Arch Pediatr Infect Dis. 2017 April; 5(2):e37680. doi: 10.5812/pedinfect.37680.

Published online 2016 September 6. **Research Article**

Diabetic Foot Infections: Antibiotic Susceptibility Patterns and Determination of Antibiotic Cross-Resistance in Clinical Isolates of *Enterococcus* Species During 2012 - 2014 in Shiraz, Iran

Mojtaba Anvarinejad,[1] Gholamreza Pouladfar,[1] Aziz Japoni,[1] Shahram Bolandparvaz,[2] Zeinab Satiary,[3] and Jalal Mardaneh[4,*]

[1]Prof. Alborzi Clinical Microbiology Research Center, Nemazee Hospital, Shiraz University of Medical Sciences, Shiraz, IR Iran
[2]Head of Trauma Research Center, Shiraz University of Medical Sciences, Shiraz, IR Iran
[3]General Surgery Ward, Nemazee Hospital, Shiraz University of Medical Sciences, Shiraz, IR Iran
[4]Department of Microbiology, School of Medicine, Gonabad University of Medical Sciences, Gonabad, IR Iran

[*]*Corresponding author*: Jalal Mardaneh, Department of Microbiology, School of Medicine, Gonabad University of Medical Sciences, Gonabad, IR Iran, E-mail: Jalalmardaneh@yahoo.com

Received 2016 March 07; **Revised** 2016 August 03; **Accepted** 2016 August 20.

Abstract

Background: Diabetic foot infections (DFIs) are an increasingly common public health problem and are associated with mortality and morbidity. The incidence of *Enterococci* in DFIs, a leading cause of hospital admission in Iran, has been increasing, possibly due to previous antibiotic use.

Objectives: The aims of this study were 1) isolation of bacteria from diabetic patients with foot ulceration, 2) characterization of the isolated bacteria, 3) confirmation of *Enterococci* and their genus, 4) determination of the susceptibility profile of the isolates, and 5) survey of the cross-resistance among *Enterococcus* spp.

Methods: A total of 86 diabetic patients with foot ulceration were investigated during 2012 - 2014 in Nemazee hospital (Shiraz, Iran). Swabs were collected from diabetic ulcers. For the isolation of bacteria, microbiological media were used. Colonies were further characterized using various biochemical tests (e.g., catalase test, oxidase reaction, growth on bile esculine [BE] agar, growth in the presence of 6.5% NaCl, growth at 45°C, motility, pyrrolidonyl arylamidase [PYR], yellow pigment, arginine dihydrolase [ADH], and sugars fermentation). Antibiotic susceptibility testing was done by standard disc diffusion method, according to the CLSI protocols. Detection of vancomycin-resistant *Enterococcus* (VRE) was performed by BHI agar screen plate.

Results: In the current study, a total of 86 diabetic patients were investigated. *Enterococcus* spp. were isolated from 34 (39.5%) patients consisting of 20 males (59%) and 14 females (41%). Twenty-five (73.5%) patients received antibiotic treatment on admission. Fifty (44.1%) cases had random blood sugar ranging between 130 - 300, and 19 (55.9%) had blood sugar of 300 - 450. Of the 34 patients, 15 (44.1%) had type 1 diabetes and 19 (55.9%) had type 2 diabetes. *Enterococcus* faecalis was the most common isolated *Enterococcus* spp. (50%). Linezolid was the most effective antibiotic against *Enterococcus* isolates, and ciprofloxacin was the least effective.

Conclusions: Our data showed that resistance to vancomycin among *Enterococcus* spp. isolates is emerging. Knowledge of the causative microorganisms in DFIs and their antibiotic susceptibility profiles is essential for proper treatment and infection eradication.

Keywords: *Enterococcus* spp., Diabetic Foot Infections, Antibiotic Susceptibility, Antibiotic Cross-Resistance

1. Background

Diabetic foot infections (DFIs) are a frequent clinical problem. Diabetic extremity ulcers develop in almost 15% of people with diabetes and are a leading cause of hospital admission and amputation among this group of patients, while 85% of major leg amputations begin with a foot ulcer in the diabetic population (1, 2). Diabetic foot ulcers (DFU) are more prone to different bacterial infections that spread quickly, leading to irreversible tissue injury. Many microorganisms, alone or as part of a polymicrobial infection, can cause DFIs, of which non-spore forming, gram-positive cocci such as *Enterococci* are the most common bacteria (3).

