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Original Article

Determination of the Resistance Pattern of Prevalent Aerobic Bacterial Infections of Diabetic Foot Ulcer

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ABSTRACT

Background and Objective: **Diabetes mellitus is one of the main problems in health systems in the world. Diabetic Foot infection (DFI) is one of the main complications and the most cause of non-traumatic lower limb amputation. This study aimed to determine the prevalence of bacteria involved in DFI and their antibiotic resistance in patients with DFI diagnosis.**

Material and Methods: **This descriptive-analytical and cross-sectional study was designed from 2007 to 2010 on 90 patients in Shahid Mostafa Khomeini Hospital, Tehran, Iran. For bacteriological analysis, all wound samples culture grown by standard methods of bacteriology and disk diffusion method was used for antibiogram. Patient's clinical and epidemiologic data were collected from recorded file. The data were analyzed using SPSS16 statistical software.**

Results: **Totally, 104 bacteria were isolated from 90 patients. 57.70% were Gram-positive and 42.30% were Gram-negative. Among Gram-positive bacteria, *Staphylococcus aureus* (60%) and Enteroccoci spp.(33.3%) and among gram-negative bacteria *E. coli* (47.73%), *Pseudomonas* aroginosa (22.73%) and *Proteus spp*. (18.18%) were the most common isolates respectively. Of isolates 75% were resistant to two antibiotics or more. Previous antibiotic therapy was significant risk factor for multidrug resistant (MDR) infections (P: 0.003). All Gram-positive isolates were sensitive to vancomycin, imipenem and amikacin had good activity against Gram-negative bacteria.**

Conclusion: **Infection with MDR bacteria in patients with diabetic foot ulcers is high and has significant association with recent antibiotic therapy. So the proper use of antibiotics in order to prevent the creation of multi-drug resistant bacteria is recommended.**

Keywords: **Diabetic Feet Infections, Bacteriology, Antibacterial Drug Resistance**

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Introduction

Diabetes mellitus (DM) is one of the main problems in health systems in Iran and the world. In worldwide are more than 140 million patients with diabetes (1). World Health Organization expects this number to increase to 300 million patients by 2025 and to 366 million by 2030 (1-3).

One of the main, common, late onset and costly complications of this disease is diabetic foot ulcers (DFU), considered the major cause of disability and hospitalization in patients with DM (4).

According to existing data, 15% of patients with DM during their lifetime suffered from the DFU (5, 6). Twenty percent cause of hospital admissions in patients with DM is diabetic foot ulcers (7). Overall, 60% of the cases of non-traumatic lower limb amputation occur in patients with DM (2, 3). The mean expense of recovery for a simple uninfected ulcer, an infected wound, and that of a great amputation surgery is $8,000, $17,000, and $45,000 respectively. More than 80,000 ablation surgeries are performed yearly on diabetic patients in the USA, and ~ 50% of the patients with amputations will suffer DFU in the opposite extremity in next 18 months. An alarming 58% have an opposite side amputation 3–5 years after the first ablation surgery. As well as, the 3-year deaths rate after a first amputation has been assessed about 20–50%, and these rates have not altered much in the 30 years ago, contrary to enormous promotion in the medical and surgical management of patients with diabetes (8).

Risk factors for diabetic foot ulcers include: 1) External factors: small and thermal trauma, smoking, alcoholism, inadequate control of blood sugar and lack of patient cooperation (1). 2) Internal factors: male sex, peripheral neuropathy, joint deformities caused by motor dysfunction, vascular insufficiency, diabetes duration, age and previous history of foot ulcers (9). Due to the increased rate of lower limb amputations, and psychological and movement problems that occur on the lives of this patients,

Accurate research to suggest possible and low cost methods for prevention and treatment will be necessary. In this regard, recognition of microorganisms causing infection is necessary to choose appropriate treatment regimen (1). The most common microorganisms in diabetic foot infections (DFI), are aerobic gram positive coccies particularly *Staphylococcus aureus* and beta hemolytic *Sreptococcus* species (2, 3). In cases of chronic and deep foot ulcers with tissue necrosis and gangrene or in patients with recent antibiotic treatment has failed, infection usually occurs with 5-3 different species of bacteria (3, 9, 10) as well as to gram-positive bacteria, Gram-negative bacteria, including *E. coli, Proteus* and *Klebsiella* species, (3) and anaerobic bacteria including *Peptostreptococcus, Bacteroides***,** etc, are involved (3,11). Antibiotic susceptibility tests results have shown that vancomycin is the most effective antibiotics against Gram-positive bacteria and imipenem is the most effective antibiotics against Gram-negative organisms (12).

