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Original Article

Investigation of the Most Common Bacterial Strains in Diabetic Foot Ulcer Patients

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Abstract

Background: Diabetes has been one of the most prevalent medical illnesses, and diabetic foot ulcer (DFU) infections are the main causes of hospitalization in diabetics. The present study aimed to investigate the factors contributing to DFUs in patients referred to Taleghani Hospital in Abadan.

Methods: In this study, the data of 316 diabetic patients with DFUs referred to Taleghani Hospital in Abadan from March 21, 2019, to March 19, 2020, were analyzed by SPSS version 22.0.

Results: The study revealed that out of the 316 patients, 212 (67.1%) were male and 104 (32.9%) were female. Additionally, 64.3% of them had type 2 diabetes. Most of the patients had third-degree ulcers (60.4%) and 26.9% had amputations. *Staphylococcus aureus* (34.5%) and *Pseudomonas aeruginosa* (30.4%) were the most prevalent bacteria isolated from DFUs. The highest frequency of antibiotic resistance was reported for Cotrimoxazole (33%) and Tetracycline (28.2%).

Conclusion: In conclusion, Gram-positive bacteria had a higher prevalence compared to gram-negative bacteria. It is essential to conduct periodic examinations to reduce the risk of health-threatening complications in diabetic patients.

Keywords: *Staphylococcus aureus*, *Pseudomonas aeruginosa,* Diabetic foot ulcer, Diabetic foot infection

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Introduction

Diabetes is a health condition that lasts a long time and affects how the body processes food for energy. It occurs when there is insufficient insulin or when cells do not respond to insulin effectively, leading to excess glucose in the bloodstream. This can lead to severe health complications such as heart disease, vision loss, and kidney disease over time (1). Currently, a significant portion of the global population is affected by either type 1 or type 2 diabetes. In addition, there is mounting evidence indicating that the number of individuals diagnosed with diabetes is expected to rise rapidly, from 425 million in 2017 to an estimated 600 million by 2030 (2,3). Type 1 diabetes is typically caused by the destruction of immune system cells, leading to a complete lack of insulin. Meanwhile, type 2 diabetes is usually caused by a gradual loss of beta-cell insulin secretion, often combined with insulin resistance (4). Diabetes can lead to various physiological issues, one of which is foot ulcers that affect many diabetics. Diabetic foot ulcer (DFU) refers to a skin break on the foot of a diabetic person that takes a long time to heal. These ulcers, which occur in around 15% of diabetic patients, typically appear on the bottom of the foot. Of these patients, 6% may require hospitalization due to infection or other complications related to the ulcer (5). Foot ulcers are the main reasons for hospitalization, amputation, reduced mobility, loss of social participation, and lower quality of life in people with diabetes (6). Diabetic foot disease poses a significant financial burden and considerable threat to health care systems (7). DFU can be caused by bacterial activity. Studies have identified several bacteria commonly found in DFU infections, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus species*, *Staphylococcus aureus*, *Klebsiella pneumonia*, and *Enterobacter* species. These bacteria can colonize and make the treatment process more difficult. Some may even form biofilms and develop resistance to antibiotics, leading to further challenges in treatment (8).

Other studies have shown that several risk factors are linked to the development of DFU. These factors include gender, diabetes duration of over 10 years, advanced age, high body mass index, and comorbidities such as retinopathy, diabetic peripheral neuropathy, peripheral vascular disease, high HbA1C levels, foot deformity, high plantar pressure, infections, and improper foot self-care habits (9). The purpose of this research was to assess the prevalence and antibiotic resistance of bacteria found in foot ulcers of diabetic patients who were referred to Taleghani Hospital in Abadan, Iran.

Materials and Methods

Study Setting

In this cross-sectional study, after obtaining the permission of the Ethics Committee, all diabetic patients with diabetic foot infections (DFIs) referred to Taleghani Abadan Hospital from March 21, 2019, to March 19, 2020, were included. A demographic profile was obtained from the patients' files using a predesigned validated checklist. It included the following variables: age, gender, duration of diabetes, type of diabetes, weight, height, family history, occupation, and level of education. The patients were also assessed clinically and the ulcers were graded according to Wagner's classification (1981) (grade 0: hyperkeratosis, grade I: superficial ulcers, grade II: deep ulcers, grade III: tendonitis, osteomyelitis, cellulitis, or abscess, grade IV: gangrene of a toe or forefoot, and grade V: gangrene of the whole foot).

Patients who had foot ulcers or foot infections were included in the study. DFU infection was defined as the presence of at least 2 of the following indicators: local swelling or induration, erythema around the wound, local tenderness or pain, local warmth, and purulent discharge (10).

The exclusion criteria were other foot ulcers and foot infections in persons without diabetes. Patients with first or second-degree infection, incomplete history or physical examination, failure to provide deep tissue samples, incomplete treatment, or failure to report radiology, pathology, and microbiology were excluded from the study.