Foot infections in individuals with diabetes are an increasingly common public health problem and are associated with mortality and morbidity. DFIs followed by amputations contribute significantly not only to the morbidity among diabetic persons, but are also associated with severe clinical dumps and remarkably increased mortality rates (2, 4).

Previous studies point toward gram-positive cocci, such as members of the *Enterococcus* genus, as the most common pathogens in DFI samples, contributing to the

persistence or severity of the disease and leading to higher morbidity and mortality rates (5).

Over the past few decades, a major problem in treating diabetic foot infections has been the increased isolation rate of antimicrobial-resistant bacteria, especially methicillin-resistant *Staphylococcus aureus* (MRSA) and, to a lesser degree, glycopeptides-intermediate *S. aureus* (GISA), vancomycin-resistant *Enterococci* (VRE), extended-spectrum β-lactamases (ESBLs), or carbapenamase-producing, gram-negative bacilli. The isolation rates of these multidrug-resistant (MDR-resistance to at least three unrelated antibiotic classes) bacteria vary widely according to geographic areas and treatment centers. The potential presence of such MDR isolates emphasizes the importance of ideal sample collection for bacterial culture and antimicrobial susceptibility testing for the infected DFIs, as well as preventing the excessive antimicrobial use that drives this resistance (6, 7). The prevalence of *Enterococci* in DFIs has been increasing. This increased incidence might be due to prior antibiotic use (8, 9).

2. Objectives

The aims of this study were the isolation and characterization of *Enterococci* from diabetic patients with foot ulceration, confirmation of *Enterococci* and their genus, determination of the susceptibility profile of the isolates, and survey of the cross-resistance among *Enterococcus* isolates.

3. Methods

3.1. Patients

A total of 86 diabetic patients with foot ulcers were surveyed during 2012 - 2014 at Nemazee Hospital in Shiraz, Iran. The study population was defined as the total number of patients with type 1 diabetes mellitus (DM) and type 2 DM with foot ulcers (i.e., suspected infection according to physician decision) at initial visit and admission to hospital. Information regarding patients' demographic and clinical features was gathered.

3.2. Isolation, Characterization, and Confirmation of Isolates

Swabs were collected from diabetic ulcers that were macroscopically examined and classified (10). Swabbing was performed on sloughy or inflamed tissue, as bacteria tend to present in greater number in such areas. From each patient, two swabs (for isolation of bacteria and wet mount microscopy) were obtained. The sterile cotton-tipped swab was moistened with sterile normal saline before sample collection. One of the prepared swabs was used for the isolation of bacteria. The other was used for wet mount microscopy. For the isolation of bacteria from collected specimens, the microbiological media used were blood, chocolate, and MacConkey agar, which were incubated for 16 - 18 hours at 35°C. Representative bacterial colonies recovered after incubation were sub-cultured on blood agar plates, which were incubated at 35°C in the presence of 5% CO2 for 24 hours (5).

The cultural characteristics of bacterial isolates on the agar plates were examined. The characterization and identification methods for the bacteria were carried out by standard procedures. Gram staining and cell morphology from air-dried, heat fixed smears were performed. The motility of the isolates was surveyed by hanging drop (HD) technique. Bacterial colonies were further characterized by different biochemical diagnostic tests, including catalase test, oxidase reaction, growth on bile esculine (BE) agar, growth in the presence of 6.5% NaCl, growth at 45°C, motility, pyrrolidonyl arylamidase (PYR), yellow pigment, and arginine dihydrolase (ADH). Final identification of different species of the *Enterococcus* genus was conducted by sugar fermentation tests (i.e., glucose, mannitol, sorbose, arabinose, sorbitol, raffinose, sucrose, and pyruvate).