This study aimed to determine the microorganisms involved in DFIs, and their antibiotic sensitivity or resistance, and association between blood sugar control as well as multi drug resistant infection, DM duration, previous history of foot ulcer or recent antibiotic therapy.

Materials and Methods

This was a descriptive-analytical and cross-sectional study of all patients admitted to Shahid Mostafs Khomeini Hospital with DFI diagnosis, Tehran, Iran, from April 2007 to 2010.

All patients with negative wound culture were excluded. Then, in patients with positive wound culture factors such as age, sex, duration of DM, previous history of DFI, resent antibiotic therapy, status of blood glucose levels control (HgbA1c), was obtained. All samples culture grown by standard methods bacteriology and disk diffusion method was used for antimicrobial susceptibility testing. Thioglycoollate culture media was used for cultivation and isolation of

obligate and facultative anaerobe microorganism. A microorganism was classified as multi drug resistant if it was resistant to two or more classes of antimicrobials.

The data were analyzed using SPSS16 statistical software and chi-square and one way ANOVA test. We considered differences significant at $P < 0.05$.

Results

Of 90 patients with DFI and positive wound cultures 52.2% were male. The average age was 62.06± 1.21 yr with range between 37-88 yr. Age and sex distribution of patients is shown briefly in Table 1.

Table 1: Age and sex distribution of patients with diabetic foot ulcers infection

Age (yr)	Male	Female	Total
≤40	1	0	1
41-50	9	10	19
51-60	9	10	19
61-70	15	10	25
71-80	12	10	22
≥81	1	3	4
total	47	43	90

Average years of DM duration was 15.66 ± 7.98 (from 1 year to 35) years and in 70% of patients blood sugar was uncontrolled (HgbA1c>7%).

62.2% of patients had previous history of developing diabetic foot ulcer infection and 55.4% had a history of recent antibiotic therapy in last 30 days. Totally, 104 aerobe pathogens were isolated from 90 patients, which on average, each ulcer had 1.15 bacteria. More than one microorganism was isolated from 22.2% of patients. No anaerobe microorganism was grown from wound culture. Of 104 isolated bacteria, 60 (57.7%) were Gram-positive and 44 bacteria (42.3%) Gram-negative. The most common isolated bacteria were: *S. aureus* 34.6%; *E. coli* 20.2%*; Enterococcus* spp**.** 19.2 % and *Pseudomonas Aeroginosa* 9.6%**.** The number and percent of organisms isolated from different specimens are summarized in Table2.

Table 2: Bacteria isolated from diabetic foot infections

Bacteria	NO. (%)
Gram-positive bacteria	
Staphylococcus aureus	36(34.6)
Enterococcus spp.	20(19.2)
Streptococcus spp.	4(3.9**)**
Gram-negative bacteria	
E. coli	21(20.2)
Pseudomonas Aeroginosa	10(9.6)
Proteus spp.	8(7.7)
Other gram negative bacteria	5(4.8)
Total	104(100)

66.7% of *S. aureus* isolates were methicillin-resistant (MRSA).

All *S. aureus* isolates were sensitive to vancomycin. The frequency of antibiotic resistance of

Gram-positive bacteria is given in Table 3. More than 80% of Gram-negative bacteria were susceptible to imipenem**.** The frequency of antibiotic resistance of Gram-negative bacteria is given in Table 4.

Seventy-five percent of isolates were resistant to two antibiotics or more. There was a significant relationship between the frequency of MDR microorganisms and recent antibiotic therapy (*P*: 0.003). There was no significant association between the frequency of multidrug-resistant microorganisms with duration of DM, controlled blood sugar and previous foot ulcer.

Table 3: Pattern of antibiotic resistance in gram-positive bacteria isolated from DFI*

Antibiotics	*Enterococcus* spp. No. (%)	*Staphylococcus aureus* No. (%)	*Streptococcus* spp. No. (%)
Amicacin	15(75)	16(44.4)	-
Cefotaxime	8(40)	19(52.8)	-
Cephalotin	19(95)	25(69.4)	0(0)
Ciprofloxacin	7(35)	19(52.8)	0(0)
Clindamycin	17(85)	23(63.9)	0(0)
Co-Trimoxazole	18(90)	27(75)	1(25)
Chloramphenicol	9(45)	5(13.9)	-
Cloxacillin	-	24(66.7)	-
Gentamicin	12(60)	19(52.8)	-
Imipenem	18(20)	24(66.7)	0(0)
Penicillin	18(90)	36(100)	0(0)
Vancomycin	0(0)	0(0)	0(0)

*Diabetic Foot Infection

Table 4: Pattern of antibiotic resistance in gram-negative bacteria isolated from DFI*

