Microbiologic characteristics and antibiotic resistance rates of diabetic foot infections
Perfil microbiológico e de resistência bacteriana no pé diabético infectado

INTRODUCTION

Diabetes Mellitus (DM) is currently one of the most prevalent diseases in the world, with about 425 million people affected and with an increase forecast to about 642 million over the next 20 years. In Brazil, in 2017, there were about 12.5 million people between 20 and 79 years of age with DM, with an estimated increase to 20.3 million by the year 2045. It is believed that the increase in the prevalence of DM is due to population aging, greater urbanization and increase in sedentary lifestyle, concomitantly leading to the increase in obesity in the Brazilian population.

An important problem of DM is the morbidity resulting from its complications. Peripheral neuropathy and circulatory complications are highly prevalent, manifesting clinically through the appearance of foot ulcers. Patients with DM are 15-25% likely to have foot ulceration throughout their lives.

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.
Wounds are often initially colonized by microorganisms from the surrounding skin microbiota, the main pathogens responsible for infections usually being Staphylococcus aureus and Streptococcus spp.\textsuperscript{5}.

In most severe cases requiring antibiotic therapy, it is necessary to start them empirically, at the moment of diagnosis. Subsequently, daily clinical evaluations and the result of culture and antibiogram allow adjustment to the suitable antimicrobial therapy\textsuperscript{3,6}.

Routine culture and antibiogram exams in a certain hospital allows knowledge of the microbiota of that population, subsidizing the development of local guidelines for initial empirical antibiotic therapy, promoting direct impact on treatment success, and even reducing costs, with a stepwise and more logical use of antimicrobials\textsuperscript{7,8}. The inappropriate use of antimicrobials during the treatment of diabetic foot infections can aggravate the infection and even lead to the development of bacterial resistance\textsuperscript{9}.

In the state of Amazonas, there is lack of studies that analyze the microbiological characteristic and bacterial resistance of infections in foot injuries of diabetic patients. In view of this knowledge gap, we set out to analyze the sociodemographic and microbiological aspects of patients with infected diabetic foot, hospitalized in a reference hospital in the State of Amazonas.

**METHODS**

Through a prospective, observational, cross-sectional, descriptive, prevalence study, we evaluated the microbiological characteristic and bacterial resistance of patients admitted to the Hospital 28 de Agosto, in the period from March to August 2018. The injuries were podal and infected. The study protocol was approved by the Ethics in Research Committee on Human Beings at the Federal University of Amazonas (No. 2.335.126).

The sample consisted of patients with DM, with infected foot lesions (infected diabetic foot), who sought emergency care through the Brazilian Unified Health System (SUS) in the vascular surgery department of the Hospital 28 de Agosto, in Manaus, capital of the state of Amazonas. We included patients of both sexes, over 18 years old, and who formally agreed to participate in the research.

We interviewed patients eligible to enter the survey using a form on demographic data, comorbidities, physical examination, and classification of infection severity. In a second step, we added the results of culture and antibiogram tests of fragments of deep tissues harvested during the first surgical procedure of the studied participant. We also recorded the type of surgical procedure performed, complications, length of hospital stay, and deaths.

During surgical procedures performed in the operating room, under anesthesia and with antisepsis and asepsis care, such as abscess drainage, debridement and amputations, we collected a tissue fragment approximately 2 cm long at the greater axis, immediately after debridement of devitalized tissues and irrigation of the wound with saline, as recommended by Sotto et al.\textsuperscript{10} and Lipsky et al.\textsuperscript{11}. We conditioned the biological specimens in vials with sterilized saline and sent them for culture and antibiogram.

