Major Article

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Aerobic bacterial profile and antibiotic resistance in patients with diabetic foot infections

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ABSTRACT

Introduction: This study aimed to determine the frequencies of bacterial isolates cultured from diabetic foot infections and assess their resistance and susceptibility to commonly used antibiotics. **Methods:** This prospective study included 41 patients with diabetic foot lesions. Bacteria were isolated from foot lesions, and their antibiotic susceptibility pattern was determined using the Kirby-Bauer disk diffusion method and/or broth method [minimum inhibitory concentration (MIC)]. **Results:** The most common location of ulceration was the toe (54%), followed by the plantar surface (27%) and dorsal portion (19%). A total of 89 bacterial isolates were obtained from 30 patients. The infections were predominantly due to Gram-positive bacteria and polymicrobial bacteremia. The most commonly isolated Gram-positive bacteria were *Staphylococcus aureus*, followed by *Staphylococcus saprophyticus*, *Staphylococcus epidermidis*, *Streptococcus agalactiae*, and *Streptococcus pneumoniae*. The most commonly isolated Gram-negative bacteria were *Proteus* spp. and *Enterobacter* spp., followed by *Escherichia coli*, *Pseudomonas* spp., and *Citrobacter* spp. Nine cases of methicillin-resistant *Staphylococcus aureus* (MRSA) had cefoxitin resistance, and among these MRSA isolates, 3 were resistant to vancomycin with the MIC technique. The antibiotic imipenem was the most effective against both Gram-positive and Gram-negative bacteria, and gentamicin was effective against Gram-negative bacteria. **Conclusions:** The present study confirmed the high prevalence of multidrug-resistant pathogens in diabetic foot ulcers. It is necessary to evaluate the different microorganisms infecting the wound and to know the antibiotic susceptibility patterns of the isolates from the infected wound. This knowledge is crucial for planning treatment with the appropriate antibiotics, reducing resistance patterns, and minimizing healthcare costs.

Keywords: Diabetic foot infection. Polymicrobial infections. Multidrug-resistant organisms.

INTRODUCTION

Diabetes is a chronic disorder that affects a large number of people globally and is a major public health problem(1). Approximately one-fourth of people with diabetes will develop an ulcer during their lifetime, and as many as half of these ulcers will become infected(2) (3). In people with diabetes and foot ulcers, several factors, such as inappropriate antibiotic treatment, the chronic nature of the wound, and frequent hospital admission, can influence the presence of multidrug-resistant microorganisms in the ulcer(4). Moreover, the specific organisms identified in diabetic foot infections can differ not only from patient to patient and hospital to hospital but also from one part of the country to another(5).

Infectious microorganisms are associated with amputation of the infected foot if not treated promptly and may increase the duration of hospital stay and the cost of management as well as morbidity and mortality(6). Most diabetic foot infections are true emergencies; therefore, antibiotic therapy should be started immediately to improve the chances of salvaging the limb. Initial empirical therapy should be based on clinical presentation, gram-staining results, and knowledge of the organisms that are most frequently isolated from a particular infection(7).

The appropriate selection of antibiotics based on the antibiograms of isolates from diabetic foot infections is extremely critical for the proper management of these infections. Therefore, the aim of the present study was to evaluate the bacteriology of diabetic foot ulcers at Hospital Geral de Palmas, Tocantins, Brazil, in order to determine the relative frequencies of bacterial isolates cultured from foot infections and to assess the *in vitro* antibiotic resistance and susceptibility of the isolated bacteria to a variety of commonly used antibiotics.

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METHODS

Study design

This prospective study included 41 consecutive patients with diabetes and foot ulcers, who were admitted to the Hospital Geral de Palmas, Tocantins, Brazil between January 2013 and June 2013. The Hospital Geral de Palmas is a 220-bed tertiary care hospital utilized by people from Tocantins and its surrounding areas. The patients underwent extensive debridement of their diabetic foot ulcers, and all patients were taking antibiotics. Demographic and lesion data, including age, sex, duration of diabetic foot, diabetes medications used, features of the lesion, and location of the lesion, were recorded for each patient.

