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Original Research Article

Bacteriological Study of Diabetic Foot Ulcer Infection in Babylon

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Abstract: This study addressed the importance of bacterial infections in diabetic foot as a serious complication that can lead to ulcers and chronic inflammation, increasing the risk of limb loss. The study reviewed the most common types of bacteria that infect diabetic foot and the role of these bacteria in complicating wounds and injuries. Thirty foot swab samples (15 patients and 15 controls) were collected and cultured in the microbiology unit at Al-Marjan Hospital in Babylon, statistical analysis was done and results shows some types of bacteria are more common, such as Staphylococcus aureus. also noted that men with diabetic foot ulcer more than women, and that middle-aged people (aged 52-62, 63-73) are more likely to be infected with diabetic foot. This study concluded that staphylococcus aureus was common pathogenic bacteria that cause diabetic foot ulcer, also male sex is regarded as risk factor for diabetic ulcer. Additionally no relation between agent species with severity of disease.

Keywords: Diabetic Foot Ulcers (DFU), Pathogenic Bacteria, Staphylococcus Aureus, Pseudomonas Aeruginosa, Catalase Test, Oxidase Test.

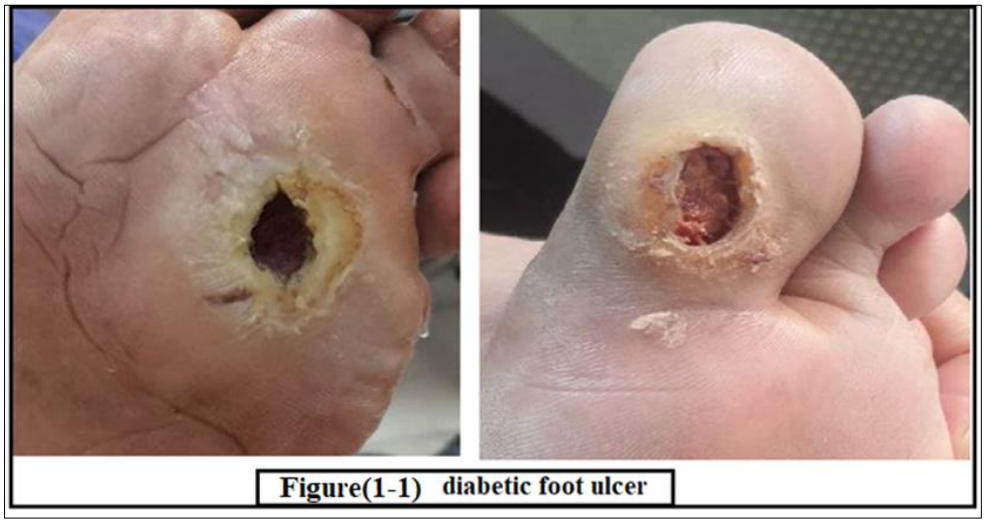
INTRODUCTION

Diabetic foot infections (DFIs) are one of the most serious complications associated with diabetes, contributing significantly to morbidity and mortality worldwide [1]. Diabetes mellitus leads to a multitude of changes in the body, including chronic hyperglycemia, impaired immune function, neuropathy (nerve damage), and poor peripheral circulation [2]. These factors collectively contribute to the vulnerability of diabetic patients to foot infections. Once an infection occurs, it can progress quickly, leading to severe complications such as ulcers, gangrene, and even limb amputation [3]. Foot infections in diabetic patients are typically caused by a wide range of bacteria. These include both aerobic and anaerobic pathogens, with some bacteria exhibiting multidrug resistance, making the infections more challenging to treat [4]. One of the most problematic aspects of diabetic foot infections is the increased risk of polymicrobial infections—where multiple different bacterial species are present in the wound. This polymicrobial nature complicates treatment options and often results in prolonged hospitalizations or even amputation in severe cases [5]. The introduction of antibiotic-resistant bacteria adds another layer of difficulty in the management of diabetic foot infections. As the prevalence of antibiotic-resistant strains, such as methicillin-resistant *Staphylococcus aureus* (MRSA), increases, conventional treatments become less effective, necessitating the development of alternative therapeutic strategies [6]. Furthermore, diabetic neuropathy leads to loss of sensation in the feet, causing patients to be unaware of the early stages of infection, which can significantly delay diagnosis and treatment [7].