The sociodemographic variables were age, sex, origin, marital status, education and occupation, to characterize the epidemiological characteristics of the studied population. We studied the clinical status during the interview by searching for the variables hypertension, peripheral arterial disease, chronic kidney disease, hemodialysis, dyslipidemia, smoking, previous hospitalizations, and previous use of antimicrobial agents. To classify wounds, we used the Wagner’s and the PEDIS (Perfusion, Extent / Size, Depth / Tissue Loss, Infection, Sensation)\textsuperscript{11} classifications. We identified the surgical procedures as debridements, minor amputations (with heel preservation) and major amputations (above the ankle). We also recorded the length of stay and the number of in-hospital deaths.

We performed the statistical analysis with simple and absolute frequencies, using the Shapiro-Wilk test for quantitative data and the Pearson’s chi-square test for qualitative variables, with a significance level of 5%.

**RESULTS**

The study sample consisted of 105 patients with complete data collection forms and with biological...
material obtained for microbiological analysis.

Regarding the sociodemographic characteristics, there was a predominance of male patients, aged between 50 and 70 years old, married, with low level of education and from the city of Manaus (Table 1).

### Table 1. Distribution of sociodemographic variables of patients.

| Variables (n = 105)       | fi  | %   |
|----------------------------|-----|-----|
| Sex                        |     |     |
| Female                     | 43  | 40.0|
| Male                       | 63  | 60.0|
| Age (years)                |     |     |
| 30 |--- 39 | 6  | 5.7 |
| 40 |--- 49 | 23 | 21.9|
| 50 |--- 59 | 31 | 29.5|
| 60 |--- 69 | 28 | 26.7|
| 70 |--- 79 | 15 | 14.3|
| 80 |--- 89 | 2  | 1.9 |
| Mean + Sd                  | 57.8±11.6 |
| Educational level          |     |     |
| Elementary/Middle school   | 61  | 58.1|
| High school                | 35  | 33.3|
| College                    | 9   | 8.6 |
| Marital Status             |     |     |
| Married                    | 54  | 51.4|
| Single/divorced            | 37  | 35.2|
| Widower                    | 14  | 13.3|
| Profession                 |     |     |
| Retired                    | 32  | 30.5|
| Freelance                  | 26  | 24.8|
| Domestic                   | 10  | 9.5 |
| Formal job                 | 20  | 19.1|
| Informal job               | 10  | 9.5 |
| Unemployed                 | 7   | 6.7 |
| Origin                     |     |     |
| Capital                    | 67  | 63.8|
| Countryside                | 35  | 33.3|
| Other state                | 3   | 2.9 |

\(fi =\) absolute frequency; \(\% =\) percentage; \(Sd =\) standard deviation.

Considering the clinical data and comorbidities (Table 2), there was a high prevalence of hypertension, as well as dyslipidemia. Peripheral arterial disease was present in 27.6% of patients.

### Table 2. Clinical variables of patients.

| Variables (n = 105)       | fi  | %   |
|----------------------------|-----|-----|
| Insulin-dependent          | 46  | 43.8|
| Chronic kidney disease on  | 3   | 2.9 |
| dialysis                   |     |     |
| Dyslipidemia               | 48  | 45.7|
| Peripheral arterial disease| 29  | 27.6|
| Hypertension               | 59  | 56.2|
| Chronic kidney disease     | 13  | 12.4|
| Osteomyelitis on radiography| 40  | 38.1|
| Lesion location            |     |     |
| Right limb                 | 58  | 55.2|
| Left limb                  | 47  | 44.6|
| Surgical procedure         |     |     |
| Major amputation*          | 7   | 6.7 |
| Minor amputation**         | 56  | 53.3|
| Debridement                | 42  | 40.0|
| Previous antibiotic use    | 62  | 59.0|
| Previous hospitalization   | 54  | 51.4|
| Death                      | 5   | 4.8 |
| Hospitalization period     |     |     |
| (days)                     | Q1 – Median – Q3 | 10 – 16 – 26 |
| Smoking                    | 17  | 16.2|

\(fi =\) absolute frequency; \(\% =\) percentage; \(Qi =\) quartiles; *major amputation = amputation above the ankle; **minor amputation = amputation at foot level (with ankle preservation).