Characterization of bacterial isolates

Culture specimens were collected using sterile cotton swabs. To eliminate the possibility of isolating colonizing bacteria, superficial ulcers were excluded from the study. After rinsing the wound area with saline and debriding the wound, swab/tissue samples were collected aseptically from the wound, conditioned in Stuart medium, and immediately taken to the microbiology laboratory. The specimens were inoculated on blood and MacConkey agar plates for the isolation of aerobic bacteria. Additionally, thioglycollate broth and mannitol salt agar were inoculated. The media plates and broth were then incubated at 37°C for 24 hours. The isolates were identified based on colony morphology, gram-staining results, motility, a catalase test, an oxidase test, a coagulase test, and biochemical tests[8]. In this study, anaerobic bacteria were not investigated owing to limited laboratory facilities.

Antibiotic sensitivity testing

Antibiotic susceptibility testing was performed using the Kirby Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines[9]. The antibiotics tested for Gram-positive bacteria were azithromycin, amoxicillin/clavulanic acid, cefoxitin, cefalexin/cefalotin, erythromycin, imipenem, oxacillin, penicillin, trimethoprim-sulfamethoxazole, and vancomycin, while the antibiotics tested for Gram-negative bacteria were amoxicillin/clavulanic acid, amoxicillin, ampicillin, aztreonam, cefotaxime, cefoxitin, gentamicin, imipenem, polymyxin B, norfloxacin, and tetracycline.

Using the broth macrodilution (tube) method [minimum inhibitory concentration (MIC)], the modified Kirby-Bauer disk diffusion method was validated for vancomycin and polymyxin B susceptibility testing of *Staphylococcus aureus* and *Pseudomonas* spp., respectively. MICs were determined and interpreted according to the criteria of the CLSI[10].

Staphylococcus spp. were tested for methicillin resistance using oxacillin and cefoxitin disks as recommended by the National Committee for Clinical Laboratory Standards[11] [12] and according to the criteria of the CLSI[10], respectively. Novobiocin disks were used to distinguish *Staphylococcus saprophyticus*, which is resistant to novobiocin in culture, from other coagulase-negative staphylococci (CONS). *Streptococcus pneumoniae* isolates were identified on the basis of standard laboratory procedures, including colony morphology on blood agar and optochin sensitivity tests[13]. *Streptococcus pyogenes* isolates were confirmed with blood agar culture and a bacitracin test, which is used in the presumptive identification of group A, beta-hemolytic streptococci.

Multidrug-resistant organisms (MDROs) were defined as bacteria that were resistant to more than one or all classes of antibiotics[14] [15].

Statistical analyses

The statistical analysis was carried out using the Stata software, version 12.0, and Fisher's Exact Test was used to verify the association between antibiotic use and Gram-negative bacteria resistance.

Ethical considerations

The study was performed after receiving approval for the Ethics Committee of our institution (number 020/2011) and after obtaining informed consent form the patients or responsible guardians. Additionally, permission to perform the study was obtained from the Health Department of the State of Tocantins (*Secretaria da Saúde do Estado do Tocantins* – SESAU).

RESULTS

The present study included 41 patients with diabetes, and of these patients, 22 (54%) were female and 19 (46%) were male. Additionally, 38 (93%) patients were taking diabetes medications and 3 (7%) were not taking any diabetes medications. The mean age of the patients was 65.8 ± 13.76 years (mean ± SD; range, 36-75 years). The duration of diabetic foot infection ranged from 1 day to more than 90 days. The types of lesions did not differ significantly among the patients; 22 (54%) exhibited superficial ulcers, 19 (46%) deep ulcers, 20 (49%) chronic wounds, and 21 (51%) acute wounds. The majority of the lesions were located on the right toe [16 (39%) patients] and in the plantar region [11 (27%) patients]. The demographic and lesion characteristics have been summarized in **Table 1**.

Among the 41 study patients, the specimens were culture-positive in 30 (73%) and were negative in the remaining 11 (27%) patients. A total of 89 bacterial isolates were obtained from the 30 patients in whom the specimens were culture-positive. In 9 (30%) patients, only 1 pathogen was isolated, while in 21 (70%) patients, more than 1 pathogen was isolated. Of these 21 patients, 10 (48%) were infected with 2 pathogens, 9 (43%) with 3 pathogens, and 2 (9.5%) with 4 pathogens. Gram-positive bacteria represented 69% (n = 61) of the isolates, and Gram-negative bacteria represented 31% (n = 28). In monomicrobial infections, Gram-positive bacteria (14%, n = 12) were more common than Gram-negative bacteria (3%, n = 3), whereas in polymicrobial infections, both Gram-positive (55%, n = 49) and Gram-negative bacteria (28%, n = 25) were common **(Table 2)**. The organisms that were isolated from the diabetic foot infections are summarized in **Table 2**. *Staphylococcus aureus* [27 (30%) isolates] was the

TABLE 1 - Characteristics of the patients and lesions.