3.3. Susceptibility Testing

The bacterial isolates were subjected to antibiotic sensitivity testing on Muller-Hinton agar by the Kirbey-Bauer standard disc diffusion method (11). All inoculated plates were incubated for 16 - 18 hours in ambient air incubators at 35°C, and the results were recorded by measuring the zone of inhibition, according to the Clinical and Laboratory Standards Institute (11) protocols. Susceptibility of *Enterococcus* isolates was tested for clindamycin (CD, 2 μg), erythromycin (E, 15 μg), linezolid (LZD, 30 μg), penicillin G (PG, 10 μg), co-trimoxazole (TS, 1.25/23.75μg), rifampicin (RP, 5 μg), oxacillin (OX, 1 μg), ciprofloxacin (CIP, 5 μg), gentamicin 120 (GMH, 120 μg), ceftriaxone (CRO, 30 μg), cefixime (CFM, 5 μg), vancomycin (VA, 30 μg), gentamicin (GM, 10 μg), ampicillin (Ap, 10 μg), imipenem (IMP, 10 μg), cefepime (CPM, 30 μg), cefoxitin (FOX, 30 μg), chloramphenicol (C, 30 μg), cefotaxime (CTX, 30 μg), ceftizoxime (CZX, 30 μg), azithromycin (ATH, 15 μg). *E. faecalis* ATCC 29212 was used as quality control.

3.4. Detection of Vancomycin-Resistant Enterococcus (VRE) by BHI Agar Screen Plate

All *Enterococcus* isolates were examined for reduced vancomycin susceptibility by agar incorporation. Ten μL of a 0.5 McFarland bacterial suspension (final concentration =10^6 CFU/mL) was spotted on the brain heart infusion (BHI)

agar (Merck, Germany), containing 6μg/mL vancomycin, allowed to air dry for almost five minutes, and incubated at 35°C (11). Culture plates were examined at 24 and 48 hours of incubation for any discernible growth.

4. Results

In the current study, a total of 86 diabetic patients were investigated. *Enterococcus* spp. were isolated from 34 (39.5%) patients. The cases consisted of 20 males (59%) and 14 females (41%), aged between 28 - 85 years. In this study, 34 strains of *Enterococcus* were isolated from diabetic foot patients referred to Nemazee Hospital (Shiraz, Iran). Diabetic foot patients' weights ranged from 45 to 100 kg, with the maximum number of cases in the weight group of more than 70 kg (n = 20, 59%) (Table 1). Nine (26.4%) patients had higher than general education. Twenty-five (73.5%) patients received antibiotic treatment on admission (24 patients received clindamycin and ciprofloxacin, 1 patient received cephalexin). Fifty (44.1%) patients had random blood sugar ranging between 130 - 300, and 19 (55.9%) had blood sugar of 300 - 450. Of the 34 patients, 15 (44.1%) had type 1 diabetes, 19 (55.9%) had type 2 diabetes (Table 2), and one patient (2.9%) died.

Table 1. Demographic Features of the Diabetic Foot Patients Infected with *Enterococcus* spp

Category	Results (%)
Gender	
Male	20 (58.8)
Female	14 (41.2)
Age, years	28 - 85
Weight, kg	45 - 100
< 75	1 (2.9)
50 - 75	13 (38.3)
> 70	20 (58.8)
Education	
Higher than general education	9 (26.4)
General education	10 (29.4)
Lower than general education	15 (44.2)

Table 2. Distribution of the Diabetic Patients Infected with *Enterococcus* spp. in Terms of the Antibiotic Treatment, Diabetic Type, Blood Sugar, Ulcer Size, Ulcer Type, Amputation, and Risk Factor (n = 34)

