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Bashar Salim Abdulraheem
Lecturer, Department of Microbiology, Basra University College of Science and Technology, Basra, Iraq

Aya Abdulhussein Khudair
B.Sc., Bachelor Senior Student, Basra University College of Science and Technology, Basra, Iraq

Dina Ali Abdalhafth
B.Sc., Bachelor Senior Student, Basra University College of Science and Technology, Basra, Iraq

Zahraa Hameed Shalal
B.Sc., Bachelor Senior Student, Basra University College of Science and Technology, Basra, Iraq

Zahraa Muhanned Sabeeh
B.Sc., Bachelor Senior Student, Basra University College of Science and Technology, Basra, Iraq

Bacterial species prevalence and their antimicrobial susceptibility in diabetic foot infection patients in single center study

Bashar Salim Abdulraheem, Aya Abdulhussein Khudair, Dina Ali Abdalhafth, Zahraa Hameed Shalal and Zahraa Muhanned Sabeeh

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Abstract
Background: One of the most dangerous and costly effects of diabetes is Diabetic Foot Infection (DFI). Many factors, such as peripheral neuropathy and peripheral artery disease, can combine to cause foot infections. Lower limb amputation is frequently necessary for patients with DFI.
Objectives: This study aimed to determine which bacteria in Basra City cause DFI and what their antibiotic sensitivity patterns were.
Methods: A cohort study was conducted on 40 patients From October 2022 until March 2023 at the AL Mashfa Diabetic Foot Clinic and Laboratory, Basra City.
Results: In the current study, there were 40 patients with DFI. Most of the patients (23) were male (57.5%), while females (17) (42.5%). Most of the cases were severe, n = 26 patients (65%), while the others were moderate, n = 14 patients (35%). 24 patients (60%) were gram negative bacteria while others were gram-positive bacteria 16 patients (40%).Most isolated cases were *Escherichia coli* 14 patients (35%) and the second most cases were *Staphylococcus aureus* 12 patients (30%).
Conclusion: Numerous pathogens can cause infections in diabetic feet. Inadequate glycemic control is the main risk factor for diabetic complications. Therefore, all problems, including DFI, will stop progressing if plasma glucose is kept under ideal control.

Keywords: Diabetic foot, infection, diabetes mellitus, *Escherichia coli*

Introduction

Diabetes Mellitus (DM) is a metabolic group of disorders characterized by chronic hyperglycemia (high blood sugar levels) resulting from a deficiency of insulin secretion, insulin action, or both. Insulin is a hormone manufactured by the beta cells of the pancreas [1].

One of the biggest new health risks of the twenty-first century is thought to be diabetes mellitus. More than 439 million people are diagnosed with DM, which causes a high rate of mortality and morbidity around the world [2]. The International Diabetes Federation (IDF) 2015 research projects that number to increase to 640 million (1 in 10) by 2040 [3].

In 2019, the straight reason of 1.5 million deaths is due to Diabetes Mellitus (DM).

48% of all deaths happened before age of 70 years [4].

The incidence of DFIs and peripheral arterial disease due to increase higher number of diabetic patients [5].

Most diabetic patients were admitted to the hospital due to DFIs. DFIs cause an increase in the use of antibiotics [6].

DFIs are the main cause of Lower Extremity Amputation (LEA) in individuals with Diabetes Mellitus (DM), especially those that reach the bone. This leads to a higher risk of death, a greater financial burden, and a worse quality of life. To escape these harmful results, it is important to protect against DFIs or, if that is not feasible, to handle wounds that have not been treated [5].

Individuals with diabetes mellitus have an increased risk of developing skin sores due to neuropathy, vascular disease, trauma, and persistent infections. Individuals with diabetes mellitus frequently experience peripheral neuropathy and peripheral artery disease.

Corresponding Author:
Bashar Salim Abdulraheem
Lecturer, Department of Microbiology, Basra University College of Science and Technology, Basra, Iraq

People with diabetes in general have immune system deficiencies that are not yet known, which limits their capacity to prevent or cure infections. The most common condition in people with DM is foot infection, because they are probably to develop this condition [7-8].

DM is classified into two major classes: Type 1 DM (T1DM) and Type 2 DM (T2DM). About 5%-10% of people with diabetes have T1DM and it is mostly diagnosed during childhood. Whereas T2DM accounting for 90%-95% of all diabetes and it is usually developing after the age of 40 years, but it may occur at any age [9-10].

DM has been linked to the traditional symptoms of the illness as well as reduced T-cell responses, disruption of humoral immunity, and neutrophil function [11-12].

Diabetic retinopathy, diabetic nephropathy, diabetic neuropathy, and DFI are among the complications that patients with uncontrolled diabetes mellitus are most likely to experience [13].