MATERIAL AND METHODS

Patients Samples

The study was conducted from Babylon in Iraq. A total of 30 specimens were collected from patients with diabetic foot infection obtained from Marjan teaching hospital in Babylon between October 2024 to march 2025. The specimens were 15 from diabetic foot ulcer. The swabs were collected using sterile cotton swab and swab media.



Figure(1-1) diabetic foot ulcer

1. Catalase Test

Catalase is an enzyme that catalysis the release of oxygen from hydrogen peroxides. Gas bubbles formation indicates a positive result.

2. Oxidase Test

The test relies on the presence of any bacterial oxidase enzyme that would catalyze the transportation of electrons between electron donors in the bacteria and a redox dye (tetramethyl-phenylene-diamineihydrochloride).

3. Preparation of Culture Media

The culture media listed in Table (3.3) were prepared according to the instructions of the company and sterilized at 121°C for 15 minutes in the autoclave.

4. Bacterial Culture

Culture Bacteria from a Foot Swab on Agar Plate is a simple procedure used to isolate and grow bacteria found on the skin or other parts of the body. Here are the steps for culturing bacteria from a foot swab on an agar plate:

Staphylococcus aureus

RESULTS AND DISCUSSION

Study Characteristics

This study of diabetic foot ulcer focuses on understanding, isolation and identification of bacterial species causes infection in DFU patients.15 specimens were collected within the scheduled date for collection, from November 2024 to March 2025 in Marjan hospital.

Teaching Hospital. The specimens were divided as follows (15 swap samples) and 15 control specimens after performing the swap culture. The culture results for 15 specimens were positive.

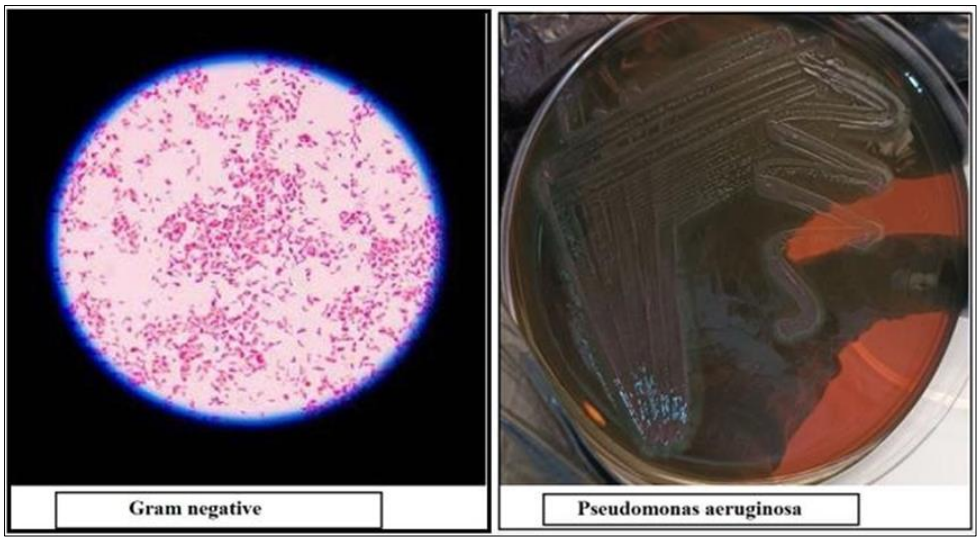
Bacteriological Study Results

1. Identification and Characteristics of Bacterial Species by Laboratory Diagnosis

The diagnosis of bacterial species in the laboratory were based on phenotypic characteristics including morphology of colonies were verified by Gram staining, the isolated colonies are streaked onto a selective and differential medium mainly used for isolating and identifying, it was mentioned previously. Bacterial Identification microscopes are utilized to examine stained bacterial specimens, such as those from Gram staining, to determine the presence and species of bacteria responsible for an infection.