Category	Results (%)
Antibiotic treatment	
Clindamycin and ciprofloxacin	24 (70.6)
Cephalexin	1 (2.9)
No antibiotic	9 (26.5)
Diabetic type	
Type 1	19 (55.8)
Type 2	15 (44.2)
Random blood sugar range	
130 - 300	15 (44.2)
300 - 450	19 (55.8)
Ulcer size	
> 4 mm	24 (70.6)
< 4 mm	10 (29.4)
Ulcer type	
Osteomyelitis	6 (17.6)
Gangrene	7 (20.6)
Cellulitis	7 (20.6)
Neuroischemic ulcer	6 (17.6)
Ischemic ulcer	4 (11.8)
Abscess	4 (11.8)
Amputation	14 (41.1)
Risk factor	
Vascular diseases	16 (47)
Hypertension	15 (44.1)
Retinopathy	6 (17.6)
Osteomyelitis	6 (17.6)
Neuropathy	5 (14.7)
Nephropathy	5 (14.7)
Isolated *Enterococcus* spp.	
E. faecalis	17 (50)
E. faecium	16 (47)
E. mundetti	1 (2.9)
Vancomycin-resistant *Enterococcus*	5 (14.7)

Enterococcus faecalis was the most common isolated *Enterococcus* spp. (50%). According to the in vitro antibiotic susceptibility testing, linezolid was the most effective antibiotic against *Enterococcus* isolates (all isolates [100%] were sensitive) and ciprofloxacin was the least effective drug (all isolates [100%] were resistant) (Figure 1). Susceptibility rates for vancomycin, imipenem, ampicillin, and gentamicin 120 were 79.4%, 64.7%, 58.8%, and 50%, respectively, and the susceptibility rate for erythromycin, rifampicin, ceftriaxone, chloramphenicol, cefotaxime, and ceftizoxime was 14.7% (Figure 1). Analysis of cross-resistance results revealed that more than 85% of the isolates were resistant to macrolides and 24 (70.5%) to chlo-

ramphenicol, clindamycin, erythromycin, azithromycin, and gentamicin (Table 3).

Table 3. Antibiotic Cross-Resistance Patterns of *Enterococcus* spp. Isolated from Diabetic Foot Patients (n = 34)[z]

Pattern	Antibiotic Resistance Patterns	Number (%)
A	E, ATH	29 (85.3)
B	CPM, CTX, CZX, CFM, CRO	28 (82.3)
C	E, ATH, CD, RP	25 (73.5)
D	C, CD, E, ATH, GM	24 (70.5)
E	GM, GM120	16 (47)
F	CIP, AP, GM, GM120	9 (26.5)
G	GM, GM 120, AP	9 (26.5)
H	IMI, VA, CIP, AP	6 (17.6)
I	VA, AP, GM	5 (14.7)
J	VA, RP, IMI	5 (14.7)
K	VA, AP, GM, GM120	4 (11.7)
L	VA, AP, GM, GM120, IMI	4 (11.7)
M	CD, VA, RP, IMI, AP,GM, GM120	4 (11.7)

Abbreviations : ATH, azithromycin; AP, ampicillin; C, chloramphenicol; CD, clindamycin; CFM, cefixime; CIP, ciprofloxacin; CPM, cefepime; CRO, ceftriaxone; CTX, cefotaxim; CZX, ceftizoxime; E, erythromycin; GM, gentamicin; GM 120, gentamicin high dose; IMI, imipenem;; RP, rifampicin; VA, vancomycin.

5. Discussion

Most DFIs have a polymicrobial etiology, with enterococcal strains being part of the complex diabetic foot microbiota. Previous studies point toward the *Enterococcus* genus as one of the most common gram-positive pathogenic bacteria in DFI samples, contributing to the persistence or severity of the disease and leading to higher morbidity and mortality rates (5).

All DFI *Enterococci* present gelatinolytic, hemolytic, and biofilm forming (which contribute to the chronicity of infection) abilities. Since the screened virulence traits are considered among the most relevant for enterococcal pathogenicity mechanisms, often detected in clinical isolates and correlated with the persistence and severity of infection (5, 12). The choice of accurate antimicrobial depends on an accurate evaluation of sepsis severity, credible microbiologic data, and consideration of host factors, such as renal and vascular impairment (13). Lower extremity infections are a serious cause of morbidity and mortality in persons with diabetes mellitus (DM) (2). Microbiologically, diabetic foot infections are generally polymicrobial, but in this study, we focused on diabetic ulcers contaminated with *Enterococcus*. *Enterococcus faecalis* and *Enterococcus faecium* were the most common isolated *Enterococcus* species from diabetic foot infections (DFI) in the present study.

