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Original Article

Microbial Profile and Antibiotic Susceptibility Pattern in Diabetic Patients with Mild, Moderate, and Severe Foot Infections in Tehran

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Abstract

It is estimated that 10-25% of diabetic patients will encounter diabetic foot ulcers (DFU) during their lifetime. This study evaluated the microbiology of DFUs and determined the antibiotic resistance pattern of bacterial isolates based on the severity of wounds and infections in different grades of ulcer. The specimens were collected from115 diabetic foot infections (DFI) deep tissue by needle aspiration and biopsy. The aerobic and anaerobic cultures and antimicrobial susceptibility testing were carried out. The presence of resistance genes including metallo-beta-lactamases (MBL), extended-spectrum β-lactamase (ESBL), *erm*A, *erm*C, and *mec*A was also determined. A total of 222 microorganisms were isolated. The prevalence of poly-microbial infections was 69.6%. Bacterial isolates comprised 64.2% Gram-positive bacteria (GPB), 33.5% Gram-negative bacteria (GNB), and five isolates of anaerobic bacteria were also detected. The most prevalent GPB and GNB were *Staphylococcus spp.* (52.2%) and *Escherichia coli* (33.3%), respectively. The prevalence of poly-microbial infections and GNB was positively associated with increased grades of Wagner and IDSA classifications. Among *Staphylococcus aureus* isolates, resistance to clindamycin (73.5%), ciprofloxacin (70.6%), and erythromycin (70.6%) were noticeable. GNB was also highly resistant to cephalosporins and ciprofloxacin. ESBL genes were detected in approximately 40% of isolates of Enterobacteriaceae. The prevalence of *erm*A, *erm*C, and *mec*A genes in *S. aureus* isolates were 8.8%, 32.3%, and 14.7%, respectively. In conclusion, our data suggest that GPBs are the most common isolates from DFIs. Furthermore, with the development of wounds and infection, the prevalence of GNB in DFIs are increased.

Keywords: Foot infections, Antimicrobial Resistance, *S. aureus*, ESBL

1. Introduction

The prevalence of Diabetes Mellitus (DM) and its health care cost is constantly growing. One of the most critical complications of DM is diabetic foot ulcer (DFU) which appears in 10-25% of patients throughout their lifetime (1). Almost half of the DFUs turn into infectious wounds, and if they are left untreated, the subsequent problems would be sepsis, lower-extremity amputation, and death (2).

Diabetic foot infections (DFI) are classified as mild, moderate, and severe stages according to the Infectious Diseases Society of America (IDSA) system and grades 0-5 based on the Meggitt-Wagner classification (3, 4). In “mild” DFUs or lower grades of ulcers, mono-

microbial infections with Gram-positive bacteria (GPB) are common (2, 5-7). Conversely, in chronic and "moderate/severe" DFIs and patients with a history of antibiotic treatment, the predominant isolates are Gram-negative bacteria in combination with other pathogens (8, 9). Different bacteria can be involved in DFIs, such as Gram-positive cocci, particularly *Staphylococcus spp.*, members of Enterobacteriaceae and *Pseudomonas aeruginosa* (10).

One of the significant problems associated with DFIs is the prompt diagnosis of infectious agents and appropriate selection for treatment. So, delay in diagnosis or treatment may prolong the healing process of chronic wounds. Chronic wound treatment imposes a tremendous economic burden on the health system. Additionally, the subsequent need for broad-spectrum antibiotics will make the patient's management more complicated. On the other hand, the emergence of multidrug-resistant (MDR) isolates such as extended-spectrum β-lactamase (ESBL) producing bacteria and methicillin-resistant *S. aureus* (MRSA) due to the long-term use of antibiotics or a history of recurrent infections and hospitalization needs to be concerned in managing of DFIs.

Despite these problems in DFIs management, there is currently little information on the prevalence and types of bacteria in different grades of diabetic ulcers in the Iranian population, in which the prevalence of DFIs is high.

The present study aims to provide information regarding the pathogens isolated from different diabetic foot ulcers and assess their antibiotic resistance based on the severity of wounds.

2. Materials and Methods

2.1. Study Design and Patients

This cross-sectional descriptive research was conducted on 115 patients, including 66 outpatients and 49 inpatients, with clinically DFIs admitted to a Tehran university of medical sciences (TUMS) teaching hospital from June 2018 to December 2019. The selected population was patients with type I or II diabetes who had DFIs.