Characteristic of patients and lesions	Value (n = 41)	
	n	%
Age, years	65.8 ± 13.76	
Sex		
male	19	46.0
female	22	54.0
Diabetic medication	38	93.0
No diabetic medication	3	7.0
Duration of foot infection		
1–30 days	23	56.0
31–60 days	7	17.0
61–90 days	2	5.0
>90 days	6	15.0
no answer	3	7.0
Type of lesion		
superficial ulcer	22	54.0
deep ulcer	19	46.0
chronic wounds	20	49.0
acute wounds	21	51.0
Location of the foot ulcer		
plantar	11	27.0
dorsal portion	8	19.0
toes (right foot)	16	39.0
toes (left foot)	6	15.0

Data are presented as mean ± standard deviation or number (percentage).

bacterial species most commonly isolated among the Gram-positive bacteria, followed by *Staphylococcus saprophyticus* [17 (19%) isolates], *Staphylococcus epidermidis* [9 (10%) isolates], *Streptococcus agalactiae* (beta-hemolytic) [6 (7%) isolates], and *Streptococcus pneumoniae* (alpha-hemolytic) [2 (2%) isolates]. On the other hand, *Proteus* spp. [10 (11%) isolates] and *Enterobacter* spp. [9 (10%) isolates] were the most common species isolated among the Gram-negative bacteria, followed by *Escherichia coli* [4 (4.5%) isolates], *Pseudomonas* spp. [4 (4.5%) isolates], and *Citrobacter* spp. [1 (1%) isolate].

The antibiotic resistance patterns of the isolated bacteria to commonly used antibiotics, obtained with the Kirby Bauer disk diffusion method, are shown in **Table 3**. Gram-positive organisms were isolated in 61 patients. There were 16 (59%) and 9 (33%) cases of methicillin-resistant *Staphylococcus aureus* (MRSA) with oxacillin resistance and cefoxitin resistance, respectively. Among the 16 and 9 MRSA cases, 7 common strains were resistant to vancomycin in the disk diffusion test. Imipenem was the most effective antibiotic against *Staphylococcus aureus* (100%), *Staphylococcus epidermidis* (22%), *Staphylococcus saprophyticus* (100%), *Streptococcus pneumoniae* (100%), and *Streptococcus agalactiae* (100%). Additionally, *Streptococcus pneumoniae* (100%) was sensitive to oxacillin, penicillin, trimethoprim-sulfamethoxazole, and vancomycin. Almost all of the members of the *Enterobacteriaceae* family (*Proteus* spp., *Enterobacter* spp., *Escherichia coli*, and *Citrobacter* spp.) and the non-fermenters (*Pseudomonas* spp.) were uniformly resistant to the majority of the antibiotics tested. However, *Escherichia coli* (100%), *Proteus* spp. (100%), and *Citrobacter* spp. (100%) were sensitive to imipenem, *Citrobacter* spp. (100%) were sensitive to polymyxin B, and *Escherichia coli* (75%), *Proteus* spp. (70%), and *Pseudomonas* spp. (75%) were sensitive to gentamicin.

Considering the incidence of antibiotic-resistant organisms, including vancomycin-resistant *Staphylococcus aureus* (26% of all *Staphylococcus aureus* isolates) and polymyxin B-resistant *Pseudomonas* spp. (100%), the modified Kirby-Bauer disk diffusion method was validated using the broth method (MIC). For vancomycin, 6 (67%), 0 (0%), and 3 (33%) MRSA isolates were classified as susceptible, less sensitive, and resistant, respectively, and for polymyxin B, 1 (25%), 2 (50%), and 1 (25%) *Pseudomonas* spp. isolates were classified as susceptible, less sensitive, and resistant, respectively, using the MIC method **(Table 4)**.

In chronic wounds, Gram-negative rods comprised 13 (65%) of the aerobic organisms **(Table 5)**. Although no

TABLE 2 - Characteristics of the culture and bacteria isolated from the diabetic foot lesions.