DFIs often start when there is a breach in the protective cutaneous envelope, typically in an area where there has been trauma or ulceration. People with peripheral neuropathy and peripheral arterial disorders are the most common cases of this [14].

Clinically speaking, DFI is the existence of signs of an inflammatory process in any tissue in a diabetic patient below the malleoli [15].

There are three types of foot infections in diabetic patients: mild, moderate and severe [16].

Acute infection in a previously untreated patient is usually caused by aerobic gram-positive cocci (*Staphylococcus aureus*), but deep or chronic wounds often harbor polymicrobial flora, including aerobic gram-negative (*Escherichia coli*) and obligate anaerobic bacteria (*Clostridium perfringens*) [17].

The biggest problem after DFI is the heightened susceptibility to many possible infections that can cause dangerous consequences like infection, gangrene, osteomyelitis, amputation, or even death [18].

Studies conducted by the Infectious Diseases Society of America (IDSA) and others indicate that individuals with DFI infection have a 50% higher risk of amputation than those with uninfected foot infections [19-20].

Around 20% of the general public who create a DFI will require LEA, each of two minor (below the ankle), major (above the ankle), or both, and 10% will die within 1 year of their first DFI diagnosis [5].

Conversely, the development of diabetic foot osteomyelitis is observed in around 44-68% of hospitalized patients, and it is the primary cause of amputation in these individuals [21].

This research aims to determine the antibiotic resistance profile and the causative microbe in Basra City DFIs.

Materials and Methods
Study design: The AL Mashfa Diabetic Foot Clinic and Laboratory is the site of this cross-sectional descriptive study in Basra City. From October 2022 until March 2023. It included 40 adult patients suffering from DFI: 23 (57.5%) were male patients and 17 (42.5%) were female patients. A questionnaire form is completed.
It includes the patient's name, age, sex, type of DM, severity, type of bacteria, and random blood sugar. The patient as a whole, the affected limb, and a local evaluation

of the wound were the three levels at which patients were examined.

Local examination of the wound
1. Diabetic foot wound classification according to the University of Texas Classification (4 grades and 4 stages).

The classification was
According to the grades
Grade 0: No ulcer,
Grade1: Superficial ulcer,
Grade 2: Deep ulcer, and
Grade 3: Osteomyelitis

According to the stages
Stage A: No infection,
Stage B: Infection,
Stage C: Vascular, and
Stage D: Infection plus vascular.

2. Clinical diagnosis of infected DFI was based on the presence of inflammatory signs and the IDSA infection severity score.

Investigations: The counts of white blood cells and hemoglobin were performed on each subject.

Samples collection
In this study, the wound swabs were collected according to the Levine technique. The Levine technique involves turning on the wound swab over a 1.2-cm section of the wound after cleaning the wound surface with copious sterile normal saline, then inserting the swab immediately into a sterile container.
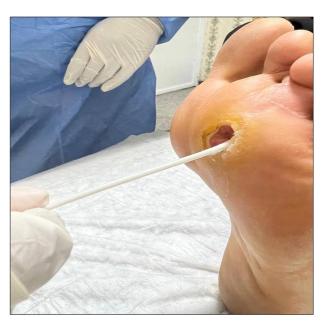

Fig 1: Shows the Samples Collection

The swabs that were taken from patients were inoculated on blood agar and MacConkey agar media, and then Petri dishes were incubated for 24-48 hours at 37 °C. After that, Gram stain was used to differentiate the growing bacterial species. Then, the cultured samples were taken to the AL

Mashfa laboratory, where they diagnosed the bacteria on the plate and did an antibiotic sensitivity test.

Statistical analysis
We used the SPSS software (version 260, SPPS, Chicago, IL, USA) for statistical analyses. Quantitative variables were expressed as mean ± Standard Deviation (SD) or median (Minimum and maximum), while categorical variables were expressed as percentages. To determine whether the distribution of the quantitative variables was normal, we performed the Kolmogorov-Smirnov test.

Results
The current study showed different species of gram positive and gram negative bacteria as shown in Table 1.

Table 1: The prevalence of bacterial species depends on the study parameters.

