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Microbiology of diabetic foot infections in a tertiary care hospital in São Paulo, Brazil

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Abstract: Diabetic foot infections (DFI) are one of the causes of hospitalization in diabetic patients and empirically antibiotic therapy is necessary. We conduct a retrospective study of patients with DFI that requiring hospitalization to evaluate microbiologic profile and susceptibility pattern of these infections. We evaluated 320 patients being 223 (69,7%) male with media age of 71 years. Gram-positive bacteria were responsible for 188 (58,7%) of the isolates while gram-negative bacilli were responsible for 88 (27,5%). *E. faecalis* was the most prevalent pathogen, followed by *S. aureus* and coagulase negative Staphylococci. Gram negative pathogens were isolated only in 27.5% of the cases, with *P. aeruginosa* being the most prevalent agent. Regarding the susceptibility profile we found ampicillin-sensitive enterococci in 89% of the cases, oxacillin-sensitive *S. aureus* in 47%, but in coagulase negative staphylococci, oxacillin was sensible only in 20%. The susceptibility profile of gram negatives was very good with 76% susceptibility of *P. aeruginosa* to ceftazidime and meropenem. The other prevalent Enterobacterales had great susceptibility to ceftazidime, piperacillin-tazobactam and 100% susceptibility to meropenem, exception for *K. pneumoniae* which had 75% susceptibility to meropenem. Knowledge of microbiological profile and susceptibility patterns of patients with DFI is useful to guide empirical therapy.

Keywords: diabetic foot infections; microbiology; epidemiology

1. Introduction

World Health Organization (WHO) published in 2016 a report to mark World Health Day in which it defined diabetes as a global epidemic. The document stated that the number of adults living with diabetes had quadrupled since 1980, reaching 422 million in 2014 and reflecting increases in the risk factors associated with it (1). The higher number of diabetic patients brought also an increase in the incidence of diabetic foot infections and peripheral arterial disease.

Diabetic foot infections (DFI) are one of the most common causes of hospitalization in diabetic patients and account for a significant portion of increased hospitalization and healthcare expenditure (2).

Foot ulcers start as a result of peripheral neuropathy, which, associated with the decreased neuroendocrine response and sometimes with atherosclerotic peripheral arterial disease, culminates in the appearance of ulcerations and secondary infection (3).

It has already been confirmed that in the overall complexity of DFI, perfusion is just one determinant of the result, while characteristics of the wound and presence and severity of infection are also factors that have a major impact on the risk of limb amputation. As amputation is a very unfavorable event that limits patient functionality and resonates in the social and economic life of society all efforts must be made to avoid this outcome (4).

There are many classifications available for diabetic foot trying to estimate the risk of amputation. Previously existing classification systems for threatened limbs are limited, because they do not generally cover all three pillars (wound, ischemia and infection) of the extremity at risk of amputation or do not differentiate ulcer from gangrene and so fail to encompass the entire heterogeneous nature of the causes and clinical presentations. Wagner's classification, which is still widely used for wounds of the diabetic foot, is not much help for differentiating between the causes of ischemic and infectious gangrene (5). Therefore, the Society for Vascular Surgery developed a new classification system that is based on the characteristics of the wound (W), on the degree of ischemia (I) and on the presence and severity of foot infection (fI) called Wound, Ischemia and foot Infection classification, or WIfI classification (6). Grade IV of WIfI classification estimates 23% amputation risk in 1 year (7).

In cases that requiring hospitalization, empirically antibiotic therapy is necessary at the moment of diagnosis and is one of the bases for the treatment of diabetic foot (8).

Clinical guidelines from the Infectious Diseases Society of America (IDSA) recommend treating clinically infected diabetic foot ulcers with empirical antibiotics until microbiological culture results are available. Empirical antibiotics should be chosen in accordance with the severity of infection, clinical presentation and the prevalence of microorganisms in the local area and their antibiotic susceptibility. Subsequently, based on daily clinical evaluations and the results of culture and antimicrobial susceptibility test, may be possible to adjust antimicrobial therapy (9).

On the other hand, broad-spectrum antibiotic regimens can contribute to antibiotic resistance and limit future treatment options. So, for minimize the emergency of bacterial resistance there is a need to know the local epidemiology, adjust antimicrobial therapy according to the results of cultures and avoid prolonged treatments by also thinking about the adverse effects of long-term administration of antibiotics (10).

The purpose of this study was to evaluate the microbiology profile and susceptibility pattern of DFI in patients that require hospitalization.

2. Results

We evaluated 320 patients being 223 (69,7%) male with media age of 71 years. The most common comorbidity was systemic arterial hypertension (77,4%), followed by peripheral occlusive arterial disease (57,3%), insulin-dependent diabetes mellitus (38,7%), chronic kidney disease (32,3%), coronary heart disease (15,1%) and smoking (14,8%).