Characteristic	**Value**	
	n	**%**
Total number of specimens	41	100.0
Number of patients with positive culture	30	73.0
Number of cultures with 1 pathogen isolated	9	30.0
Number of cultures with 2 or more pathogens isolated	21	70.0
Total number of pathogens isolated	89	100.0
Gram-positive bacteria	61	69.0
Gram-negative bacteria	28	31.0
Monomicrobial infections with gram-positive bacteria	12	14.0
Monomicrobial infections with gram-negative bacteria	3	3.0
Polymicrobial infections with gram-positive bacteria	49	55.0
Polymicrobial infections with gram-negative bacteria	25	28.0
Bacteria isolated	**Value**	
	n	**%**
Escherichia coli	4	4.5
Enterobacter spp.	9	10.0
Proteus spp.	10	11.0
Pseudomonas spp.	4	4.5
Citrobacter spp.	1	1.0
Staphylococcus aureus	27	30.0
Staphylococcus epidermidis (CONS)	9	10.0
Staphylococcus saprophyticus (CONS)	17	19.0
Streptococcus pneumoniae (alpha-hemolytic)	2	2.0
Streptococcus agalactiae (beta-hemolytic)	6	7.0

CONS: coagulase-negative staphylococci.Data are presented as number or number (percentage).

significant statistical differences ($p > 0.05$) were noted among antibiotic resistances, chronic wounds, and Gram-negative rods, *Escherichia coli* and *P. aeruginosa* were resistant to the majority of the antibiotics tested except imipenem, *Proteus* spp. (80%) and *Enterobacter* spp. (67%) were sensitive to imipenem, and *Citrobacter* spp. showed susceptibility to imipenem and polymyxin B.

The ulcer classification system, which is used to grade the severity of diabetic foot ulcers, provides prognosis on healing and aids in the formulation of treatment plans. However, these were not analyzed in the present study. Five (17%) patients had undergone amputation. Of these 5 patients, 2 (40%) had chronic wounds caused by monomicrobial Gram-negative bacterial infections (*Proteus* spp. and *Escherichia coli*, respectively), 2 (40%) had chronic wounds caused by polymicrobial infections (*Staphylococcus aureus*, *Streptococcus agalactiae*, and *Enterobacter* spp. in 1 patient and *Staphylococcus saprophyticus* and *Pseudomonas* spp. in the other patient), and 1 (20%) had an acute wound caused by polymicrobial infection (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Proteus* spp., and *Pseudomonas* spp.) (data not shown).

DISCUSSION

Foot infections in patients with diabetes are a common, complex, and costly problem[16]. In the present study, we found that elderly patients (>60 years of age) constituted the majority of patients with foot infections. This may be explained by the fact that foot lesions occur commonly among patients with diabetes, particularly the elderly and those with sensory neuropathy[17]. Previous studies have shown that the susceptibility to foot infections is greater in male patients than in female patients[1] [5]. However, in our study, we did not find differences between male and female patients, which may be because of the limited number of patients.

TABLE 3 - Antibiotic resistance patterns of 61 Gram-positive and 28 Gram-negative bacteria.

Antibiotic	*Staphylococcus aureus* (n = 27)		*Staphylococcus epidermidis* (CONS) (n = 9)		*Staphylococcus saprophyticus* (CONS) (n = 17)		*Streptococcus pneumoniae* (n = 2)		*Streptococcus agalactiae* (n = 6)	
	n	%	n	%	n	%	n	%	n	%
Azithromycin	16	59.0	9	100.0	10	59.0	1	50.0	4	75.0
Amoxicillin/clavulanic acid	13	48.0	6	67.0	10	59.0	1	50.0	4	75.0
Cefoxitin	9	33.0	4	44.0	10	59.0	2	100.0	6	100.0
Cefalexin/cefalotin	13	48.0	9	100.0	8	47.0	2	100.0	6	100.0
Erythromycin	18	67.0	9	100.0	16	94.0	2	100.0	6	100.0
Imipenem	0	0.0	2	22.0	0	0.0	0	0.0	0	0.0
Oxacillin	16	59.0	8	89.0	16	94.0	0	0.0	6	100.0
Penicillin	19	70.0	9	100.0	16	94.0	0	0.0	6	100.0
Trimethoprim-sulfamethoxazole	14	52.0	9	100.0	7	41.0	0	0.0	6	100.0
Vancomycin	7	26.0	ND		ND		0	0.0	5	90.0
Antibiotic	***Escherichia coli*** **(n = 4)**		***Enterobacter* spp. (n = 9)**		***Proteus* spp. (n = 10)**		***Pseudomonas* spp. (n = 4)**		***Citrobacter* spp. (n = 1)**	
Amoxicillin/clavulanic acid	3	75.0	3	33.0	6	60.0	2	50.0	1	100.0
Amoxicillin	3	75.0	9	100.0	9	90.0	2	50.0	1	100.0
Ampicillin	3	75.0	9	100.0	9	90.0	4	100.0	1	100.0
Aztreonam	2	50.0	7	78.0	6	60.0	3	75.0	1	100.0
Cefotaxime	2	50.0	8	89.0	6	60.0	3	75.0	1	100.0
Cefoxitin	2	50.0	6	67.0	3	30.0	3	75.0	1	100.0
Gentamicin	1	25.0	7	78.0	3	30.0	1	25.0	1	100.0
Imipenem	0	0.0	4	44.0	0	0.0	2	50.0	0	0.0
Polymyxin B	3	75.0	5	56.0	4	40.0	4	100.0	0	0.0
Norfloxacin	2	50.0	7	78.0	4	40.0	2	50.0	1	100.0
Tetracycline	3	75.0	9	100.0	5	50.0	3	75.0	1	100.0