The diagnosis of foot infection was determined using the criteria as recommended by the International Working Group on the Diabetic Foot (IWGDF) Guideline (11), and the information on laboratory markers related to inflammation was acquired from the patient's medical records.

The severity of the foot ulcer was classified using Meggitt-Wagner, and the severity of DFI was assessed based on IDSA/IWGDP classification systems (3, 4, 11). All wounds were categorized as Neuropathic (N), Ischemic (I), and Neuro-Ischemic (NI) DFU, and the status of chronic wounds were assessed according to Martinengo (12). Abnormal peripheral neuropathy was defined with the Semmes-Weinstein 10-g monofilament test, and the presence of PAD (peripheral arterial disease) was confirmed by ABI (Ankle Brachial Index) test and Doppler ultrasonography (4). The diagnosis of osteomyelitis (OM) approved by the combination of PTB (Probe to Bone) test, the results of inflammation markers and bone culture (8).

2.2. Sampling of Wounds and Microbiological Cultures

As reported by IDSA recommendation(4), the wounds were cleaned with sterile normal saline first, then the necrotic tissues and calluses were removed to avoid skin microbiota contamination. The specimens were collected from deep tissue and the base or margin of the wound by needle pus aspiration of infected wounds.Curettage/biopsy was used for wounds without purulent exudate. The samples were sent to the microbiology laboratory for aerobic, and anaerobic culturing within 30 minutes after collection and inoculated on thioglycolate broth, 5% sheep blood agar, and brucella blood agar supplemented with hemin and vitamin K. For anaerobic bacteria, phenotypic identification of isolates was done by the VITEK®2 microbial identification system (bioMérieux, Marcy l' Etoile, France) based on the manufacturer's instructions.

2.3. Antibiotic Susceptibility Pattern

The antibiotic resistance pattern of bacteria was tested by disk agar diffusion (DAD) as recommended by the Clinical and Laboratory Standards Institute (CLSI)(13). A disk containing antibiotics (Mast Group, Merseyside, UK) was used to determine the susceptibility of bacterial isolates according to the CLSI guidelines. *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were control strains. Isolates that showed resistance to at least one agent in three or more antimicrobial categories were considered (14). The ESBLs genes (*bla*SHV, *bla*TEM, *bla*OXA, *bla*KPC), Metallo-beta-lactamases (MBLs) (*bla*VIM), *erm*A, *erm*Cand*mec*A genes were detected by Polymerase chain reaction (PCR) using specific primers (15-17).

2.4. Statistical Analysis

All statistical analyses were performed using SPSS 25.0 software (IBM Inc., USA). Continuous variables are described as mean ± SD, and categorical variables are demonstrated as (%) of indicator value. The chi-square or two-sided Fisher's exact test was employed to identify significant discrimination between intended categorical variables. It was regarded as statistically significant whether the two-side *P*-value < 0.05.

3. Results

3.1. Patient Demographical Outcomes

One hundred fifteen patients were involved in this study, including 83 males (72.2%) and 32 females (27.8%). As it is shown in table 1, the majority of patients (85.2%) were affected by type II diabetes, and 71.3% of patients had poor blood glucose control (HbA1c ≥ 7.5%). Among all diabetic patients, 32 (27.8%) used insulin therapy alone or in combination with oral medications to control blood sugar levels. The most common comorbidities associated with patients were dyslipidemia and heart disease, observed in 67% and 56.5% of patients, respectively. In 67 patients (58.3%), white blood cell count was >10.000 /µL and 71 patients (61.7%) had an erythrocyte sedimentation rate >70 mm/h.