Tabel (4-1):The Bacterial Species dignosed by Gram stain and Biochemical Test:

Bacterial Species	Gram Stain	Catalase Test	Oxidase Test
Staphylococcus aureus	+	+	-
Pseudomonas aeruginosa	-	+	+
Staphylococcus epidermidis	+	+	-
Klebsilla pneumonia	-	+	-
Escherichia coli	-	+	-



Catalase test is an important feature in differentiating certain group of bacteria, particularly Gram-positive cocci, rapid bubbling due to the release of oxygen gas indicates the presence of catalase enzyme, the catalase test distinguishes between catalase-positive and catalase-negative bacteria, that produce catalase enzyme will quickly break down hydrogen peroxide, leading to the release of oxygen bubbles [8]. The modified oxidase test is a rapid method that differentiates Staphylococcus from Micrococcus. Differentiation among Staphylococci can also be achieved by the coagulase test. The oxidase test assesses an organism's ability to produce cytochrome oxidase enzymes. It employs a phenylenediamine reagent

to create a deep blue compound, indophenol, in the presence of atmospheric oxygen and cytochrome oxidase. Two commercially available reagents are tetramethyl-p-phenylenediamine dihydrochloride and dimethyl-p-phenylenediamine dihydrochloride.

Detection of the test is possible by adding drops of the reagent directly onto 18- to 24-hour-old cultures on a plate or using filter paper saturated with the reagent [9]. All bacterial isolates were identified by morphological, microscopical and biochemical tests, percentage Gram positive and Gram negative by end conculcated that suspected results. As shown in the Table (4-1).

2. Bacterial Species Isolated from Diabetic Foot Ulcer Patients

The distribution of bacterial species isolated from diabetic foot ulcers is shown in figure (4-1), 60% represented by mix species with (klebsilla, Staphylococcus epidermidis..), 27% represented by Staphylococcus aureus, 6.7% represented by E.coli ,6.7% represented by Pseudomonas aeruginosa. Show As in below figure(4-1)

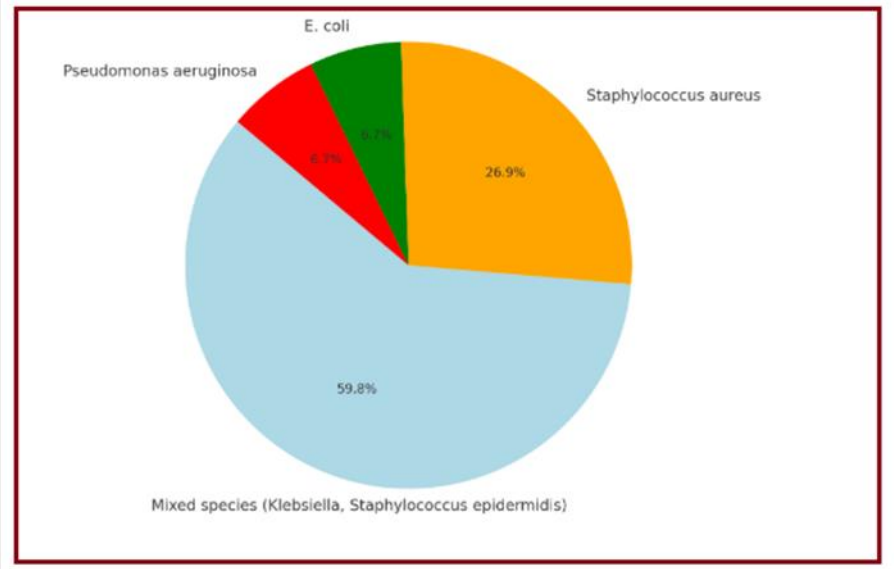


Figure (4-1): Bacterial species distrbution isolated from DFU patients

The variety of bacteria present in chronic wounds is regarded as a significant factor contributing to the persistence and severity of diabetic foot ulcer, other past researches have shown that S.aureus is the primary causative pathogen; however, there are also reports indicating a significant presence of Gram-negative aerobes [10].