CONS: coagulase-negative staphylococci; **ND:** not detected. Data are presented as number (percentage). Antibiotic susceptibility pattern determined using the Kirby-Bauer disc diffusion method.

TABLE 4 - MIC values of vancomycin and polymyxin B for *Staphylococcus aureus* and *Pseudomonas* spp., respectively.

MIC (μg/mL)	*Staphylococcus aureus* (MRSA) broth dilution for vancomycin		*Pseudomonas* spp. broth dilution for polymyxin B	
	n	%	n	%
Susceptible	6	67.0	1	25.0
Less sensitive	-	-	2	50.0
Resistant	3	33.0	1	25.0
Total	9	100.0	4	100.0

MIC: minimum inhibitory concentration; **MRSA:** methicillin-resistant *Staphylococcus aureus*. Data are presented as number (percentage). Antibiotic susceptibility pattern determined using the macrodilution (tube) broth method.

TABLE 5 - Antibiotic resistance patterns of 13 Gram-negative bacteria isolated from chronic wounds.

Antibiotic	*Escherichia coli* (n = 2)		*Enterobacter* spp. (n = 3)		*Proteus* spp. (n = 5)		*Pseudomonas* spp. (n = 2)		*Citrobacter* spp. (n = 1)		p-value*
	n	%	n	%	n	%	n	%	n	%	
Amoxicillin/clavulanic acid	2	100.0	3	100.0	4	80.0	2	100.0	1	100.0	1,000
Amoxicillin	2	100.0	3	100.0	4	80.0	2	100.0	1	100.0	1,000
Ampicillin	2	100.0	3	100.0	4	80.0	2	100.0	1	100.0	1,000
Aztreonam	1	50.0	3	100.0	3	60.0	2	100.0	1	100.0	0,685
Cefotaxime	2	100.0	3	100.0	3	60.0	2	100.0	1	100.0	0,808
Cefoxitin	2	100.0	3	100.0	4	80.0	2	100.0	1	100.0	1,000
Gentamicin	1	50.0	3	100.0	1	20.0	1	50.0	1	100.0	0,225
Imipenem	0	0.0	1	33.0	1	20.0	0	0.0	0	0.0	1,000
Polymyxin B	2	100.0	2	67.0	1	20.0	2	100.0	0	0.0	0,132
Norfloxacin	2	100.0	3	100.0	3	60.0	1	50.0	1	100.0	0,685
Tetracycline	2	100.0	3	100.0	3	60.0	2	100.0	1	100.0	0,808

Data are presented as number (percentage). Antibiotic susceptibility pattern determined using the Kirby-Bauer disc diffusion method. *Fisher's exact test.

Diabetic foot ulcers are colonized by pathogenic bacteria that may predispose a susceptible patient to a lower extremity infection, defined as the invasion and multiplication of microorganisms in body tissues associated with tissue destruction or host inflammatory responses[18]. In the present study, we found that the majority of lesions were located on the right toe and plantar region, and varied in duration from 1 day to more than 90 days. Additionally, recent lesions (1-30 days) were the most common. Our findings are in accordance with the results of Donoso et al. (2013)[19].