Previous use of antimicrobials within 90 days prior to hospitalization was observed in 87 (27,1%) patients.

Previous amputation and osteomyelitis was seen both in 97 (30.3%) patients.

168 (52,8%) patients had IWGDF classification categorized as 3 and 127 (39,8%) had grade 4 WIfI classification.

Gram-positive bacteria were responsible for 188 (58,7%) of the isolates while gram-negative bacilli were responsible for 88 (27,5%) of the isolates. 44 (13,7%) of the cultures did not have growth of microorganisms. The prevalence of isolated pathogens is described in Table 1.

Table 1. Isolated pathogens in patients admitted with DFI (N=320).

Gram positive	N	%	Gram negative	N	%
E. faecalis	68	21,3	*Pseudomonas aeruginosa*	21	6,6
S. aureus	47	14,7	*Proteus sp*	13	4,1
Other staphylococci coagulase negative	40	12,5	*Outras Enterobacterales*	13	4,1
S. epidermidis	21	6,6	*Klebsiella pneumoniae*	12	3,8
E. faecium	5	1,6	*E. coli*	9	2,8
Enterococos sp	4	1,3	*Enterobacter sp*	8	2,5
S. agalactiae	3	0,9	*Acinetobacter sp*	6	1,9
			Stenotrophomonas maltophilia	5	1,6
			Burkolderia cepacia	1	0,3

The susceptibility profile of the pathogens Gram-positive and Gram-negative is described in Tables 2 and 3, respectively.

Table 2. Susceptibility pattern of the pathogens Gram-positive.

Pathogen (N)		
	Susceptibility to oxacillin	
	N	%
S. aureus (44)	21	47,7
S. epidermidis (20)	3	15,0
Other staphylococci coagulase negative (36)	2	5,5
	Susceptibility to ampicillin	
Enterococcus faecalis (64)	57	89
Enterococcus faecium (5)	0	0
Enterococos sp (3)	3	100

Table 3. Susceptibility pattern of the pathogens Gram-negative.

Pathogen (N)	Susceptibility											
	ciprofloxacin		ceftriaxone		ceftazidime		cefepime		piperacillin-tazobactam		meropenem	
	N	%	N	%	N	%	N	%	N	%	N	%
P. aeruginosa (21)	10	47,6	-	-	16	76,1	15	71,4	12	57,1	16	76,1
Acinetobacter sp (6)	3	50	-	-	3	50	3	50	2	33,3	3	50
Proteus sp (13)	8	61,5	7	53,8	7	53,8	11	84,6	11	84,6	13	100
Others *Enterobacterales* (13)	6	46,1	7	53,8	7	53,8	8	61,5	11	84,6	13	100
Klebsiella pneumoniae (12)	4	33,3	5	41,6	5	41,6	6	50	6	50	9	75
E. coli (9)	5	55,5	7	77,7	7	77,7	8	88,8	9	100	9	100
Enterobacter sp (8)	5	62,5	4	50	4	50	5	62,5	8	100	8	100

3. Discussion

We conducted a study to evaluate the prevalence of pathogens and the susceptibility profile of microorganisms isolated from ulcers in diabetic feet infections. This is an essential prevalence study to know the local epidemiology and thus be able to guide empirical therapy in cases of DFI.

In our study, we found a higher prevalence of males with a mean age of 71 years denoting the existence of this condition in the elderly population. We found many comorbidities present, being more than half with chronic arterial occlusive disease, which

certainly aggravates the circulatory conditions of these patients that can be considered severe arteriopaths.

An epidemiological study conducted in Brazil analyzing 172 diabetic patients demonstrated systemic arterial hypertension present in 84.3% of cases, coronary heart disease in 30.2% of cases, peripheral vascular disease in 8.9%, peripheral neuropathy in 37.9% of cases, foot wound in 10% of cases, amputation in the last 6 months in 6.0% of the cases, hypoglycemia in 21% of cases, ketosis in 2.4% of cases and infection in 17.1% of cases. This Brazilian cohort was compared with a French cohort that demonstrated statistically significant differences between the two populations revealing better conditions among the French participants, which highlights the importance of the scientific evidence found in the French study for developing public health actions targeted at Brazilian older people with diabetes mellitus (11).