Table 1. Demographic and clinical feature of patients

Patients (N=115)	N (%)
Demographic	
Mean Age (SD; Min-Max)	59.3 (12.1; 15-8)
Male Gender[a]	83 (72.2)
BMI (kg/m2)[b]	26.8±4.3
Mean Year of DM (SD; Min-Max)	16.3 (8.7; 0.5-35)
Type of diabetes[a]	
Type I	17 (14.8)
Type II	98 (85.2)
Insulin use[a]	32 (27.8)
HbA1c (%)[b]	7.8±1.1
Comorbidities[a]	
Hypertension	55 (47.8)
Renal Failure	29 (25.2)
Dyslipidemia	77 (67)
Heart disease	65 (56.5)
Death	5 (4.3)
Laboratory Data[b]	
ESR (mm/hr)	80.4±33.9
CRP (mg/dL)	58.37±29.1
WBC (K/µL)	11.09±3.5

a: Expressed as Number (%), b: Expressed as mean±SD, BMI: Body Mass Index, DM: Diabetes Mellitus, HbA1c: Hemoglobin A1c, CRP (C- Reactive Protein), ESR (Erythrocyte Sedimentation Rate), WBC (White Blood Cell count)

3.2. Characteristics of Wounds and Isolated Microorganisms

The DFIs were mainly organized in the moderate to severe groups (Wagner's 3~5 grades), and in 61(53.0%) patients, a history of previous ulcers was registered. Of the 115 clinical samples processed, 222 microorganisms, including 215 (96.8%) bacteria and seven (3.2%) funguses, were obtained (Table 2). Bacterial isolates consisted of 138 (64.2%) GPB and 72 (33.5%) GNB. Most GPB were *Staphylococcus spp.*(52.2%, 72 /138), followed by *Enterococcus spp.* (22.5%, 31/138). The most prevalent GNB bacteria were Enterobacteriaceae, including *E. coli* (47.1%, 24/51), *Klebsiella spp.* (19.6%, 10/51), *Proteus spp.* (15.7%, 8/51), *Citrobacter spp.* (13.7%, 7/51) and *Enterobacter spp.* (3.9%, 2/51). Five anaerobic bacteria were also detected, including two *Clostridium spp.*, two *Bacteroides spp.*, and one *Peptoniphilus spp.*.

Among 51 isolates of Enterobacteriaceae, 44 (86.3%) and 36 (70.6%) isolates were identified as moderate/severe and Wagner's 3, 4-grade ulcers,

respectively (Table 2). These results match those observed for *P. aeruginosa* that typically infected moderate/severe DFIs (90.5%) and Wagner's 3 and 4 grades wounds (85.8%). No *P. aeruginosa* isolates were recovered from mild infections. Among 34 *S. aureus* isolates, 29 (85.3%) and 25 (73.5%) strains were isolated from Wagner's G2 and G3 grade and mild/moderate infections, respectively. No *S. aureus* strains were detected from Wagner's 5 grade. Moreover, the more significant number of *Enterococcus spp.* isolates (58.1%) were detected from Wagner's 4 and 5 grades.

The prevalence of poly-microbial infections and mono-microbial infections was 69.6% (80/115) and 30.4% (35/115), respectively (Table 3). The most prevalent poly-microbial infection was observed in moderate/severe (87.6%) DFIs and Wagner's 3, 4 grade (77.6%), respectively. The prevalence of Enterobacteriaceae (88.2%), *P. aeruginosa* (90.5%), *S. aureus*, and *Enterococcus spp.* isolates in poly-microbial infections were considerably higher than in mono-microbial infections.

The frequency of OM in our study was 40.9% (47 cases); nearly all of them belonged to moderate/severe infections, and only one case with bone involvement belonged to mild infections. The lower extremity amputation was done for 30 (26.1%) patients. A considerable number of patients (66.7%) had Wagner's 4 and 5 grades ulcers, and all patients with Wagner's 5-grade were forced to amputate lower limbs (Table 4).

