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Original research

Evaluation of aerobic bacterial etiology and antibiotic susceptibility pattern of diabetic foot infections in Rasht, the North or Iran

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Abstract

Diabetic foot ulcers (DFU) are one of the most frequently occurred complications of patients with poor-controlled diabetes mellitus. Diabetic foot infection (DFI) is increasing as a common problem and more than half of DFUs will be eventually infected. Here, we aimed to evaluate aerobic bacterial etiology and antibiotic susceptibility pattern of DFIs. This descriptive cross-sectional study was conducted at Razi educational and remedial Hospital in the North of Iran. From March to August, 2020 patients who were diagnosed as diabetic foot ulcer and attended to Razi hospital included in the study. In this study samples were collected from infected areas of diabetic foot ulcers. Standard microbiological methods were used to identify the isolates. The disc diffusion method was used to determine antimicrobial susceptibility on Mueller-Hinton agar following the Clinical and Laboratory Standards Institute (CLSI) recommendations. Thirty-one patients enrolled in this study. They included 17 females (54.8%) and 14 males (45.2%). The mean age of patients was 62.8 years, ranging from 40 to 93 years old. Totally, six types of aerobic bacteria were isolated from patients. The most prevalent type was *Escherichia coli* (41.9%), followed by *Klebsiella* spp. (16.1%). The most effective antibiotic against Gram-negative bacteria were aminoglycosides. While, *Staphylococcus aureus* isolates mostly susceptible to tested antibiotics. Also, none of the isolated *S. aureus* were methicillin-resistance. The results of antimicrobial sensitivity showed that aminoglycosides might be suitable agents for empirical therapy in Iran. Regular monitoring of culture and sensitivity reports is required to select drugs for empiric treatment.

Keywords: Diabetic foot ulcer, Wound, Antibiotic resistance, Iran

1. Introduction

Diabetic foot ulcers are one of the most frequently occurred complications of patients with poor-controlled diabetes mellitus and it leads to hospitalization of diabetic patients, which significantly increases the costs of this disease [1]. Diabetics are 25% more likely to develop diabetic foot ulcers, and almost every 30 seconds, one diabetes-affected limb occurs worldwide. Global prevalence of diabetic foot is 6.3%; however, this rate greatly varied geographically [2]. Association of several risk factors are documented with higher occurrence of diabetic foot, including male gender, type 2 diabetic, longer diabetic duration,

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elderly, low body mass index, hypertension, and smoking history [3].

Although diabetic foot ulcers are multifactorial, the most common causes are poor blood sugar control, calluses, foot deformity, improper foot care, improper footwear, peripheral neuropathy and poor blood circulation, dry skin and so on [4]. In Iran, diabetes is progressive and studies show a high prevalence of peripheral neuropathy in diabetic patients ranging from 28% to 75%. Despite the high rate of premature mortality in diabetic foot patients, they fear of severe amputation than death [5].

Diabetic foot infection is increasing as a common problem and more than half of DFUs will be eventually infected. Several studies have shown, in the development of mild infections, mono microbial causes and in severe cases, poly microbial causes have played a role [6]. Namely, aerobic Gram-positive cocci, Gram-negative bacilli (including *Pseudomonas* spp., *Escherichia coli*, *Enterobacter* spp. and *Citrobacter* spp.) are known as a common cause in mono microbial infection. Also, several anaerobes including *Bacteriodes* spp., *Peptostreptococcus* spp., *Fusobacterium* spp. and *Clostridium* spp. are reported as causes of poly microbial factors. During the last decades the inappropriate use of commercial antibiotics in humans and agriculture, along with the evolution and expansion of mobile genetic resistance elements resulting in the increase of multidrug resistance (MDR) [7].

Early diagnosis of bacterial etiology of DFIs and the targeted use of appropriate antibiotics promote wound healing and reduce the risk of amputation and mortality in diabetic patients. So we aimed to evaluate aerobic bacterial etiology and antibiotic susceptibility pattern of DFIs in Rasht, the North or Iran.