This study is limited by the fact that cultures for anaerobic bacteria could not be performed. In diabetic foot infections, the role of anaerobic bacteria is particularly unclear; some studies have reported that anaerobic bacteria play a minor role[20] [21], while other studies found a high incidence of anaerobic bacteria[5] [22].

The most common pathogens isolated were Gram-positive cocci, such as *Staphylococcus aureus* and *Staphylococcus saprophyticus* (CONS) and Gram-negative rods, such as *Proteus* spp. and *Enterobacter* spp. Although the findings of our study are consistent with the results of previous studies showing that Gram-positive bacteria were predominant in diabetic foot infections[13] [22], other studies have reported that Gram-negative bacteria were predominant in particular regions[23] [24]. These results suggest, in part, differences in the type and severity of infections[5] [25]. Aerobic Gram-negative bacteria (mainly *Enterobacteriaceae* and sometimes *Pseudomonas aeruginosa* or other Gram-negative species) are usually isolated in conjunction with Gram-positive cocci in patients with chronic or previously treated infections[26], which is consistent with our findings. Polymicrobial infections accounted for 70% of all infections. Although polymicrobial etiology has been implicated in diabetic foot infections[24], a previous study reported the predominance of monomicrobial infections[27]. These discrepancies suggest differences in diabetic foot infections, with severe infections usually having polymicrobial isolates and mild infections usually having monomicrobial isolates[25] [28].

The prognosis of diabetic foot infections remains poor, and the outcomes have been reported to be worse with MDROs than with non-MDROs in patients with diabetic foot infections[2]. Our study showed that MDROs were common in hospitalized patients with chronic and acute wounds. An increase in the occurrence of chronic wound infections with MDROs in the diabetes mellitus population has been noted over the last decade and has been primarily attributed to MRSA, but antibiotic-resistant Gram-negative organisms, particularly *Pseudomonas aeruginosa*, have also been implicated[4] [29]. In our study, few patients underwent some type of amputation. However, almost all patients had chronic wounds caused by monomicrobial infections of Gram-negative bacteria and polymicrobial infections. Moderate to severe infections often necessitate empirical regimens with activity against commonly isolated Gram-negative bacilli, MRSA, and perhaps *Enterococcus* spp.[30]. Mild infections are often managed with local wound care strategies and/or prophylactic measures. It is important to note that the decisions relating to the antibiotic treatment of wounds are influenced by clinical evidence, the availability of appropriate antibiotic interventions, patient's requirement, and practitioner's expertise[31].

Because the Mueller Hinton agar-based antibiogram-resistogram pattern study of Gram-positive bacteria isolated from the foot ulcers of patients with diabetes showed that *Staphylococcus aureus* was the predominant pathogen, *Staphylococcus* spp. were tested for methicillin resistance using oxacillin and cefoxitin. Our study found that different proportions of *Staphylococcus aureus* isolates were methicillin-resistant. Previous studies have shown that almost 50% of

Staphylococcus aureus isolates were methicillin-resistant, and MRSA is being increasingly isolated from diabetic foot ulcers[13] [32]. Other studies have identified MRSA in as many as 15-30% of diabetic wounds[33] [34]. In our study, among all of the MRSA isolates, 7 (26%) were resistant to the glycopeptide antibiotic vancomycin. The modified Kirby-Bauer disk diffusion method was therefore validated using MIC, and only 3 isolates were found to be resistant to vancomycin. Our results indicate that any resistant bacteria of clinical importance identified with a diffusion test should be confirmed using other dilution methods. Although antibiotic treatment with vancomycin is often the standard protocol for diabetic foot infections, even when there are no risk factors for MRSA[35], during the last decade, treatment failures with non-vancomycin-susceptible MRSA have been reported in the clinical setting[36]. Therefore, further genetic studies should be performed to analyze the methicillin-resistant, non-vancomycin-susceptible *Staphylococcus aureus* found in this study.