Table 2. Distribution of microorganisms in DFIs classified in different systems

	Type of DFU			*Wagner's Grade*				IDSA Grade		
Microorganisms, N (%)	**I**	**N**	**NI**	***G2***	***G3***	***G4***	***G5***	**Mild**	**Moderate**	**Severe**
Coagulase Negative Staphylococci	3 (7.9)	14 (36.8)	21 (55.3)	10 (26.3)	12 (31.6)	13 (34.2)	3 (7.9)	7 (18.4)	9 (23.7)	22 (57.9)
Staphylococcus aureus	1 (2.9)	19 (55.9)	14 (41.2)	15 (44.1)	14 (41.2)	5 (14.7)	0	9 (26.5)	16 (47.0)	9 (26.5)
Enterococcus spp.	3 (9.7)	7 (22.6)	21 (67.7)	4 (12.9)	9 (29.0)	14 (45.2)	4 (12.9)	3 (9.7)	8 (25.8)	20 (64.5)
Corynebacterium spp.	0	7 (38.9)	11 (61.1)	3 (16.7)	7 (38.9)	6 (33.3)	2 (11.1)	2 (11.1)	5 (27.8)	11 (61.1)
Streptococcus spp.	0	10 (58.8)	7 (41.2)	5 (29.4)	6 (35.3)	5 (29.4)	1 (5.9)	3 (17.6)	8 (47.1)	6 (35.3)
Escherichia coli	2 (8.3)	8 (33.3)	14 (58.4)	3 (12.5)	10 (41.7)	6 (25)	5 (20.8)	3 (12.5)	10 (41.7)	11 (45.8)
Pseudomonas aeruginosa	1 (4.8)	8 (38.1)	12 (57.1)	2 (9.5)	11 (52.4)	7 (33.3)	1 (4.8)	0	9 (42.9)	12 (57.1)
Klebsiella spp.	0	5 (50)	5 (50)	2 (20)	4 (40)	4 (40)	0	1 (10)	5 (50)	4 (40)
Proteus spp.	4 (50)	1 (12.5)	3 (37.5)	1(12.5)	3 (37.5)	3 (37.5)	1(12.5)	1(12.5)	4 (50)	3 (37.5)
Citrobacter spp.	0	3 (42.9)	4 (57.1)	2 (28.6)	0	4 (57.1)	1 (14.3)	2 (28.6)	2 (28.6)	3 (42.8)
Enterobacter spp.	0	0	2 (100)	0	1 (50)	1 (50)	0	0	1 (50)	1 (50)
Anaerobe	0	2 (40)	3 (60)	0	2 (40)	3 (60)	0	0	2 (40)	3 (60)
Fungi	0	2 (28.6)	5 (71.4)	1 (14.3)	2 (28.6)	3 (42.8)	1 (14.3)	0	1 (14.3)	6 (85.7)
Total	14 (6.3)	86 (38.7)	122 (55)	48 (21.6)	81 (36.5)	74 (33.3)	19 (8.6)	31 (14)	80 (36)	111 (50)

Table 3. The relation between Wagner's and IDSA grade and poly-microbial infection

Type of Infection	**IDSA/IWGDP Grade**				**Wagner's Grade N (%)**				
	Mild n=35	**Moderate n=45**	**Severe n=35**	***P value***	**G2 n=36**	**G3 n=38**	**G4 n=33**	**G5 n=8**	***P value***
Mono-microbial	25 (71.4)	6 (13.3)	4 (11.4)	0.000	26 (72.2)	5 (13.2)	4 (12.1)	0	0.000
Poly-microbial	10 (28.6)	39(86.7)	31 (88.6)		10 (27.8)	33 (86.8)	29 (87.9)	8 (100)	

Table 4. Predisposing factors related to the severity of wound and infection associated with OM, Amputation, and Death in patients

Classification of wounds	**Osteomyelitis N= 47**		**Amputation N= 30**		**Death N= 5**	
	N (%)	***P value***	**N (%)**	***P value***	**N (%)**	***P value***
Wagner's Grade						
G2	2 (4.3)	0.000	3 (1)	0.000	0 (0)	0.024
G3	4 (8.5)		7 (23.3)		0 (0)	
G4	33 (70.2)		12 (40)		4 (80)	
G5	8 (17.0)		8 (26.7)		1(20)	
IDSA/IWGDP Grade						
Mild	1 (2.1)	0.000	2 (6.7)	0.000	0 (0)	0.043
Moderate	13 (27.7)		11 (36.7)		1 (20)	
Severe	33 (70.2)		17 (56.7)		4 (80)	

3.3. Profile of Antibiotic Resistance

According to the definition of MDR isolates, 90.2% of Enterobacteriaceae, 67.6% of *S. aureus*, 61.9% of *P. aeruginosa*, and 16.1% of *Enterococcus spp.* were MDR strains. Tables 5 and 6 presents the DAD method's results from the phenotypic antibiotic susceptibility tests.

The high rates of antibiotic resistance in Enterobacteriaceae were against ampicillin (88.2%), ciprofloxacin (80.4%), and amoxicillin-clavulanate (72.5%). Moreover, high resistance to cephalosporins such as ceftazidime, cefepime, and ceftriaxone was observed. The MDR strains were isolated from moderate/severe DFIs and Wagner's 3 and 4 grades wounds.

