident from the current study that *E. Coli*'s highest rates of resistance have been to beta-lactam antibiotics: 100% of the isolates have shown resistance to ampicillin (AMP). Alghoribi *et al.* have reported similar results (95.2%, 94.2%) in numerous additional investigations conducted worldwide [19] and Zhong *et al.* [20] respectively. Resistance to amoxicillin/clavulanate (100%) agreement with another work has reported higher resistance in the works that have been performed by [21, 22, 23, 24], who demonstrated that (100%, 72%, 59.7% and 72%), respectively of isolates were resistant to AMC. Other studies conducted by Alghoribi *et al.* [19]; Li *et al.* [25] and Namaei *et al.* [26] were not in matching line with the present study. Furthermore, high resistance rates (50%, 90 % and 70%, respectively) were noted against 3rd generation cephalosporins: ceftazidime (CAZ), cefotaxime (CTX) and ceftriaxone (CRO), respectively. investigations by Namaei *et al.* [26]; Harris *et al.* [22]; Hojabri *et al.* [23] and Ali *et al.* [24]; Zhong *et al.* [20] and Huang *et al.* [27] revealed that *E. Coli* isolates had high rates of resistance against third-generation cephalosporins. These findings were similar to those of other investigations.

According to *K. pneumoniae* antibiotics are often recommended medications in both community and hospital settings for *K. pneumoniae*. However, there is a global concern in modern medicine about the emergence of antibiotic resistance bacteria, which is considered a significant problem [16]. According to the current findings, azithromycin 13 (92.9%) and amoxicillin-clavulanic acid (100%) were the antibiotics with the highest resistance to *K. pneumoniae*, whereas imipenem 14(100%) was the antibiotic with the highest susceptibility. Because *Klebsiella pneumoniae* is involved in multiple drug resistance mechanisms, it is a highly problematic pathogen [28]. The results of Ameen *et al.* [29], who discovered that *K. pneumoniae* was susceptible to imipenem and meropenem but showed strong resistance to amoxiclav, erythromycin, trimethoprim, ampicillin, and azithromycin, are consistent with the current data. It is possible for a group of genes linked to antibiotic resistance in *K. pneumoniae* to propagate horizontally to other Gram-negative bacteria [30]. Furthermore, the third generation cephalosporins cefotaxime (94.4%), ceftriaxone (72.2%), and ceftazidime (61.1%) were the most resistant to the test, according to the results of an antibiotic sensitivity test. These findings are consistent with the research conducted in Najaf (86.5%), (85.1%), and (82.4%) by Al-Hasnawi *et al.*, which found a significant rate of resistance to third-generation cephalosporins [31]. *K. pneumoniae* had a resistance rate of

61.1% to trimethoprim-sulfamethoxazole, which was consistent with findings from Hao et al. [32].

## 5. Conclusion

The study concluded the following:

1. The most common bacterial infection associated with diabetic foot infection was *P. aeruginosa* followed by *E. coli*.
2. Antibiotic resistant to penicillin group was most prominent in all isolated microbe from diabetic foot infection.
3. Most of the isolated bacteria were sensitive to Carbapenem group specifically to imipenem antibiotic.

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