The results of antimicrobial susceptibility testing showed that linezolid is the most effective agent against *Enterococcus* spp. Third and fourth generation cephalosporins were ineffective against more than 82% of *Enterococci* isolates. According to CLSI recommendations, for *Enterococcus* spp., cephalosporins, aminoglycosides (except for high-level resistance screening), clindamycin, and trimethoprim-sulfamethoxazole may appear active in vitro, but are not effective clinically and should not be reported as susceptible (11).

Ciprofloxacin was the least effective drug against isolates; therefore, it should not be used empirically as a single agent. The data analysis indicates that antibiotics such as gentamicin, used extensively in the treatment of different infections caused by *Enterococcus* spp. in hospitals, were active only against about 20.6% of total *Enterococcus* species tested, but gentamicin 120 was effective against 50% of the isolates. In some studies, rifampicin exhibited good activity against *Enterococcus* species (14), but this was not the case in this study, in which only 18% of the isolates showed sensitivity. Approximately 85% of *Enterococcus* species were resistant to erythromycin, rifampicin, ceftriaxone, chloramphenicol, cefotaxime, and ceftizoxime, in contrast to studies of diabetic foot isolates in Saudi Arabia (15).

Given the alarming types of resistance (i.e., resistance to vancomycin) among *Enterococcus* spp. (16), our data showed that resistance to the vancomycin tested was found to be 20.6% among *Enterococcus* spp. As revealed, significant resistance to gentamicin 120 (50%) and imipenem (35.3%) were alarming. The analysis results of cross-resistance showed that 85.3% were resistant to macrolides. Four (11.4%) isolates were co-resistant to common antibiotics (including vancomycin, ampicillin, and gentamicin) used for the treatment of infections with *Enterococcus*.

Knowledge of the causative microorganisms (such as bacteria) in diabetic foot infections (DFI) and their antibimicrobial susceptibility profiles is essential for appropriate treatment and infection eradication. In patients with serious infections, the antibiotic therapy may have to be initiated empirically to prevent systemic invasion by infecting bacteria in a formerly debilitated patient while awaiting microbiology laboratory results (17).

In the present study, *Enterococci* were found in 39.5% of the patients, which is higher, compared to the report by Citron (39.5% versus 35.7%) (18). Our results for carbapenem (imipenem) resistance among *Enterococcus* spp. (35.3%) are not in agreement with some reports from Cit-