Standard procedures were utilized for isolating, processing, and identifying all isolates, including Staphylococcus aureus, S.epidermidis, and Staphylococcus haemolyticus. Identification was confirmed using a PCR method. S. haemolyticus was isolated either alone or in combination with other Staphylococcus species such as S. aureus and S. epidermidis. Although S. haemolyticus is a less common Staphylococcus species, its prevalence was notably high [11].

In contrast [12], reported a lower prevalence of S. haemolyticus It was found that of S. haemolyticus isolates were from DFU, S. haemolyticus at a rate of 9% from diabetic foot ulcers. These variations in prevalence may be attributed to differences in sample types, the number of people studied, highlighting the significance of atypical microorganisms in the infection of DFU.

In the previous study by the most predominant bacteria were Staphylococcus aureus at 25.19%, followed by Pseudomonas spp at 18.89%, and Escherichia coli at 16.53%, overall, infections were caused by a single bacterium in 31.66% of cases and were polymicrobial in nature for the remaining samples [13].

A recent study confirmed that Enterobacteriaceae was the most dominant group among the Gram-negative bacteria, representing of the total bacterial isolates; Klebsiella pneumoniae; Proteus mirabilis, and Pseudomonas aeruginosa were identified as the most prevalent Gram- negative bacteria, followed by Morganella morganii and E.coli, it produces various virulence factors, including urease, which can increase the pH of the wound environment and contribute to tissue damage and inflammation [14].

Gram-negative rods are commonly found in foot ulcers, including bacteria such as Klebsiella spp., Acinetobacter spp., Enterobacter spp., Pseudomonas aeruginosa, and E.coli [15].

These findings align with those reported by [16], the species most isolated from patients with diabetic foot.

3. The Impact of Age on the Risk of Diabetic Foot Ulcers (DFU)

Our findings indicate that the risk of DFU increases with age, aligning with results from other studies. The result is illustrates that the age distribution of the patients with DFU in the studys , the highest percentage of patients exposed to infection which was (33.4%), falling in the two age group of 52-62 years and of 64-73 with 10 patients out of a total of 15 patients. As shown in below figure (4-2).

- ✓ 13.3% in the age group 30-40
- ✓ 13.3 % in the age group 41-51
- ✓ 33.4% in the age group 52-62
- ✓ 33.4% in the age group 63-73
- ✓ 6.6 % in the age group 74 -84

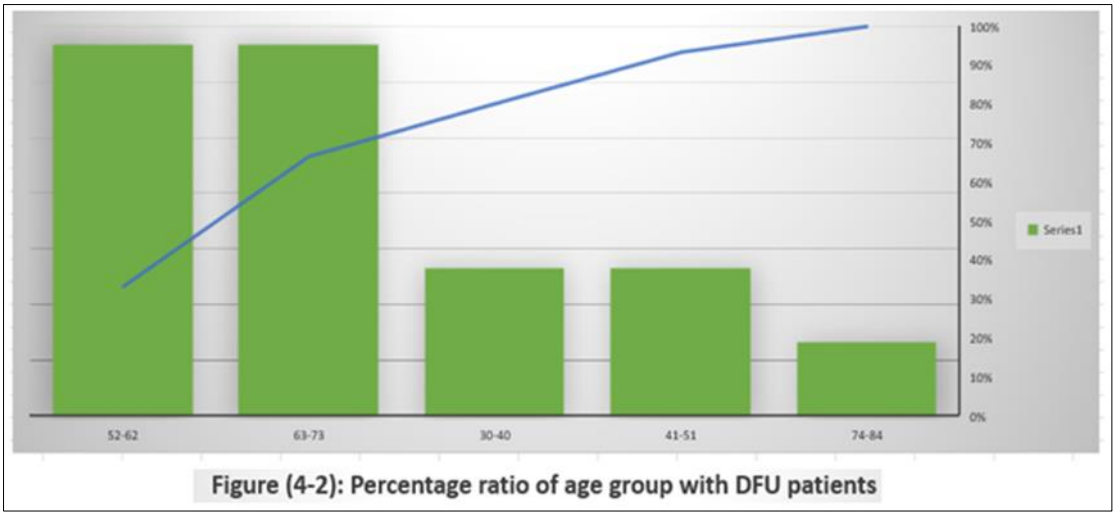


Figure (4-2): Percentage ratio of age group with DFU patients

In the study by Edmonds *et al*., (2021) found the prevalence rate of infected diabetic foot ulcers and amputations is 1.6% among individuals aged 18-44 years, 3.4% in those aged 45-64 years, and 3.6% in persons older than 65 years.