2. Materials and Methods

2.1 Study subjects

This descriptive cross-sectional study was conducted at Razi educational and remedial Hospital in the North of Iran. From March to August, 2020 patients who were diagnosed as diabetic foot ulcer and attended to Razi hospital included in the study. Both male and female patients with known diabetes at any age, regardless of their foot ulcer stage were included. The exclusion criteria were patients with immunodeficiency or who receive immunosuppressive drugs (like corticosteroids), taking broad-spectrum antibiotics for the last two days before sampling, missed information, contaminated samples and patients who do not want to be included in this study. Approved consent forms were obtained from patients. The study design was approved by the Ethics Committee of the Guilan University of Medical Sciences (Reg No. IR.GUMS.REC.1398.410) and followed the declaration of Helsinki.

2.2 Microbiological methods

In this study samples were collected from infected areas of diabetic foot ulcers. Before sampling debrided wounds with a sterile scalpel and then rinsed with normal saline. After a few minutes when normal saline was dried on wound, we used a sterile swab to collect the sample from depth of the necrotic wounds or from the lateral margin of them and then swabs were placed into sterile tubes. Samples was cut into a sterile container contained 1 mL tryptic soy broth and transferred to the laboratory as soon as possible. Following that, 10 µL of tube content was cultured into a blood agar, chocolate agar, and MacConkey agar. The blood agar and MacConkey agar plates were incubated aerobically at 37°C for 24-48 hours. At 37°C, chocolate agar plates were incubated in an atmosphere supplemented with carbon dioxide (a candle jar). Standard microbiological methods were used to identify the isolates, including morphological analysis, Gram staining, catalase, oxidase, coagulase tests, sugar fermentation, and other available biochemical tests.

2.3 Susceptibility testing

The disc diffusion method was used to determine antimicrobial susceptibility on Mueller-Hinton agar (Merck, Germany) following the Clinical and Laboratory Standards Institute (CLSI) recommendations. CLSI recommendations were followed to select antimicrobial discs (Padtan Teb, Iran), control strains, and interpretation of results for each pathogen. Plates were incubated at 35-37° C for 16-18 hours. We expounded the results of antibiotic susceptibility based on standard instructions of CLSI guidelines.

2.4 Statistical analysis

Statistical analysis was performed by the SPSS™ software (version 21). The results are presented in the form of descriptive statistics based on relative

frequency. Frequencies and percentages were used to summarize categorical variables, while median and interquartile range (IQR) values were used to describe continuous variables.

3. Results

Thirty-one patients enrolled in this study. They included 17 females (54.8%) and 14 males (45.2%). The mean age of patients was 62.8 years, ranging from 40 to 93 years old. Totally, six types of aerobic bacteria were isolated from patients. Table 1 shows the frequency and percent of each organism. The most prevalent type was Escherichia coli (41.9%), followed by Klebsiella spp. (16.1%). Antibiotic susceptibility pattern for each bacteria is presented in Table 2. The most effective antibiotic against Gram-negative bacteria were aminoglycosides. While, *Staphylococcus aureus* isolates mostly susceptible to tested antibiotics. Also, none of the isolated S. aureus were methicillin-resistance.

Table 1. The aerobic bacterial etiology of DFI

Bacteria	Frequency	Percent
E. coli	13	41.9
Klebsiella	5	16.1
Pseudomonas spp.	2	6.5
Staphylococcus aureus	2	6.5
Micrococcus spp.	1	3.2
Enterobacter spp.	1	3.2
No growth	4	12.9
Contamination	3	9.7
Total	31	100.0