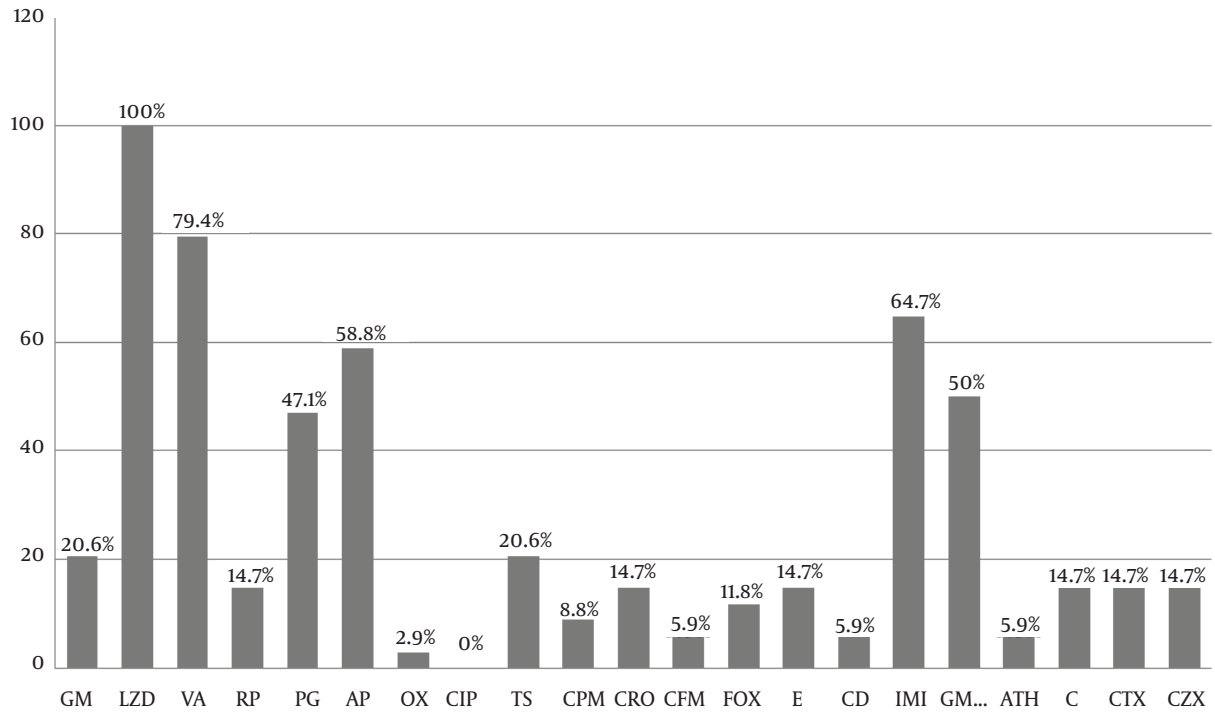

Figure 1. Antibiotic susceptibility patterns of *Enterococcus* spp. isolated from diabetic foot patients (n = 34).

ron et al., which showed resistance to other carbapenems (ertapenem) to be 90% (18). *Enterococcus* spp. may show different response to members of the carbapenem class of antibiotics. Clinicians should consider the results of bacterial culture and susceptibility testing in the light of the clinical outcome of the infection for the empirical therapy regimen. Knowledge of the characteristics of infection, i.e., the type of bacteria commonly found and the clinical evidence of infection, can help in choosing an appropriate antibiotic, even if the culture reports are not available at the time of initiation of antibiotic therapy (19, 20).

In our study, 58.8%, 79.4%, and 47.1% of isolates were susceptible to ampicillin, vancomycin, and penicillin, respectively. In El-Tahawy (15), the *Enterococci* were fully sensitive to ampicillin and vancomycin, while 16% were resistant to penicillin. This may be due to factors such as the differences in treatment regimens used for infected patients in healthcare settings. Also, the majority of antibiotics are used in regional agricultural settings and food-producing animals; therefore, different resistance patterns can emerge and spread globally. In the current study, isolates with resistance to quinolones were seen, consistent with what was reported by Goldstein and colleagues (21).

It should also be noted that, in many cases, antimicrobial resistance is transmitted to the human population, hospitalized patients, and the hospital environment through other sources including animals, plant-based foods, fish, poultry, and other industries in which antibiotics are used for different purposes and may lead to emerging resistant strains of bacteria (22-25). The multidrug resistant (MDR) status attributed to the majority of the *Enterococci* continues to be highly relevant, especially in chronic severe *Enterococci* infections such as DFIs, since antimicrobial resistance often results in treatment failure. The presence of MDR diabetic foot ulcer *Enterococci* is of major importance, also due to the possibility of transmitting those MDRs to other bacteria sharing the same ecological niche, highly impairing the implementation of successful antibiotic therapy (5).

5.1. Conclusion

Isolation, identification, and antimicrobial susceptibility of pathogens can be helpful in optimizing antimicrobial use. Because these bacteria are often resistant to the prescribed antimicrobials, the physicians must decide if the superiority of clinical and laboratory evidence suggests they are invasive pathogens that require targeted antibiotic therapy. If the patient with DFI has not adequately responded to the empirical therapy regimen, treatment

should be broadened to include all recovered microorganisms.

Acknowledgments

Our thanks go to Hassan Khajehei, PhD, for copyediting of the manuscript.

Footnotes

Authors' Contribution: All co-workers listed have contributed sufficiently to the project to be included as authors, and all those who are qualified to be authors are listed in the author byline. To the best of our knowledge, no conflict of interest, financial or other, exists.