No.	Sex	Age	Gram stain	Type of bacteria	Severity
1	F	42	Positive	*Staphylococcus aureus*	Severe
2	M	52	Positive	*Staphylococcus aureus*	Severe
3	F	57	Positive	*Staphylococcus epidermidis*	Severe
4	F	71	Negative	*Escherichia coli*	Severe
5	F	50	Positive	*Staphylococcus epidermidis*	Moderate
6	F	51	Positive	*Staphylococcus aureus*	Moderate
7	F	48	Negative	*Proteus* spp.	Moderate
8	M	45	Negative	*Escherichia coli*	Moderate
9	M	56	Negative	*Escherichia coli*	Moderate
10	F	57	Negative	*Escherichia coli*	Moderate
11	F	60	Positive	*Staphylococcus aureus*	Moderate
12	F	50	Negative	*Proteus* spp.	Severe
13	M	55	Negative	*Escherichia coli*	Moderate
14	M	65	Negative	*Pseudomonas* spp.	Severe
15	M	40	Negative	*Proteus* spp.	Severe
16	M	60	Negative	*Escherichia coli*	Severe
17	F	55	Negative	*Pseudomonas* spp.	Moderate
18	F	52	Positive	*Staphylococcus aureus*	Severe
19	F	56	Negative	*Escherichia coli*	Severe
20	M	76	Negative	*Proteus* spp.	Severe
21	F	53	Positive	*Staphylococcus aureus*	Severe
22	M	56	Negative	*Escherichia coli*	Severe
23	M	54	Positive	*Staphylococcus aureus*	Moderate
24	M	62	Positive	*Staphylococcus aureus*	Severe
25	M	60	Positive	*Staphylococcus aureus*	Severe
26	F	35	Negative	*Proteus* spp.	Severe
27	M	63	Negative	*Proteus* spp.	Moderate
28	F	75	Positive	*Staphylococcus epidermidis*	Severe
29	M	51	Negative	*Proteus* spp.	Moderate
30	M	63	Positive	*Staphylococcus aureus*	Moderate
31	M	70	Positive	*Staphylococcus aureus*	Severe
32	M	53	Positive	*Staphylococcus aureus*	Severe
33	M	70	Negative	*Escherichia coli*	Moderate
34	F	66	Negative	*Escherichia coli*	Severe
35	M	57	Negative	*Proteus* spp.	Severe
36	M	53	Negative	*Escherichia coli*	Severe
37	M	61	Negative	*Escherichia coli*	Severe
38	M	56	Negative	*Escherichia coli*	Severe
39	M	62	Negative	*Escherichia coli*	Severe
40	F	55	Positive	*Staphylococcus epidermidis*	Severe

Demographic characteristics of the study population
In current study there were 40 patients with DFI, most of the patients (23) were males (57.5%) while female (17) (42.5%), The Mean ±SD age is (56.83±8.83) as shown in Table 2.

Table 2: Demographic characteristics of the study population.

Sex	Frequency	Percent
Female	17	42.5
Male	23	57.5
	Mean ± SD	**Median (Min._Max.)**
Age	56.83±8.83	56 (35-76)
Total	40	100.0

Severity of DFI, results of culture gram stain, and type of bacteria cultured: Most of the cases were severe n=26 patients (65%) while the others were moderate n=14 patients (35%).

24 patients (60%) had gram-negative bacteria, while others had gram-positive bacteria, including 16 patients (40%). Most isolated cases were *Escherichia coli* 14 patients (35%) and the second most cases were *Staphylococcus aureus* 12 patients (30%) as shown in Table 3.

Table 3: Severity of DFI, results of gram stain, and type of bacteria cultured

Variable	Frequency	Percentage
Severity		
Moderate	14	35.0
Severe	26	65.0
Gram stain		
Positive	16	40.0
Negative	24	60.0
Type of bacteria		
Escherichia coli	14	35.0
Staphylococcus aureus	12	30.0
Proteus species	8	20.0
Staphylococcus epidermidis	4	10.0
Pseudomonas species	2	5.0
Total	40	100.0

Table 4: Antimicrobial susceptibility testing

Antibiotic	No. of Sensitive (%)	No. of Resistant (%)
Imipenem	35 (100)	0 (0)
Meropenem	34 (97)	1 (3)
Amikacin	16 (52)	15 (48)
Levofloxacin	18 (60)	12 (40)
Ciprofloxacin	17 (59)	12 (41)
Piperacillin	9 (39)	14 (61)
Gentamycin	11 (52)	10 (48)
Colistin	3 (14)	18 (86)
Cefepime	0 (0)	21 (100)
Vancomycin	19 (74)	5 (26)
Ceftriaxone	2 (12)	14 (88)
Azetronam	3 (23)	10 (77)
Cefixim	1 (8)	12 (92)
Rifampicin	0 (0)	12 (100)
Trimethoprim	0 (0)	12 (100)
Tetracycline	1 (10)	9 (90)
Tazobactam	4 (67)	2 (33)
Teicoplanin	0 (0)	4 (100)
Polymexin B	0 (0)	2 (100)
Clindamycin	1 (100)	0 (0)

The most frequent sensitive antibiotics were imipenem, meropenem, and vancomycin. The most common resistance antibiotics in order were Cefepime and Amikacin.