Antibiotic	*Proteus spp.* No. (%)	*Pseudomonas Aeroginosa* No. (%)	*E. coli* No. (%)	Other gram negative No. (%)
Amikacin	1(12.5)	2(20)	5(23.8)	60))3
Ampicillin	7(87.5)	10(100)	20(92.2)	100))15
Cehpalotine	7(87.5)	10(100)	18(85.7)	100))5
Cefteriaxon	2(25)	5(50)	13(61.9)	100))5
Cefotaxime	0(0)	9(90)	12(57.1)	80))4
Ceftazidime	2(25)	12(20)	12(57.1)	60))3
Ciprofloxacin	3(37.5)	1(10)	9(42.9)	60))3
Co-Trimoxazole	6(75)	8(80)	3(14.3)	60))3
Chloramphenicole	6(75)	9(90)	4(19)	100))5
Gentamicin	2(25)	3(30)	9(42.9)	40))2
Imipenem	0(0)	2(20)	4(19)	20))1
Tetracycline	4(50)	9(90)	11(52.4)	60))3

*Diabetic Foot Infection

Discussion

In our study, 22.2% of wounds contained polymicrobial infections. Totally 64 patients were infected with one microorganism and polymicrobial infections was present in 20 patients. An average of 1.15 types of bacteria

was isolated from each wound which is lower compared to other studies (13-15). In other studies poly microbial infection in the wounds was 83.8% and 64.4% respectively (10, 16). Multiple bacterial infections have been reported in 43% and 46% respectively (13, 17). This difference could be due to the absence of severe and deep wounds, and also the low virulence of micro-organisms isolated in our study.

Organisms with low virulence such as *S. aureus, Streptococcus viridance, S. epidermidis*, *Enterococcus* spp. and certain Gram-negative bacteria cause 2/3 of the mild infections in diabetic foot ulcers (18). Gram-positive bacteria were the predominant isolates in our study (57.7%). 80.3% of aerobic and 57.2% of anaerobic isolated microorganisms were Gram-positive, which is in agreement with our study (16). The increased prevalence of *Enterococcus* spp. in our study might be due to previous use of antibiotic. Other studies had similar results (7, 17). In some studies, Gram-negative bacteria were reported higher than Gram-positive bacteria in DFU (15, 19). In our study, from isolated Gram-negative bacteria, *E. coli* (47.73%), *P. Aeroginosa* (22.73%) and *Proteus spp.* 18%) respectively had the highest frequency, while in other studies, *P. Aeroginosa , Proteus* spp.*, Escherichia coli* and *Proteus* spp. , respectively had the highest frequency (7, 20).

Hospitalization, resent use of broad-spectrum antibiotics, history of surgery and chronic wounds, can prone the patients to infection with antibiotic-resistant organisms like MRSA and vancomycin-resistant *Enterococcus* spp. (VRE) and *P. aeruginosa*. A British study compared the prevalence of pathogenic organisms isolated from foot ulcer in diabetic patients at a diabetic foot clinic. MRSA was isolated in 30.2% of the patient in 2001 almost double the proportion carrying this organisms in 1998 (21). DFI rate with MRSA has been 56% (20) while this rate was higher in our study and 66.7% of isolated *S. aureus* were MRSA. This difference could be due to the incorrect or contamination of the sampling producer, inappropriate antibiotic therapy or the overall increase in incidence of MRSA in our population study. Antimicrobial susceptibility testing results of this study showed that 75% of isolated microorganisms were resistant to two or more types of antibiotic. This rate compared with another study shows more multi-drug resistant (MDR) infections (17). The most probable explanation for this difference is that the incorrect use of antibiotics, incorrect diseases identifying, incorrect doses of antibiotics, inappropriate treatment duration (less or more than been recommended time), arbitrary use of antibiotics, prescription of antibiotics by unaware persons, inappropriate formulation and low quality of some of antibiotics in our population study.

In our study, infection with microorganisms resistant to multiple drugs was associated with history of recent antibiotic therapy. In those infected with multidrug-resistant microorganisms' use of antibiotics within last 1 month were more than others. A similar result was obtained by ozlem kandrmir *et al.* (22). All isolated Gram-positive bacteria sensitive to vancomycin, imipenem and amikacin had acceptable sensitivity against Gram-negative bacteria. That this is compatible with results of other studies (12, 13, 15).

Conclusion

We found that fewer antibiotic alone were able to cover all microorganisms are involved in diabetic foot ulcers and determine the wound culture and antibiotic susceptibility test of isolated microorganisms are required for proper treatment. We also believe previous antibiotic therapy is significant risk factor for infections with multidrug resistant microorganisms. So the proper use of antibiotics in order to prevent the creation of multi-drug resistant bacteria is recommended.

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