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Bacterial Etiology and Antibiotic Resistance Pattern of Diabetic Foot Infection in Patients Admitted to Shiraz Hospitals, Iran

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ABSTRACT

Backgrounds: A common problem among diabetic patients is foot ulcers and infections, impacting up to 15% of diabetic patients over their lifetime. The aim of this study was to investigate the frequency of bacterial agents and their antimicrobial resistance pattern in patients with diabetic foot infection in Namazi and Shahid Faghihi hospitals in Shiraz.

Materials & Methods: This cross-sectional study was conducted in Namazi and Shahid Faghihi hospitals in Shiraz. The collected samples were transferred to the laboratory for culture and biochemical tests. After accurate identification of bacterial agents, antibiotic susceptibility of all isolated bacteria was evaluated by disk diffusion method based on CLSI guidelines. Data were analyzed using SPSS software (Version 19).

Findings: In this study, 166 patients with diabetic foot ulcers were evaluated. The mean age of patients was 55.8± 13.2 years, and 109 (66.4%) cases were male. Also, 62% of patients had an underlying disease, while most of them had hypertension (27%). The most prevalent isolated bacterium was *Staphylococcus epidermidis*. The most effective antibiotics against isolated Gram-positive and Gram-negative bacteria were vancomycin and amikacin, respectively.

Conclusion: In this study, it was concluded that the frequency of Gram-negative bacteria in diabetic foot ulcer infections was higher than that of Gram-positive bacteria.

Keywords: Diabetic foot infection, Bacterial etiology, Antibiotic resistance pattern, Iran.

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Introduction

According to the World Health Organization, diabetes affects 150 million people worldwide each year and continues to spread. It is estimated that the incidence of this disease will double by 2025 [1]. Problems associated with the quantity and quality of life constitute an important part of the problems of diabetics. These problems are caused by acute and chronic complications of diabetes, one of the most harmful of which is diabetic foot infection [2]. The most common and costly late complication of diabetes is diabetic foot ulcer, which has worse complications and mortality than many cancers. Research has shown that one in three diabetic people develops a diabetic foot ulcer during their lifetime. Incurable diabetic foot ulcers are the leading cause of hospitalization, amputation, and death in diabetic patients [3, 4]. About 40 to 80% of diabetic foot ulcers become infected. In fact, due to their high susceptibility to infection, these injuries rapidly become infected and spread to adjacent tissues, leading to extensive tissue damage and eventually amputation [5]. Previous studies have shown that Gram-positive cocci, mostly *Staphylococcus aureus*, are the most common cause of diabetic foot ulcer infection. Infections in chronic wounds treated with antibiotics are generally polymicrobial [6]. In these infections, organisms have distinct characteristics compared to situations where Gram-negative pathogens or anaerobes alone cause infection. Polymicrobial infection causes the interaction between bacteria and the generation of different virulence factors. These factors that cause injury and delay treatment include hemolysis, proteases, collagenase, and short-chain fatty acids [7]. Most diabetic infections are extremely urgent; thus, antibiotic therapy should be initiated immediately to accelerate recovery and prevent organ loss. The initiation of experimental treatment should be based on clinical signs, results of Gram staining, and knowledge of the most common organisms in wound infections in this region. One of the most important tasks in the proper management of a wound infection is to perform susceptibility testing for organisms isolated from the diabetic wound and to choose proper antibiotics [8].

Objectives: The objective of this study was to study the bacteriology of diabetic wound infections in patients admitted to Namazi and Shahid Faghihi hospitals in Shiraz in order to determine the prevalence of bacterial agents isolated from diabetic foot infections and to assess their antibiotic resistance pattern.