4. Discussion

Foot infection in diabetic patients is a common, complex, and costly problem [8]. 20% of cases of hospitalization of diabetic patients are related to diabetic foot ulcers, and about 50% of these ulcers are likely to become infected [9]. Diabetic foot infections are usually polymicrobial, and aerobic and anaerobic bacteria are isolated from these infections [10]. Various studies have shown that *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus*, *Klebsiella*, *Enterobacter*, and *Enterococcus* are the most common bacteria isolated from diabetic foot ulcers. However, the pattern of antibiotic resistance of pathogens can vary depending on the geography, prevalence of microorganisms, and antibiotic consumption [11]. This study evaluated microbiological characteristics, and antimicrobial susceptibility of infected diabetic foot ulcers in diabetic patients referred to a hospital in North of Iran. A total of 31 patients who met the inclusion criteria were included in the study for five months (March to August 2020). Our study showed that various aerobic bacteria could be isolated from DFI samples. All our patients were older than 40 years. Most of the patients with foot infections in the study of Lipsky, Zhang, and Rastogi were older than 50 years, and in the study of Perim, they were more aged than 60 year [8, 12-14]. In general, foot lesions commonly occur among elderly patients with diabetes and those with sensory neuropathy [8]. Previous studies have shown that the prevalence of foot infection is higher among male patients than in female patients [15-17]. Differences in lifestyle, occupation, and more physical activity of men than women may cause their feet to bear more pressure [18]. However, following Perim's study, we also did not find a difference between the prevalence of infection in male and female patients, which may be due to the limited number of patients. Aerobic gram-negative bacteria (mainly Enterobacteriaceae and occasionally *Pseudomonas aeruginosa* or other gram-negative species) are usually isolated along with gram-positive cocci in patients with chronic infections [8]. In our study, the prevalence of aerobic gram-negative bacteria was 67.7%, and aerobic gram-positive bacteria was 9.7%. The most common microorganisms isolated were *Escherichia coli* and *Klebsiella*. In two studies conducted on patients with diabetic foot ulcers in India and Kuwait, aerobic gram-negative organisms (51.4% and 51.2%) and aerobic gram-positive organisms (33.3% and 32.3%) were the most common pathogens isolated respectively [19]. In other studies, most bacteria isolated from diabetic foot ulcers were Gram-negative [17, 20-22]. while 80.3% of the aerobic microorganisms isolated from DFI were gram-positive in Amini's study [23]. Similar to our results, the most common bacteria isolated in several studies was *Escherichia coli* [9, 17, 19, 23, 24]. While several studies in Iran have reported Gram-positive bacteria, especially *Staphylococcus aureus*, as the most common organism isolated from DFI [10, 18, 23, 25]. Differences in the profile of microbes infecting diabetic foot ulcers can be due to differences in environmental factors such as hygiene habits, geographic region, and study time [9, 19]. Also, the

Table 2. Antibiotic susceptibility pattern

Antibiotic	Pattern	*Enterobacterales*		*P. aeruginosa*		Staphylococci	
		No.	%	No.	%	No.	%
CFZ	S	3	15.8	-	-	-	-
	I	4	21.1	-	-	-	-
	R	12	63.2	-	-	-	-
CAZ	S	3	15.8	1	50	-	-
	I	1	5.3	0	0	-	-
	R	15	78.9	1	0	-	-
IMI	S	4	21.1	0	0	-	-
	I	3	15.8	0	0	-	-
	R	12	63.2	2	100	-	-
AMK	S	10	52.6	2	100	-	-
	I	3	15.8	0	0	-	-
	R	6	31.6	0	0	-	-
GEN	S	10	52.6	1	50	2	100
	I	1	5.3	0	0	0	0
	R	8	42.1	1	50	0	0
CIP	S	2	10.5	0	0	2	100
	I	0	0	0	0	0	0
	R	17	89.5	2	100	0	0
SXT	S	5	26.3	-	-	2	100
	I	1	5.3	-	-	0	0
	R	13	68.4	-	-	0	0
TAZ	S	7	36.8	1	50	-	-
	I	4	21.1	0	0	-	-
	R	8	42.1	1	50	-	-
PEN	S	-	-	-	-	2	100
	I	-	-	-	-	-	0
	R	-	-	-	-	-	0
ERY	S	-	-	-	-	-	0
	I	-	-	-	-	-	0
	R	-	-	-	-	2	100
TET	S	-	-	-	-	2	100
	I	-	-	-	-	-	0
	R	-	-	-	-	-	0
FOX	S	-	-	-	-	2	100
	I	-	-	-	-	-	0
	R	-	-	-	-	-	0