For *P. aeruginosa*, the resistance to cefepime, amikacin, aztreonam, gentamicin, ceftazidime, and piperacillin-tazobactam was approximately 50%, and for ciprofloxacin, it was 71%. The MDR strains were detected from Wagner's 3, 4 grade and moderate/severe infections.

Among *S. aureus*, resistance to clindamycin (73.5%), ciprofloxacin (70.6%), and erythromycin (70.6%) were notable. The most effective antibiotics were linezolid and rifampin. Methicillin resistance in *S. aureus* was seen in nine (26.5%) of 34 isolates. The MDR strains were identified in Wagner's 2, 3 grade, and most infections were moderate.

The high resistance rates to rifampin (58.1%), penicillin (41.9%), and ampicillin (38.7%) were observed in *Enterococcus spp.*, while for other antibiotics such as linezolid, teicoplanin, and vancomycin, the frequency of susceptible strains was more than resistant strains. Additionally, a total of 12 isolates were resistant to teicoplanin and vancomycin.

Table 5. Antibiotic resistance pattern of Gram-negative bacteria

Antibiotics	Resistance N (%)					
	***Escherichia coli* n=24**	***Klebsiella spp.* n=5**	***Citrobacter spp.* n=7**	***Enterobacter spp.* n=2**	***Proteus spp.* n=8**	***Pseudomonas aeruginosa* n=21**
Ampicillin	21 (87.5)	10 (100)	7 (100)	2 (100)	5 (62.5)	-
Penicillin	-	-	-	-	-	-
Cefepime	18 (75)	7 (70)	3 (42.86)	1 (50)	2 (25)	11 (52.4)
Ceftriaxone	15 (62.5)	7 (70)	4 (57.14)	2 (100)	2 (25)	-
Ceftazidime	16 (66.67)	7 (70)	6 (85.71)	2 (100)	2 (25)	10 (47.6)
Cefoxitin	-	-	-		-	-
Amoxicillin-clavulanate	17 (70.83)	7 (70)	6 (85.71)	2 (100)	5 (62.5)	-
Piperacillin-tazobactam	5 (20.83)	6 (60)	4 (57.14)	0	2 (25)	9 (42.9)
Amikacin	2 (8.33)	3 (30)	1 (14.28)	1 (50)	2 (25)	11 (52.4)
Gentamicin	8 (33.33)	4 (40)	2 (28.57)	2 (100)	2 (25)	10 (47.6)
Tetracycline	16 (66.67)	5 (50)	4 (57.14)	1 (50)	8 (100)	-
Trimethoprim-sulfamethoxazole	18 (75)	6 (60)	4 (57.14)	1 (50)	6 (75)	-
Ciprofloxacin	21 (87.5)	7 (70)	6 (85.71)	1 (50)	6 (75)	15 (71.4)
Imipenem	7 (29.17)	3 (30)	3 (42.86)	1 (50)	3 (37.5)	10 (47.6)
Aztreonam	-	-	-	-	-	11 (52.4)

Table 6. Antibiotic resistance pattern of Gram-positive bacteria

Antibiotics	**Resistance N (%)**	
	S. aureus	***Enterococcus spp.***
Total Isolates	34	31
Ampicillin	-	12 (38.7)
Penicillin	-	13 (41.9)
Cefoxitin	9 (26.5)	-
Gentamicin	4 (11.8)	-
Tetracycline	20 (58.8)	-
Trimethoprim-sulfamethoxazole	4 (11.8)	-
Ciprofloxacin	24 (70.6)	-
Clindamycin	25 (73.5)	-
Erythromycin	24 (70.6)	-
Rifampin	3 (8.8)	18 (58.1)
Linezolid	0	2 (6.5)
Vancomycin	-	5 (16.1)
Teicoplanin	-	7 (22.6)

The *bla*SHV, *bla*OXA, and *bla*TEM genes were detected in 20 (39.2%), 15 (29.4%) and 13 (25.5%) of Enterobacteriaceae respectively. Moreover, *bla*KPC and *bla*VIM genes were not detected in any isolates. Among 51 isolates of Enterobacteriaceae, 14 strains (27.4%) simultaneously had two ESBL genes. Consequently, these results, along with the phenotypic determination of antibiotic susceptibility test, showed that most ESBL-positive strains had notable resistance to ampicillin, cefepime, ceftazidime, and ceftriaxone.