Materials and Methods

This cross-sectional study was conducted in Namazi and Shahid Faghihi hospitals affiliated to Shiraz University of Medical Sciences in 2019 and 2020. This study was reviewed by the Ethics Committee of Shiraz University of Medical Sciences and approved by the Code Number 789901. Inclusion criteria included type 1 and type 2 diabetic patients over 18 years of age, who were hospitalized due to diabetic foot ulcers and required antibiotic treatment. Patients who were unable to answer the questions and also had non-diabetic neuropathies were excluded from the study. Wound samples were collected from patients. After washing with saline, wound samples were collected from the base and deep part of the wound or by needle aspiration from the abscess before antibiotic treatment. The collected samples were submitted to the microbiology laboratory in thioglycollate tubes and incubated at 37 °C for 24 hours. Following Gram staining, both blood agar and MacConkey agar culture media were incubated under aerobic and anaerobic

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conditions at 37 °C for 48 hours. For Gram-negative bacteria, IMViC tests were used to accurately identify the isolates. For Gram-positive bacteria, confirmatory tests were performed for accurate diagnosis. Antibiotic sensitivity testing was performed for the isolates using disc diffusion method on Mueller-Hinton agar plates (transferred from a broth with a visual turbidity of 0.5 McFarland). The following antibiotic disks were used to evaluate the antibiotic susceptibility of the isolates: ciprofloxacin (5 mcg/disk), ceftriaxone (30 mcg/disk), imipenem (10 mcg/disk), chloramphenicol (10 mcg/disk), meropenem (10 mcg/disk), gentamicin (10 mcg/disk), amikacin (30 mcg/disk), clindamycin (2 mcg/disk), ampicillin (10 mcg/disk), vancomycin (30 mcg/disk), and cefepime (30 mcg/disk). Patients' demographic information, including age, sex, duration of illness, and underlying diseases, was collected by a questionnaire. Descriptive statistics were represented as mean and percentage, and Chi-square test was used for analysis. Data were analyzed using SPSS software (Version 19).

Findings

In this study, 166 patients with diabetic foot ulcers were evaluated to determine the patterns of bacterial infection and antibiotic susceptibility. The mean age of patients admitted to these hospitals was 55.813.2± years. Of 166 patients evaluated, 109 (66.4%) were male, and 57 (33.6%) were female. Also, 63 (38%) patients did not have underlying conditions, 45 (27%) had hypertension, 35 (21.1%) had cardiovascular disease, and 23 (13.9%) had kidney disease. Also 122 patients (73.4%) were diagnosed with infections caused by one type of microorganism, while the other 44 patients (26.6%) were diagnosed with polymicrobial infections. Out of 166 bacteria isolated, 81 (49%) isolates were Gram-positive, and 85 (51%) isolates were Gram-negative. In the current study, the highest frequency among Gram-positive bacteria was associated with *S. epidermidis* (20.4%), followed by *S. aureus* (14.4%) and *Enterococci* (13.8%), respectively. The most prevalent Gram-negative bacteria were *Escherichia coli* (16.9%), *Pseudomonas aeruginosa* (9.6%),

Table 1) Frequency and antimicrobial susceptibility pattern of Gram-positive bacteria isolated in this study

Antibiotics	MSSA N=12		MRSA N=12		*Staphylococcus epidermidis* N=34		*Enterococcus* N=23	
	Susceptible (%)	Resistant	Susceptible (%)	Resistant	Susceptible (%)	Resistant	Susceptible (%)	Resistant
Vancomycin	100	0	100	0	100	0	33.3	66.7
Gentamycin	100	0	100	0	57.7	43.3	23.8	76.2
Ciprofloxacin	25	75	25	75	30.3	69.7	4.3	95.7
Clindamycin	25	75	25	75	11.8	88.2	0	100
Ampicillin	-	-	0	100	20	80	40	60
Amoxicillin	0	100	0	100	0	100	0	100
Chloramphenicol	54.5	45.5	100	0	89.7	10.3	45.5	54.5

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Acinetobacter baumannii (9.6%), *Klebsiella pneumoniae* (8.3%), and *Proteus* (7.1%), respectively. Vancomycin was the most effective antibiotic against Gram-positive organisms, while they showed the highest resistance to amoxicillin. Other effective antibiotics against Gram-positive organisms are presented in Table 1. The most efficient antibiotic against Gram-negative organisms was amikacin, and the greatest resistance was observed to ceftriaxone. Table 2 lists other effective antibiotics against Gram-negative organisms.