Funding/Support: This work was supported by a research grant from the Professor Alborzi clinical microbiology research center (PACMRC).

References

1. Lipsky BA, Richard JL, Lavigne JP. Diabetic foot ulcer microbiome: one small step for molecular microbiology . . . One giant leap for understanding diabetic foot ulcers?. *Diabetes.* 2013;**62**(3):679–81. doi: 10.2337/db12-1325. [PubMed: 23431007].
2. Dowd SE, Wolcott RD, Sun Y, McKeehan T, Smith E, Rhoads D. Polymicrobial nature of chronic diabetic foot ulcer biofilm infections determined using bacterial tag encoded FLX amplicon pyrosequencing (bTEFAP). *PLoS One.* 2008;**3**(10):3326. doi: 10.1371/journal.pone.0003326. [PubMed: 18833331].
3. Lipsky BA. Empirical therapy for diabetic foot infections: are there clinical clues to guide antibiotic selection?. *Clin Microbiol Infect.* 2007;**13**(4):351–3. doi: 10.1111/j.1469-0691.2007.01697.x. [PubMed: 17359317].
4. Ismail K, Winkley K, Stahl D, Chalder T, Edmonds M. A cohort study of people with diabetes and their first foot ulcer: the role of depression on mortality. *Diabetes Care.* 2007;**30**(6):1473–9. doi: 10.2337/dc06-2313. [PubMed: 17363754].
5. Semedo-Lemsaddek T, Mottola C, Alves-Barroco C, Cavaco-Silva P, Tavares L, Oliveira M. Characterization of multidrug-resistant diabetic foot ulcer enterococci. *Enferm Infecc Microbiol Clin.* 2016;**34**(2):114–6. doi: 10.1016/j.eimc.2015.01.007. [PubMed: 25704893].
6. Lipsky BA, Berendt AR, Cornia PB, Pile JC, Peters EJ, Armstrong DG, et al. 2012 Infectious Diseases Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. *Clin Infect Dis.* 2012;**54**(12):132–73. doi: 10.1093/cid/cis346. [PubMed: 22619242].
7. Anvarinejad M, Pouladfar G, Japoni A, Bolandparvaz S, Satiary Z, Abbasi P, et al. Isolation and Antibiotic Susceptibility of the Microorganisms Isolated from Diabetic Foot Infections in Nemazee Hospital, Southern Iran. *J Pathog.* 2015;**2015**:328796. doi: 10.1155/2015/328796. [PubMed: 26843987].
8. Ki V, Rotstein C. Bacterial skin and soft tissue infections in adults: A review of their epidemiology, pathogenesis, diagnosis, treatment and site of care. *Can J Infect Dis Med Microbiol.* 2008;**19**(2):173–84. [PubMed: 19352449].
9. Peters EJ, Lipsky BA, Aragon-Sanchez J, Boyko EJ, Diggle M, Embil JM, et al. Interventions in the management of infection in the foot in diabetes: a systematic review. *Diabetes Metab Res Rev.* 2016;**32 Suppl 1**:145–53. doi: 10.1002/dmrr.2706. [PubMed: 26344844].
10. Bondor CI, Veresiu IA, Florea B, Vinik EJ, Vinik AI, Gavan NA. Epidemiology of Diabetic Foot Ulcers and Amputations in Romania: Results of a Cross-Sectional Quality of Life Questionnaire Based Survey. *J Diabetes Res.* 2016;**2016**:5439521. doi: 10.1155/2016/5439521. [PubMed: 27019852].
11. CLSI . Clinical and Laboratory Standards Institute. 31. United States: Clinical and Laboratory Standards Institute; 2011. pp. M100–S21.
12. Tiwari S, Pratyush DD, Dwivedi A, Gupta SK, Rai M, Singh SK. Microbiological and clinical characteristics of diabetic foot infections in northern India. *J Infect Dev Ctries.* 2012;**6**(4):329–32. [PubMed: 22505442].