Another study added that advanced age is a significant factor contributing to the increased severity of diabetic foot ulcer, consequently, their studies aimed to assess the interaction between the location of diabetic foot ulcers and age in presentations of the condition, to enhance understanding of how ulcer location impacts prognosis across various age groups [17].

The younger the age at which (T2DM) is diagnosed, the greater the risk of developing Diabetic Foot Ulcers (DFU). Furthermore, the longer the duration of DFU, the higher the risk of infection with mixed bacterial isolates. Gram-negative bacteria were found to be more prevalent in DFU cases. However, Staphylococcus aureus was the most common bacterial isolate, followed by Escherichia coli [18].

Another study reported that wound healing in older patients with diabetic foot ulcer was more challenging, this difficulty might be due to a decline in vascular function as people age, leading to a higher rate of infections in the elderly compared to younger individuals [19].

4. The Incidence of Diabetic Foot Varies from Men to Women. We Found That:

- 72 % male
- 28 % female

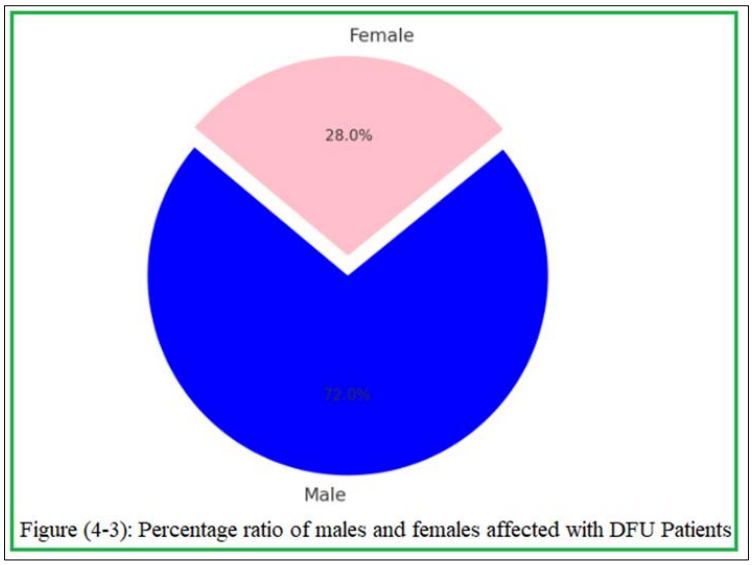


Figure (4-3): Percentage ratio of males and females affected with DFU Patients

Patients with diabetic foot ulcer often have a high prevalence of comorbidities and are exposed to accelerated aging, this combination can lead to increased frailty, making the management of DFU more complex and challenging (Maltese *et al.,* 2022); with increase in age, P. aeruginosa and S. aureus became more frequent, while Streptococci decreased and ischemic and/or deep wounds were more likely to bear Gram-negative species [20].

The result pressented in figure (4-3) show the sex distribution of patients with a diabetic foot ulcer in this study,with 11 patients (72%) being male and 4 patients (28%) begin female. Diabetic foot ulcer are predominantly associated with male, showing a higher prevalence in male despite this, past studies indicate that females may face a risk of adverse outcomes following DFU, a complication commonly seen in males with diabetes appears to have severe repercussions for females too [21].

A previous study conducted by [22], showed that male, peripheral vascular disease is more common, and the arterial tree is often more severely affected, displaying the morphological characteristics typical of diabetic arteriopathy, conversely, females tend to exhibit a more widespread involvement of the microcirculation and a pro-inflammatory profile.