We also found 52.8% of patients with IWGDF considered severe and 39.8% with grade 4 WIfI, also severe, where the chance of amputation is about 23 % in one year (7). However, some studies observed that major amputation rates at stage 2 were the same or even higher than rates at stage 3 (12, 13, 14, 15). Recently, a group of researchers from Johns Hopkins Hospital conducted a retrospective analysis of 217 patients with 279 limbs affected by DFI who were seen at their multidisciplinary clinic from 2012 to 2015 and found that the incidence of major amputation at 12 months was similar across WIfI classification stages. They concluded that this classification system was not predictive of risk of major amputation at 1 year for diabetic patients with DFI (16).

Although the risk of amputation may not be estimated by classification systems, the fact is that patients with DFI who requiring hospitalization are severe, have comorbidities other than diabetes, and have already experienced antimicrobial treatments. In our study we found 27.1% of patients with previous antimicrobial use. This data was collected in order to try to correlate the use of antimicrobials with bacterial resistance, but it also serves to demonstrate the unsuccessful strategy of outpatient treatment in cases of DFI that evolve to moderate or severe due to the conditions of vascularization of the limbs (17,18).

Another indicator of severity in the population studied is because 30% of patients had previous amputations and osteomyelitis, which is a difficult infection to treat because of antimicrobial bone penetration in patients with arterial disease (19).

The gram-positive pathogens were the majority, with *E. faecalis* being the most prevalent pathogen, followed by *S. aureus* and coagulase negative Staphylococci. Gram negative pathogens were isolated only in 27.5% of the cases, with *Enterobacterales* being the most prevalent agent accounting for 17,3% of cases and *P. aeruginosa*, responsible for only 6.6% of cases.

In a recent review that analyzes global literature relating to incidence, risk factors, resistance patterns and geographic distribution of the microorganisms isolated from diabetic foot infections, *Staphylococcus aureus* was a significant pathogen, with a growing incidence of *Pseudomonas aeruginosa* and multi drug resistance gram-negative bacilli (3).

In one study conducted at Indonesia Gram-negative bacteria were obtained in 112 (85.5%) subjects with *Enterobacter sp* as the predominant bacteria. Gram-positive bacteria were found in 19 (14.5%) subjects with *Staphylococcus* spp. as the predominant bacteria (20).

Another study conducted in Turkey that analyzed the microbiological profile of DFI by conducting a systematic review of articles published in 20 years and comparing two periods (1989 to 2007 vs 2007 to 2011) identified 31 studies. Overall, these studies reported 2.097 patients, from whom 1.974 microorganisms were isolated. The total percentage of gram-negative and gram-positive aerobic bacteria were similar in each of the assessed periods. The rate of isolation of *Staphylococcus aureus* during the entire period, compared with just the past 5 years, was 23.8% and 19.1%, respectively, while the rate of methicillin-resistant *S. aureus* was 7.8% and 5.7%, respectively. The isolation rate of *Pseudomonas aeruginosa* was 13.7% for the entire period and 14.9% for the past 5 years. While linezolid, vancomycin and teicoplanin were the most active agents against gram-positive

microorganisms, imipenem and cefoperazone-sulbactam were the most active against gram-negative microorganisms (21).

On the other, the few Brazilian studies published show a high prevalence of the Enterobacterales family (51,5%) with *Staphylococcus aureus* isolated in 20% and *Enterococcus faecalis* in 17,9% of cases (22).

Another Brazilian study found a predominance of Gram-positive bacteria with the most commonly isolated *Staphylococcus aureus*, followed by *Staphylococcus saprophyticus*, *Staphylococcus epidermidis*, *Streptococcus agalactiae* and *Streptococcus pneumoniae*. Between Gram-negative bacteria, the most commonly isolated were *Proteus spp* and *Enterobacter* spp, followed by *Escherichia coli*, *Pseudomonas spp* and *Citrobacter spp* (23).

While the few Brazilian studies show predominance of *S. aureus*, we found a predominance of Enterococci. These discrepancies demonstrate the importance of knowledge local epidemiology and resistance patterns that are essential for antibiotic treatment considerations (24).

Regarding the susceptibility profile we found ampicillin-sensitive enterococci in 89% of the cases and oxacillin-sensitive *S. aureus* in 47% of the cases, but in coagulase negative staphylococci, the sensitivity to oxacillin was only 20%. It is worth noting that coagulase-negative staphs may be colonization or contamination (25). Although coagulase-negative staphylococci may be colonizing in wounds, in these cases they were considered pathogens because they were collected from deep tissue fragments during surgical procedures (26). The great susceptibility of enterococci to ampicillin is due to the fact that the vast majority is of the species *faecalis*. As we know, this species has much less resistance when compared to specie *faecium* (27).

Study conducted in Brazil studied thirty-four individuals with DFI and *S. aureus* was isolated in 17 (50% of cases), of these only 5/17 (29.4%) were resistant to oxacillin (28).