13. Grayson ML. Diabetic foot infections. Antimicrobial therapy. *Infect Dis Clin North Am.* 1995;**9**(1):143–61. [PubMed: 7769215].
14. Senneville E, Yazdanpanah Y, Cazaubiel M, Cordonnier M, Valette M, Beltrand E, et al. Rifampicin-ofloxacin oral regimen for the treatment of mild to moderate diabetic foot osteomyelitis. *J Antimicrob Chemother.* 2001;**48**(6):927–30. [PubMed: 11733482].
15. El-Tahawy AT. Bacteriology of diabetic foot. *Saudi Med J.* 2000;**21**(4):344–7. [PubMed: 11533815].
16. Shaghaghian S, Pourabbas B, Alborzi A, Askarian M, Mardaneh J. Vancomycin-Resistant Entrococci colonization in chronic hemodialysis patients and its risk factors in southern Iran (2005-2006). *Iran Red Crescent Med J.* 2012;**14**(10):686–91. [PubMed: 23285424].
17. Anvarinejad M, Japoni A, Rafaatpour N, Mardaneh J, Abbasi P, Amin Shahidi M, et al. Burn Patients Infected With Metallo-Beta-Lactamase-Producing Pseudomonas aeruginosa: Multidrug-Resistant Strains. *Arch Trauma Res.* 2014;**3**(2):18182. doi: 10.5812/atr.18182. [PubMed: 25147779].
18. Citron DM, Goldstein EJ, Merriam CV, Lipsky BA, Abramson MA. Bacteriology of moderate-to-severe diabetic foot infections and in vitro activity of antimicrobial agents. *J Clin Microbiol.* 2007;**45**(9):2819–28. doi: 10.1128/JCM.00551-07. [PubMed: 17609322].
19. Karakus A, Ozkan M, Karcioglu M, Ozden R, Ustun I, Caliskan K, et al. Diabetic foot due to anaphylactic shock: a case report. *Arch Trauma Res.* 2014;**3**(2):17610. doi: 10.5812/atr.17610. [PubMed: 25147776].
20. Poorabbas B, Mardaneh J, Rezaei Z, Kalani M, Pouladfar G, Alami MH, et al. Nosocomial Infections: Multicenter surveillance of antimicrobial resistance profile of Staphylococcus aureus and Gram negative rods isolated from blood and other sterile body fluids in Iran. *Iran J Microbiol.* 2015;**7**(3):127–35. [PubMed: 26668699].
21. Goldstein EJ, Citron DM, Nesbit CA. Diabetic foot infections. Bacteriology and activity of 10 oral antimicrobial agents against bacteria isolated from consecutive cases. *Diabetes Care.* 1996;**19**(6):638–41. [PubMed: 8725864].
22. Mardaneh J, Soltan-Dallal MM. Isolation and Identification of E. cowanii from Powdered Infant Formula in NICU and Determination of Antimicrobial Susceptibility of Isolates. *Iran J Pediatr.* 2014;**24**(3):261–6. [PubMed: 25562018].
23. Soltani J, Poorabbas B, Miri N, Mardaneh J. Health care associated infections, antibiotic resistance and clinical outcome: A surveillance study from Sanandaj, Iran. *World J Clin Cases.* 2016;**4**(3):63–70. doi: 10.12998/wjcc.v4.i3.63. [PubMed: 26989670].
24. Anvarinejad M, Pouladfar GR, Pourabbas B, Amin Shahidi M, Rafaatpour N, Dehyadegari MA, et al. Detection of Salmonella spp. with the BACTEC 9240 Automated Blood Culture System in 2008 - 2014 in Southern Iran (Shiraz): Biogrouping, MIC, and Antimicrobial Susceptibility Profiles of Isolates. *Jundishapur J Microbiol.* 2016;**9**(4):26505. doi: 10.5812/jjm.26505. [PubMed: 27284396].
25. Mardaneh J, Soltan Dallal MM. Isolation and Identification Enterobacter asburiae from Consumed Powdered Infant Formula Milk (PIF) in the Neonatal Intensive Care Unit (NICU). *Acta Med Iran.* 2016;**54**(1):39–43. [PubMed: 26853289].