The frequency of *bla*SHV, *bla*OXA, *bla*TEM and *bla*VIM genes in *P.aeuroginosa* was (4.7%), (9.5%), (4.8%) and (19.1%) respectively. One ESBL-producing *P. aeruginosa* with three resistance genes simultaneously, including *bla*SHV, *bla*OXA, and *bla*VIM, was isolated from a 75-old female diagnosed unexpectedly with DFU two days before the test, whose wound was classified to Wagner's 2 grade/Moderate infections. Interestingly, her wound infection was polymicrobial with *P.aeruginosa*, *E. coli* (*bla*SHV, *bla*OXA positive), and *S. aureus*.

The *erm*A, *erm*C, and *mec*A genes were detected in 3 (8.8%), 11 (32.3%), and 5 (14.7%) of *S. aureus* isolates, respectively. Among isolates containing *erm*A or *erm*C genes, nine *S. aureus* strains were simultaneously resistant to clindamycin and erythromycin.

4. Discussion

This study has provided new data on the prevalence and antimicrobial resistance patterns of bacteria isolated from DFU in Tehran, Iran. In our study, the most frequent isolated bacteria (64.2%) were Gram-positive, especially *Staphylococcus spp.* that match with the previously reported data in other populations such as the UK (63%), Portugal (60%) and another study in Iran (62.1%) (18-20). In the current study, the frequency of *S. aureu*s decreased with the severity of the wound (*P*=0.017) and severity of infection (*P*=0.006) which is similar to reports from Saudi Arabia and China (5, 7). In concordance with the mentioned studies showing that *S. aureus* is the most prevalent GPB isolated from Wagner's grade 1 and 2 ulcers, in the current study, *S. aureus* was more common in mild/moderate infections and Wagner's grade 2 and 3 wounds. In this way, Saeed, Esposito (10) reported that mild infections are associated with GPB, while in moderate/severe infections, GPB, along with Gram-negatives, has a more important role.

In contrast with our observation, some reports revealed that the prevalence of GNB in diabetic wounds is higher than GPB, particularly in Asian and African countries (21-23), which may reflect the differences in the geographical area and the health system of the countries with the treatment and control of DFIs. One of the important results of this study was the presence of GNB (77.3%) in Wagner's 3-5 grades, which is similar to the results of Wu, Pan (7) (72.8%) and Xie, Bao (23) (53.6%). In our study, there was a significant relationship between the severity of the infection and GNB (*P*=0.02).In other words, the detection of Enterobacteriaceae and *P. aeruginosa* increased with a greater level of IDSA and Wagner classification.

The prevalence of poly-microbial infection in this study (69.6%) was higher than those reported in China (59.6%) and Mexico (48.3%) (23, 24). In line with other studies (7, 21), our study showed that the higher frequency of polymicrobial infections was in severe wounds and Wagner's grade 4 and 5 ulcers. We observed that as Wagner's grade and severity of infection increased, the possibility of polymicrobial infection was significantly raised (*P*=0.000). It is predicted that the extended use of antibiotics, immense necrosis, and gangrene condition of the chronic wound in severe infections provide the tendency for the presence of several bacterial isolates simultaneously (25, 26).

It is important to highlight that 20-60% of DFIs may lead to OM (10). In this study, the rate of OM and amputation was significantly higher in severe DFIs and increased Wagner's grade wounds (*P*=0.000). In a gradual process, more severe infections are more likely to develop into subcutaneous and bone tissue. Unlike hematogenous OM, DFIs-related OM occurs due to recurrent and chronic wound infections and bacteria or

products (toxins) penetration into deeper tissues (27, 28). The prevalence of mild/moderate DFIs-related OM is about 10-20%, whereas this percentage is even higher (50-60%) in severe diabetic foot infections (29).

The next major issue about severe DFIs is the ineffectiveness of antibiotic treatment and wound healing due to vascular problems and immune system failures, which eventually force the physicians to amputate the injured limbs (30). Similar to Rastogi, Sukumar (31), we observed that as the severity of infection increased, the possibility of amputation was significantly raised (P=0.000). A systematic review in 2020 remarked that the fate of twenty percent of patients with moderate/severe foot infections was amputation (32). Moreover, the presence of GNB in wounds, OM, vascular diseases, Wagner's 4, 5-grade ulcers, and IDSA 3, 4 grades was mentioned as risk factors for amputation (33).