Bacterial Sample Collection and Isolation

A total of 316 patients were sampled over the course of a year after receiving their informed consent. To collect samples, two sterile swabs were used to obtain material from the depth of the wound. One swab was used for hot staining, while the other was inoculated in blood agar, chocolate agar, McConkey agar, and *Thioglycolate agar*, which were provided by Merck (Germany). After incubating at 37 °C for 24 hours, the samples were inspected for identification of suspicious colonies using bacterial biochemical tests. Then, the identified samples were preserved in LB medium with 20% glycerol (Merck Co., Germany) and stored at -80°C until they were ready to be used.

Phenotypic Detection

In phenotypic studies, specific tests and media were used to identify the bacteria involved in this infection. The first steps in identifying the bacteria included gram staining, motility analysis, endospore staining, and capsule staining. Biochemical investigations were carried out to confirm the presence of gram-negative bacteria. Tests such as catalase (CAT), oxidase, indole, methyl red (MR), Voges-Proskauer (VP), citrate utilization, nitrate reductase (NR), triple sugar iron, sugar fermentation, and aminoacyl decarboxylase were conducted. Gram-positive bacteria were identified using tests including CAT, MR, VP, NR, sugar fermentation, DNase, and coagulase (11).

Antibiograms

The pattern of antimicrobial resistance was determined using the Kirby-Bauer antibiogram method and the results were interpreted according to CLSI 2019 guidelines.The antibiotics used in the gram-negative group included cotrimoxazole (400 mg), cefazolin (30 µg), gentamicin (10 µg), amikacin (30 µg), ciprofloxacin (5 µg), cefepime (30 µg), and imipenem (10 µg).

The antibiotics used in the gram-positive group included tetracycline (30 µg), penicillin (15 µg), azithromycin (15 µg), clindamycin (2 µg), ciprofloxacin (5 µg), and linezolid (30 µg).

Molecular Analysis

Genomic DNA was extracted from bacteria grown in Mueller-Hinton broth (Merck Co., Germany) overnight at 37 °C. Pelleted bacterial cells were suspended in 200 µL of phosphate-buffered saline (PBS). DNA extraction was performed using the High Pure PCR Template Preparation Kit (Roche Co., Germany) following the manufacturer's instructions. The concentration and purity of extracted DNA were measured by NanoDrop spectrophotometer (DeNovix Inc., USA). In the following molecular assessments, specific primers were used for the final detection of *S. aureus*, *P. aeruginosa*, and *E. coli* isolated from DFU infections in diabetic individuals who participated in this study, respectively:

F: GCGATTGATGG TGATACGGTT/ R: AGCCA AGCCTTGACGAACTAAAGC (12), F-GGGGGATC TTCGGACCTCA/ R-TCCTTAGAG TGCCCACCCG (13), and F-CCGATACG CTGCCA ATCAGT/ R-ACG CAGACCGTAGGCCAGAT (14).

Statistical Analysis

In the current study, quantitative variables were presented as mean and standard deviation and qualitative variables as numbers and percentages. The data analysis was carried out using SPSS version 22.0.

Results

Population Characteristics

In this study, of 316 patients with DFU, 67% (n = 212) were male and 33% (n = 104) were female, with the dominance of the male gender. Additionally, 24.4% of the patients (n = 77) had a family history of DFUs. Moreover, of 316 patients, 64.3% (n = 203) had type 2 diabetes, and 46.5% (n = 147) of the patients developed DFUs within 1-3 years after diagnosis of diabetes mellitus (Table 1). Furthermore, 60.4% (n = 191) had third-degree infections (Table 2) and 26.9% (n = 85) had amputations. In addition, the mean height, weight, and age of patients are shown in Table 3.

Bacteriological Results

All of the samples were positive for bacterial culture and were mono-microbial infections (Table 4). Gram-negative pathogens represented 64.87% (n = 205) of the isolates. Among them, 53.17% (n = 109) were Enterobacteriaceae, with *E. coli* being the most commonly isolated pathogen (55.96%, n = 61), followed by *Enterobacter* spp. (23.85%, n = 26). Non-fermenting gram-negative bacilli represented 46.82% (n = 96) of the isolates of *P. aeruginosa* strains. Among gram-positive bacteria, the most commonly isolated species was *S. aureus* (98.19%, n = 109), followed by *Anthracosis* spp. (1.80%, n = 2).

The results of the antibiotic resistance test are shown in Table 5. We found a causative association between the different mechanisms of resistance and antibiotic prescriptions in patients. For example, Enterobacteriaceae and Staphylococci showed resistance to aminoglycosides.