The susceptibility profile of gram negatives in our study was very good with 76% susceptibility of *P. aeruginosa* to ceftazidime and meropenem. The other prevalent Enterobacterales had great susceptibility to ceftazidime, piperacillin-tazobactam and 100% susceptibility to meropenem with the exception of *K. pneumoniae* which had 75% susceptibility to meropenem.

Ciprofloxacin was the agent with the lowest susceptibility to gram negatives, most likely because it is an oral antibiotic, which patients must have used before hospitalization and whose previous use induced resistance. In a Brazilian study conducted at Amazonas, 43,5% of the Gram-negative isolated germs were resistant to ciprofloxacin (22).

In a recent study conducted at China, more than 50% of Gram negative bacteria were resistant to third-generation cephalosporins, while the resistance rates of piperacillin/tazobactam, amikacin, meropenem, and imipenem were relatively low (29).

The finding of several microorganisms with different susceptibility profiles corroborates the strategy of broad-spectrum therapy with coverage for gram-positive, gram-negative and anaerobic (30, 31).

Another important issue in the treatment of these infections is the duration of antimicrobial treatment. We know that there is a tendency of prolonged treatments due to the delay of surgical treatment and also the tendency to use antimicrobials until the closure of the lesion. In a randomized, controlled pilot trial, post-debridement antibiotic therapy for soft tissue DFI for 10 days gave similar (and non-inferior) rates of remission and adverse effects to 20 days (32).

Our study has some limitations. First of all, the retrospective nature of this study precludes the inclusion of information such as glycosylated hemoglobin level, diabetic decompensation and which antibiotics were taken by patients prior to admission. Also, we do not correlate the severity of the diabetic infected foot with the isolated agents. We also did not evaluate the duration of treatment or clinical outcomes. This is also a study conducted in a single referral center for the treatment of the elderly and the main objective of this study was to evaluate the prevalence of pathogens and their susceptibility profile.

Despite the limitations mentioned, we are able to implement a new treatment protocol based on the information of the study, where the proposed treatment regimen for cases without previous use of antimicrobials was ampicillin plus ceftazidime plus metronidazole and for cases with previous use of antimicrobials, vancomycin plus piperacillin-tazobactam.

Knowledge of microbiological profile and susceptibility patterns of patients with DFI is useful to guide empirical therapy in these serious infections, difficult to treat and that requiring hospitalizations to avoid amputations and to improve the living conditions of these patients. Nevertheless, each hospital should obtain its own microbiologic profile of patients with diabetic foot infections and treat them with the most appropriate empirical antibiotic.

4. Methods

This is a retrospective study conducted through the analysis of medical records of patients with diabetic foot infections that requiring hospitalization from Jan/2017 to Dec/2019 at Hospital do Servidor Público Estadual (HSPE) de São Paulo, located in São Paulo, Brazil.

The HSPE is a tertiary teaching hospital, with 823 beds and 77 beds for intensive care units. It also has clinical and surgical hospitalization wards of various specialties. The Department of Vascular Surgery has an inpatient unit with 24 beds for the treatment of vascular diseases and DFI is responsible for about 40% of hospitalized cases.

The inclusion criteria were all patients hospitalized with DFI who required debridement. All debridements were performed in an operating room environment with aseptic techniques and fragments of tendon or deep tissue were removed and sent for culture in a sterile dry tube. The microbiology laboratory upon receiving the material sown the fragments in blood agar and chocolate agar or medium containing thioglycolate. After growth, bacterial colonies were identified and submitted to susceptibility test in an automated Phoenix®.

The variables studied were demographic data; presence of comorbidities; previous use of antimicrobials until 90 days before; previous amputation; presence of osteomyelitis diagnosed by imaging; identified etiologic agent and susceptibility test.

All patients were classified according classification of diabetic foot ulcers by International Working Group on the Diabetic Foot (IWGDF) and by system using risk stratification based on wound, ischemia and foot infection (WIfI) (33,6).

All information regarding patients was stored in a database using the Excel 5.0 program and frequency analysis and percentage were estimated.

Author´s contribution: ATP: including patients, data collection and data analysis; APMP: conceptualization, data collection and data analysis; MFM; RAS; CP; RS: conceptualization and validation; AIP: providing and performing the laboratory tests; NH: providing and performing the laboratory tests; CMG; CLF; DKSP: conceptualization and validation; AY; JSM; SFC: revision and writing manuscript; TG: data collection, statistical analysis and writing manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of HSPE (protocol code 39425620.6.2004.5463 and date of approval in 19-Dec-2019).

Informed Consent Statement: Patient consent was waived due to retrospective character of research based on medical records data with guarantee of confidentiality.

Conflict of interest: The authors declare no conflict of interest.

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