The *Enterobacteriaceae* family was highly resistant to the majority of antibiotics tested, which is partially consistent with the findings of a study conducted by Banashankari et al. in 2012[37]. Additionally, *Enterobacter* spp. were resistant to the majority of antibiotics tested, which is consistent with the findings of a previous study[37]. Moreover, *Proteus* spp. were resistant to all betalactamics except imipenem, cefoxitin (a cephamycin), and gentamicin (an aminoglycoside antibiotic). *Proteus* spp. are known to produce a unique β-lactamase (cefuroximase) that has high activity against antibiotics, primarily cefotaxime[38], a third-generation cephalosporin. Furthermore, *Escherichia coli* were resistant to the majority of antibiotics tested, except gentamicin and imipenem. Therefore, in our study, gentamicin and imipenem were the most effective antibiotics against almost all bacteria from the *Enterobacteriaceae* family, which is partially consistent with the results of previous studies[28] [39]. It is important to consider that some Gram-negative bacteria from the *Enterobacteriaceae* family have the ability to produce highly effective ß-lactamase enzymes, making them resistant to all ß-lactam antibiotics, except cephamycins (cefoxitin, cefotetan) and carbapenems[40].

It has been reported that imipenem is the most effective antibiotic against Gram-negative organisms, including *Pseudomonas aeruginosa*[41]. In our study, 50% of the *Pseudomonas* spp. isolates were resistant to imipenem, which is consistent with the results of a previous study[35]. In 2011, Sivanmaliappan and Sevanan[1] reported that 100% of *Pseudomonas aeruginosa* isolates were resistant to ampicillin and norfloxacin, 83.3% were resistant to tetracycline, 66.6% were resistant to gentamicin and imipenem, and 16.6% were resistant to cefotaxime. These findings are partially consistent with our results, where 100% of *Pseudomonas* spp. isolates were resistant to ampicillin, 50% were resistant to norfloxacin, 75% were resistant to tetracycline, and 75% were resistant to cefotaxime. Additionally, we found that 75% of *Pseudomonas* spp. isolates were regularly sensitive to only gentamicin. Differences in the results obtained in many studies shows that the patterns of microbial infection are not consistent in patients with diabetic foot infections; therefore, repeated evaluation of microbial characteristics and the antibiotic sensitivity is necessary for the selection of appropriate antibiotics[24]. *Pseudomonas* spp. isolates were also resistant to polymyxin B, as assessed using the Kirby Bauer disk diffusion method. Some studies have demonstrated a poor correlation among the results of different susceptibility test methods for polymyxins, possibly because of the poor diffusion of polymyxins in agar[42]. Additionally, the *in vitro* activity of polymyxins may be affected by the levels of cations in agar[43]. Therefore, the resistance determined with a diffusion test was validated using the broth dilution method, and we found that only 1 (25%) *Pseudomonas* spp. isolate was resistant to polymyxin B. Despite the relatively low incidence of polymyxin-resistant microorganisms in our study, the increased use of these antibiotics for the treatment of multi-resistant strains has led to a high frequency of resistant clinical isolates[44].

A common risk factor for the development of highly resistant bacteria is the previous use of broad-spectrum antibiotics[45]. In our study, all patients had received antibiotic therapy prior to surgical debridement, and this may explain the higher rate of multidrug-resistant bacteria present in the diabetic foot lesions in our study than in previous studies[1] [41]. Patients with diabetic foot infections are usually hospitalized multiple times and are often exposed to multiple courses of antibiotics[46], which may influence antibiotic resistance. Therefore, the potential presence of such resistant strains emphasizes the importance of obtaining optimal specimens from diabetic foot infections for culture and sensitivity testing[47] [48] as well as the need to avoid excessive antibiotic therapy that promotes this resistance.

In conclusion, the present study report has some limitations because cultures for anaerobic bacteria could not be performed and sample size was small. However, it confirmed the high prevalence of multidrug-resistant pathogens in diabetic foot ulcers. Diabetic foot infections were predominantly due to Gram-positive bacteria, such as *Staphylococcus aureus*, or were polymicrobial infections. Many studies on the bacteriology of diabetic foot infections have reported results that vary and are often contradictory[22] [23] [25]. In such cases, application of molecular techniques may lead to more accurate microbial characterizations and targeted antibiotic therapy. Therefore, it is necessary to evaluate the different microorganisms infecting the wound on a routine basis and to know the antibiotic susceptibility patterns of the isolates from the infected wound in patients with diabetic foot lesions. This knowledge is crucial for planning the treatment of these patients with the appropriate antibiotics, reducing resistance patterns, and minimizing healthcare costs. We hope the data presented on this article can assist the clinicians in determining the multidrug-resistant pathogens in diabetic foot ulcers.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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