Discussion

In the current study which was conducted in the Taleghani hospital in Abadan, it was observed that the incidence of diabetic wound infection was higher in men (67%) than in women (33%). Our study also observed that diabetic patients with a mean age of 52.73 years suffered from DFU, which is in line with the results of the study conducted by Ogba et al. They reported that the mean age of the patients was 55.4 ± 10.1 years (15). In our study, grade 3 ulcers had the highest prevalence (60.4%), while in another study, all subjects had grade 4 wounds (15). In the current research, the ulcer microbiota was dominated by gram-positive species, primarily *S. aureus* (34.5%). The Gram-negative bacteria included *P. aeruginosa* (30.4%), *E. coli* (19.3%), *Enterobacter* (8.2%), and *Proteus* spp. (7%). However, another study conducted by Noor et al showed that the most prevalent bacteria among 50 DFU samples was *K. pneumonia* (44.6%), while the lowest prevalence was observed for *E. coli* (9.2%) (16). However, the study conducted by Dörr et al demonstrated that among 353 individuals with infected DFU, the ulcer microbiota was dominated by gram-positive species, primary *S. aureus* (20.6%), and among the gram-negative ones, *P. aeruginosa* (5.6%) was the most prevalent (17). Additionally, the study conducted in teaching hospitals of Sari revealed that 54% of patients were male and 46% were female with a mean age of 58.3 + 10.2 years, and *S. aureus* (28%) had the highest prevalence, followed by *P. aeruginosa* (14.5%) (18). For further analysis, the antibiogram studies were performed to determine the prevalence of antibiotic resistance among gram-negative and gram-positive bacteria. In the classification of gram-negative bacteria, cotrimoxazole had the highest frequency

Table 1. Frequency Distribution of Length of Diabetes in Patients

Length of Diabetes	Frequency Distribution No. (%)
Less than 1 year	43 (13.6%)
Between 1 and 3 years	147 (46.5%)
More than 3 years	126 (39.9%)
Total	316

Table 2. Frequency Distribution of Infection Degree in Patients

Degree of Infection	Frequency Distribution No. (%)
Grade three	191 (60.4%)
Grade four	125 (39.6%)
Total	316

Table 3. Mean Height, Weight, and Age of Patients

Variables	Height (cm)	Weight (kg)	Age (y)
Mean	160.70	69.58	52.73
Standard deviation	15.81	12.89	8.92
Minimum	113	45	35
Maximum	190	96	86

Table 4. Frequency Distribution of Bacteria Isolated from Diabetic Foot Ulcer Patients

Bacteria Isolated From Infection	Frequency Distribution No. (%)
Staphylococcus aureus	109 (34.5)
Pseudomonas aeruginosa	96 (30.4)
Escherichia coli	61 (19.3)
Enterobacter spp.	26 (8.2)
Proteus spp.	22 (7)
Anthracosis spp.	2 (0.6)
Total	316 (100)

Table 5. Prevalence of Antibiotic Resistance of Organisms Isolated from Diabetic Foot Ulcer Patients

	Antibiotic	Resistance
Gram-negative	Cotrimoxazole	177 (33)
	Cefazolin	146 (27.2)
	Gentamicin	87 (16.2)
	Amikacin	81 (15.1)
	Ciprofloxacin	28 (5.4)
	Cefepime	14 (2.6)
	Imipenem	3 (0.5)
Gram-positive	Tetracycline	98 (28.2)
	Penicillin	93 (26.8)
	Azithromycin	91 (26.2)
	Clindamycin	34 (9.7)
	Ciprofloxacin	26 (7.7)
	Linezolid	5 (1.4)

of resistance (33%) and imipenem (0.5%) had the lowest resistance rate, followed by cefepime (2.6%), ciprofloxacin (5.4%), amikacin (15.1%), and gentamycin (16.2%). These results were similar to those reported by Manikandan and Jaikumar. Additionally, gram-positive organisms were less resistant to Linezolid (1.4%) and ciprofloxacin (7.7%), which is in line with the results of a study by Manikandan and Jaikumar (19). However, in the study conducted by Thanganadar Appapalam et al, the results indicated a big difference in the prevalence of resistance to antibiotics. All strains showed 100% resistance to cotrimoxazole and amikacin antibiotics, while in our study, resistance rates of 33% and 15% were reported, respectively. In addition, based on the results of a study performed by Thanganadar Appapalam et al, the prevalence of Tetracycline resistance in the group of Gram-positive bacteria was reported to be 54%, while it was 28.2% in our study (20).

One limitation of this study is the lack of a control group and comparison of results with it. Additionally, the sample size was small. Therefore, it is recommended that future studies should include a control group and a larger sample size. Comparisons across different communities should also be considered if feasible.

Conclusion

The present study found that the most common bacteria causing DFU infections is *S. aureus*. The isolated organisms showed resistance to cotrimoxazole (gram-negative bacteria) and tetracycline (gram-positive bacteria). This study highlights the importance of recognizing risk factors in diabetes and DFUs. Patients and their companions should receive proper training and solutions to prevent and reduce complications. Periodical examinations can greatly reduce the occurrence of life-threatening complications in diabetic patients. Additionally, reducing hospital stays and avoiding financial waste are important considerations for patient care.

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Authors' Contribution

Conceptualization: Sara Mobarak.
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Competing Interests

No conflict of interests existed.

Ethical Approval

This study was approved by the Clinical Research Development Unit of Taleghani Educational Hospital, Abadan University of Medical Sciences (Ethical approval ID: IR.ABADANUMS.REC.1398.061).

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