According to the article, the mortality rate is higher in diabetic patients with OM (10), all five patients who died in this study had OM, and an amputation procedure was performed for them.

The resistance rate of *S. aureus* to clindamycin (73.5%), ciprofloxacin (70.6%), and erythromycin (70.6%) were noticeable. This probably could be explained by the fact that in our country, these are the first choices of antibiotics for mild infections. This finding agrees with Najari's research which reported that the resistance rate of GPB to ciprofloxacin, clindamycin, and erythromycin was 62.7%, 80.5%, and 82.1%, respectively (20). Furthermore, another study from Malaysia and China reported high rates of resistance to these antibiotics (21, 23). Therefore, it should be noted that empiric antibiotics for diabetic wounds are at risk of becoming useless. Although the prevalence of MRSA in this study (26.5%) was lower than those reported in Portugal (43.2%) and Iran (44%) (19, 20), in a meta-analysis published in 2019, the prevalence of MRSA in DFIs was reported 16.8% (34).

In the current study, GNB were highly resistant to ciprofloxacin. Additionally, in agreement with other studies from Iran (20), which have reported a high resistance rate to ceftazidime (87.5%), cefepime (83.3%), and ceftriaxone (59.3%), GNB in our study were also highly resistant to cephalosporins. In recent years, many reports of GNB resistance to third and fourth-generation cephalosporins (19, 21, 23, 24). Interestingly, the presence of ESBL genes in our study in approximately 40% of Enterobacteriaceae isolates can limit the effect of many cephalosporins in treating DFIs. Similar studies in China and Portugal have reported 40% and 57% of ESBL genes, respectively (7, 19). A promising point in this study was the lower resistance of GNB to amikacin, imipenem, and piperacillin-tazobactam, which can still be used as treatment options.

The evidence of antibiotic susceptibility testing indicated that the empiric regimen used in this area, including clindamycin and ciprofloxacin for mild DFIs and cephalosporins and carbapenems for moderate/severe infections, had probably resulted in the emergence of resistant strains. Based on our findings, several antibiotics frequently used for empiric therapy would not be optimal in DFIs treatment. So, locally evaluating the bacterial profile and antibiotic susceptibility can be effective in selecting the appropriate treatment.

We faced some limitations in this study, which we can mention as follows: first, inaccessible history of antibiotic use in patients especially hospitalized patients, which could help assess resistant bacterial strains. Second, we examined a limited population of DFIs patients in two diabetic centers in Iran. So, more research on this topic needs to be undertaken locally before generalizing the microbiological information and resistance patterns. Third, although the number of severe wounds in the IDSA classification system was proper, the number of specimens with Wagner's 5 grade (completely ischemic and necrotic wounds) did not reach the quorum.

5. Conclusion

In conclusion, our data showed that GPB was the most common isolate in diabetic foot ulcers. The prevalence of

poly-microbial infections was high (69.6%) and associated with the severity of wounds and DFIs. Additionally, the prevalence of GNB and ESBL-producing Enterobacteriaceae in moderate/severe DFIs was high. We observed that empiric therapy with clindamycin, ciprofloxacin, and cephalosporins might result in resistant strains of GPB and GNB. In mild infections, careful sampling is essential to find the correct infectious agent, distinguish infections from colonization, and prevent antibiotic resistance. To treat moderate to severe diabetic wound infections, the presence of multidrug-resistant bacteria (ESBL-positive GNB and MRSA) and their antibiotic sensitivity pattern should be considered.

Authors' Contribution

E. T. conducted the experiments, collected data, and drafted the manuscript. M. M. F., M. R. M. T., and R. B. participated in the study's design and advised in all parts. M. E. and F. J. supervised all parts of the study. All authors read and approved the final manuscript.

Ethics

The Ethics Committee approved the study of Tehran University of Medical Sciences (IR.TUMS.MEDICINE.REC.1398.509), and all participants were informed about the working process and endorsed consent forms.

Conflict of Interest

The authors declare that they have no conflict of interest.

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