Discussion

One of the most significant complications for people with diabetes is foot ulcers. These injuries are not restricted to specific subcutaneous tissues and are the result of uncontrolled diabetes and poor health care [9, 10]. In this study, the mean age of patients was 55.8± 13.2 years, which is similar to the results reported in other studies by Anvarnejad et al. (2015) [11] and Akhi et al. (2015) [5] in Iran as well as Sundresh et al. (2014) [12] and Shareef et al. (2018) [13] in India. In this study, the prevalence of infection was higher among males than females, which is consistent with other studies in this field [5, 11, 13, 14]. This could be due to the fact that men are more active outdoors than women and are more likely to engage in physical activity, increasing the risk of injury for men. Of all patients with diabetic foot ulcers, 62% had an underlying illness. The highest frequency was associated with hypertension (27%), followed by heart disease (21.1%) and kidney disease (13.9%), respectively. Other studies have also found that hypertension is the most important comorbidity in these patients [13, 15, 16]. In the current study, 52% of the isolated organisms were Gram-negative, and 48% were Gram-positive, which is similar to previous studies results [17, 18]. Contrary to this study results, Anvarnejad et al. (2015) in Shiraz reported a higher prevalence rate for Gram-positive bacteria [11]. Unlike most previous studies,

Table 2) Frequency and antimicrobial susceptibility pattern of Gram-negative bacteria isolated in this study

Antibiotics	*Escherichia coli* N=28		*Klesbsiella pneumoniae* N=14		*Acinetobacter baumannii* N=16		*Pseudomonas aeroginosa* N=16		*Proteus memorabilia* N=12	
	Susceptible (%)	Resistant	Susceptible (%)	Resistant	Susceptible (%)	Resistant	Susceptible (%)	Resistant	Susceptible (%)	Resistant
Imipenem	88	12	42.9	57.1	0	100	31.3	68.7	83.3	16.7
Gentamycin	79.2	20.8	50	50	13.3	86.7	26.7	73.3	60	40
Ciprofloxacin	14.8	85.2	23.1	76.9	12.5	87.5	23.3	67.7	58.3	41.7
Amikacin	88	12	71.4	28.6	6.7	93.3	43.8	56.2	100	0
Cefepim	0	100	33.3	66.7	0	100	50	50	100	0
Cefteriaxon	0	100	50	50	0	100	0	100	100	0
Meropenem	82.6	17.4	60	40	7.7	92.3	41.7	58.3	72.7	27.3
Chloramphenicol	44	56	45.5	54.5	7.1	92.9	7.1	92.9	45.5	54.5

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the most prevalent bacterium isolated in the present study was *S. epidermidis* [5, 11, 13, 19, 20]. In line with other studies in Iran, the most commonly isolated Gram-negative organism in this study was *E. coli* [5, 11, 18, 21]. All isolates were assayed for susceptibility to different antibiotics. This research showed that Gram-positive and Gram-negative bacteria were most susceptible to vancomycin as well as amikacin and imipenem, respectively. In a study by Talebi et al. (2010), all *S. aureus* isolates were susceptible to vancomycin, but resistance of *E. coli* isolates to ceftriaxone was 88%, compared to 100% resistance observed in this study [22]. In a study by Ahmadishooli et al. (2020) [23], *E. coli* isolates were most sensitive to meropenem and least sensitive to ciprofloxacin, while in the present study, *E. coli* isolates showed the highest sensitivity to amikacin and imipenem and the lowest sensitivity to ceftriaxone. In a study by Dwedar et al. (2015) in Egypt, the antibiotic susceptibility pattern was similar to that obtained in the present study, and the highest susceptibility of Gram-positive and Gram-negative bacteria was reported to vancomycin as well as imipenem and amikacin, respectively [24]. Contrary to the present study findings, Perim et al. (2015) in Brazil showed that the most effective antibiotic against both Gram-positive and Gram-negative bacteria was imipenem [8].

Conclusion

The most common bacterial infection in diabetic foot ulcers in patients admitted to Namazi and Shahid Faghihi hospitals was associated with *S, epidermis*, followed by *E. coli*. Moreover, half of *S. aureus* isolates were MRSA. Gram-positive bacteria were most susceptible to vancomycin, and Gram-negative bacteria were most susceptible to amikacin, followed by imipenem.

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