The literature indicates a greater prevalence of comorbidities and complications among men; social studies have demonstrated lower adherence to health habits and disease follow-up in men, furthermore, in secondary prevention men are less likely to adopt self-protective behaviors, such as wearing therapeutic insoles and footwear [23].

5. Diabetic Foot May be Associated with Chronic Diseases. The Results Indicate That:

- 46% Absent
- 41% Hypertension
- 13 % MI and Hypertension

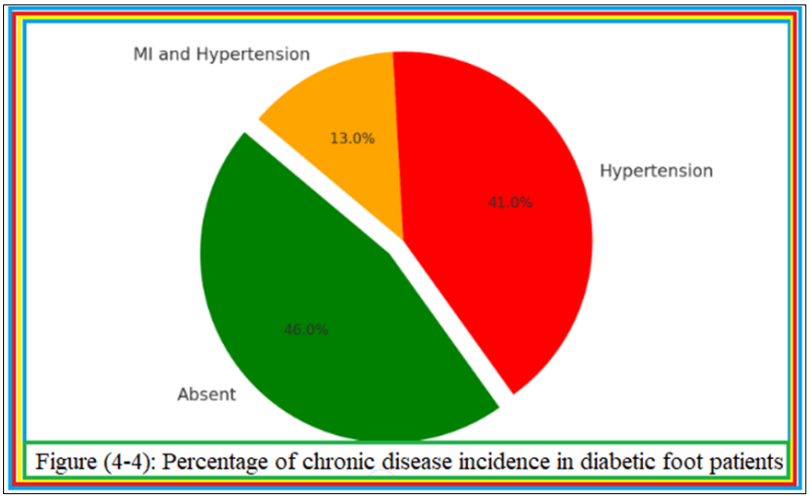


Figure (4-4): Percentage of chronic disease incidence in diabetic foot patients

The presence of DFU is linked to significant adverse cardiac events and may serve as an indicator of cardiovascular complications, warranting prompt clinical investigation and management, patients with DFU have a notably higher incidence of heart failure compared to those without DFU [24].

The study found that ischemic heart disease is commonly observed in patients with diabetic foot ulcer and has pinpointed multiple risk factors. Patients suffering from both heart failure and DFU experience poorer outcomes than those with just one of these conditions, additionally, heart failure may hinder the healing process of DFUs due to inadequate perfusion and oxygenation of the affected tissues [25].

The previous study also indicated a high prevalence of hypertension and hyperlipidemia as cardiovascular risk factors among DFU patients, additionally, patients with DFU may present with diabetic peripheral neuropathy, which heightens the risk of developing silent myocardial ischemia [26].

Systemic arterial hypertension heightens the risk of microvascular and macrovascular injuries, thereby increasing the likelihood of peripheral arterial disease [27].

The data suggest that hypertension may be a significant factor affecting the prognosis of patients with diabetic foot ulcer. However, findings indicate that hypertension does not increase the risk of amputation in DFU patients, nevertheless, it remains essential for DFU patients to manage their blood pressure, as hypertension can result in adverse cardiovascular and cerebrovascular events [28].

Septic arthritis can lead to chronic conditions, such as hammer toe ulceration and the recurrence of a diabetic foot ulcer or the failure to heal indicates a chronic DFU, which is defined by the inability to heal within the expected timeframe [29].

The comorbidities we examined were all linked to a heightened risk of developing DFU, such as hypertension, hyperlipidemia, coronary artery disease, heart failure, stroke, diabetic retinopathy, diabetic kidney disease, and diabetic neuropathy; several baseline characteristics have been associated with a higher risk of developing diabetic foot ulcers [30].

Numerous factors, including age, sex, severity of the ulcer, and duration of the ulcer, can influence the types of bacteria involved in diabetic foot infections [31].

CONCLUSION

Staphylococcus aureus is the most common bacterium causing diabetic foot ulcers, highlighting its significant role in infection development. The infection rate in men is significantly higher than in women, indicating possible gender-related risk factors. Delayed diagnosis and inappropriate treatment can lead to severe complications, emphasizing the need for effective healthcare strategies.

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