Table 5: Sensitive and Resistance Antibiotic for *E. coli*

Sensitive antibiotic for *E. coli*	Patient number	Resistance antibiotic for *E. coli*	Patient number
Meropenem	14	Cefepime	8
Imipenem	13	Ceftriaxone	7
Ciprofloxacin	8	Piperacillin	5

Discussion
The current study was carried out in Basra City to assess the susceptibility profile and pathogen prevalence of microorganisms isolated from infected wounds in patients with diabetic foot disease.

For defense, the immune system is essential. The conditions of hyperglycemia and hyperinsulinemia in diabetes modify the innate immune system's typical functioning, but they have little to no influence on the adaptive immune system. In DM, there are several changes to immune cell activities [22]. In the current study, most patients were males, as compared with other studies, which reported a higher frequency of DFI in males than in females. In our study, the mean age group was 56.83±8.83, while eight of the 12 published studies documented that the maximum number of DFI mostly occurred within the age group of 40-60 years. Both male gender and age were considered risk factors for DFI. Male predominance was explained in several previous studies due to outdoor activity by males, hard physical activity, being at higher risk for trauma, higher alcohol consumption, higher smoking behavior, and less compliance with foot care practices. The elderly patients who have spent longer with DM will have decreased immunity and nutritional deficiencies and are at risk for the development of certain complications such as peripheral neuropathy and vascular diseases [23-26].

60% of the microorganisms in our investigation were gram negative, which is in line with findings from a study done at the Haji Adam Malik General Hospital in Medan by Bulolo *et al.*, which showed that gram-negative bacteria were the most often detected in DFI [27].

In contrast to a prior research conducted in Ethiopia that found *Klebsiella* species to be the most common bacterium at 23.9%, followed by *Proteus* species at 18.47%, the current investigation found that *E. coli* was the predominant isolate in 35% of cases [28].

According to another Egyptian research, *Proteus mirabilis* (16.8%) is the most prevalent isolate [29].

In Saudi Arabia studies found *Pseudomonas* species in 15.6% of cases, while the most prevalent bacteria in the South America study was *Pseudomonas* species 18.8%.

In Kenya research 17.5% of cases was *Pseudomonas* also *Pseudomonas* found in Nigeria cases 32.9%, India 24.42%, 65.2% in China and 28% in Iran [30-36].

This demonstrates how various environments may have different prevalent bacteria that cause DFI illnesses.

The results of this study can be taken into account while creating an empirical antibiotic treatment plan for the local management of DFI.

The most common microorganism was *E. coli*, while the most sensitive antibiotics were Meropenem (14 cases), Imipenem (13 cases), and Ciprofloxacin (8 cases), while the most resistant antibiotics were Cefepime (8 cases), Ceftriaxone (7 cases), and Piperacillin (5 cases).

Gram-negative bacteria outer membrane performs the vital function of providing an additional layer of defense without interfering with the flow of materials necessary to maintain life [37].

Bacterial porins are a prominent topic of study in bacterial pathogenesis due to their functional significance in microbe-host interactions throughout various bacterial infections [38].

The beta-lactam antibiotic family includes the carbapenem class of antibiotics, which includes the antimicrobials meropenem and imipenem. Because most gram-negative bacteria are resistant to these drugs, they have a demonstrated clinical efficacy against a variety of pathogens, including extended-spectrum beta-lactamase-producing organisms like *Escherichia coli* [39].

Conclusion
1. Various microorganisms can cause diabetic foot infections.

2. The most common isolate was *E. coli*, which was followed by other gram-positive bacterial species.
3. A current study showed that isolated bacterial species are more sensitive to imipenem, meropenem, amikacin, and levofloxacin.
4. The results indicate the importance of bacteriological culture and sensitivity tests before starting imiprical antibiotic therapy for DFIs.
5. Theemergence of resistance appeared in the use of antibiotics, alerting the careful use of antibiotics.

Recommendation

1. Having poorly controlled blood sugar is the primary cause of problems from diabetes. As a result, all problems, including DFI, will stop progressing if plasma glucose is optimally controlled.
2. Therefore, the patient must follow the doctor's instructions optimum control of blood sugar, follow a healthy diet and drink water.
3. Exercising, protecting the foot from wounds and trauma, wearing appropriate shoes, cutting nails, taking care of wounds immediately upon injury, and seeing a doctor to regulate sugar.
4. Doing analyzes to ensure the patient's health and adherence to medications and all the doctor's instructions.
5. In order to start treatment as soon as possible with the right medicines, it is required to identify the particular bacteria and their susceptibility pattern.

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