difference in the microbial profile of diabetic foot ulcers and their sensitivity pattern can be due to the difference in sample volume, microbial detection method, source of infection, prescribing inappropriate doses of antibiotics, arbitrary use of antibiotics, and prescribing antibiotics by non-specialists [10, 19, 23]. Based on our findings, the Enterobacteriaceae family was resistant to most of the tested antibiotics (31-89%), which is partially consistent with the results of the study conducted by Perim et al. [8]. Aminoglycosides had an acceptable sensitivity against Gram-negative bacteria, which is consistent with the results of other studies [8, 23, 24]. Therefore, this antibiotic can be suitable for use in experimental treatment. Amikacin was the most effective antibiotic against *Pseudomonas* isolates. Also, similar to our results, in some recent studies, high resistance of gram-negative bacilli to ciprofloxacin has been reported [9, 18]. Therefore, Ciprofloxacin as an experimental antibiotic may not be appropriate in this situation. It should be considered that most E. coli strains isolated are probably non-pathogenic and do not require treatment. Therefore, clinicians should focus on pathogenic organisms (especially

Staphylococcus aureus and *Pseudomonas aeruginosa*) and use high-dose and narrow-spectrum antibiotics recommended in international guidelines [18]. To prevent wound infection, proper management of diabetes, foot care, and compliance with hygiene principles are essential [24]. Antimicrobial treatment without determining the cause of infection eradicates sensitive microorganisms and can cause the selection and spread of resistant organisms [18]. Therefore, it is necessary to evaluate different micro-organisms infecting the wound on a routine basis and to know the antibiotic sensitivity patterns of infectious wound isolates in patients with a diabetic foot infection. This knowledge is crucial to achieving optimal clinical outcomes, planning treatment of these patients with appropriate antibiotics, limiting the emergence of AMR strains, and reducing healthcare costs [8, 26].

The report of the current study has limitations because this study was conducted in only one hospital in Iran and the sample size was small. Culture was not performed for anaerobic bacteria. Also, the wounds were not examined for multi-microbial or mono-microbial infections, and there was no history of previous antibiotic use by the patients. Therefore, larger-scale validation studies with more data should be conducted before generalizing the findings.

The difference in the microbial pattern of diabetic foot infection in various studies shows that experimental treatment in each country should be selected according to the most common pathogen specific to the region and its antimicrobial sensitivity. Also, considering the isolation of all types of microorganisms from these wounds, it is essential to start experimental antibacterial treatment to cover Gram-positive and Gram-negative bacteria. The results of antimicrobial sensitivity showed that aminoglycosides might be suitable agents for empirical therapy in Iran. Regular monitoring of culture and sensitivity reports is required to select drugs for empiric treatment. Similar studies can help to understand the diversity of microorganisms responsible for DFI and the antibiotic resistance pattern of each region.

Authors' contributions

Concept and Study design: HH, ME, EM, HS. Methods, data collection, and experimental work: SK, EM, HH, ME. Results analysis and conclusions: MH, MM, RT, HS. Manuscript preparation and editing: MH, MM, RT, HS. All authors read and approved the final version of the manuscript.

Conflict of interests

No potential conflict of interest was reported by the authors.

Ethical declarations

The study design was approved by the ethical committee at the Guilan University of Medical Sciences [IR.GUMS.REC.1398.410].

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