All patients underwent radiological examination of the affected foot, with presence of radiological osteomyelitis in 40 patients (38.1%).

When analyzing the type of surgical procedure, just over half the patients underwent minor amputations (with heel preservation), 40% of them underwent surgical debridement, and seven patients (6.5%), major amputations (Table 2).

There were five deaths (4.8%), septic shock being the most recorded cause. The median length of hospital stay was 16 (10-26) days (Table 2).
Considering the distribution of injuries severity according to the PEDIS and Wagner classifications (Figures 1 and 2), there were no injuries characterized as Grades 0 and 1 by either one, since the sample was composed exclusively of patients with infected diabetic foot who demanded surgical treatment. As for the PEDIS classification, we found a higher prevalence of Grade 3 injuries (79.0%); as for the Wagner’s one, there was a higher prevalence of Grade 2 (54.3%) and Grade 3 injuries (34.3%).

Of the 105 samples of tissue fragments collected for culture and susceptibility testing, 95 were positive for growth of a single germ. There was a predominance of Gram-negative bacteria from the Enterobacteriaceae family (51.5%) and a low incidence of Gram-negative bacteria from the Pseudomonadaceae family (4.2%). Among the Gram-positive bacteria isolated, there was a higher incidence of germs from the Staphylococcaceae and Enterococcaceae families.

Although the group of Gram-negative enterobacteria was more prevalent, the germs most frequently isolated in this study were the Gram-positive S. aureus (20.0%) and Enterococcus faecalis (17.9%). Proteus mirabilis (12.6%) and Klebsiella pneumoniae (10.5%) were the most isolated Gram-negative specimens, whereas Pseudomonas aeruginosa showed a low incidence (4.2%) in this population (Table 3).

**Table 3.** Distribution of culture results according to isolated germ.

| Isolated germ                        | fi | %    |
|--------------------------------------|----|------|
| Achromobacter xylosoxidans           | 1  | 1.1  |
| Citrobacter freundii                 | 2  | 2.1  |
| Citrobacter youngae                  | 1  | 1.1  |
| Complexo de Enterobacter cloacae     | 2  | 2.1  |
| Enterobacter cloacae ssp cloacae     | 1  | 1.1  |
| Enterococcus avium                  | 1  | 1.1  |
| Enterococcus faecalis               | 17 | 17.9 |
| Enterococcus faecium                | 1  | 1.1  |
| Escherichia coli*                    | (4*)| 6.3  |
| Klebsiella oxytoca                   | 4  | 4.2  |
| Klebsiella pneumoniae*              | (4*)| 10.5 |
| Morganella morganii ssp. Morganii    | 5  | 5.3  |
| Proteus mirabilis                    | 12 | 12.6 |
| Proteus penneri                     | 4  | 4.2  |
| Providencia stuartii                | 1  | 1.1  |
| Pseudomonas aeruginosa              | 4  | 4.2  |
| Serratia marcescens                 | 1  | 1.1  |
| Staphylococcus aureus**             | (12**)| 20.0|
| Staphylococcus saprophyticus        | 1  | 1.1  |
| Stenotrophomonas maltophilia        | 1  | 1.1  |
| Streptococcus agalactiae            | 1  | 1.1  |

*fi = absolute frequency; % = percentage; * = frequency of ESBL (extended spectrum beta-lactamase); ** = frequency of MRSA (methicillin-resistant S. aureus).

Regarding the bacterial resistance in vitro, we found high rates of S. aureus resistant to methicillin (63.2%) and to ciprofloxacin (55.5%), and 43.5% of Gram-negative bacteria were resistant to ciprofloxacin. P. aeruginosa, as well as all other Gram-negative bacteria, were sensitive to carbapenems. We highlight the presence of four strains of Klebsiella pneumoniae...
and four strains of Escherichia coli that were multi-drug resistant organisms (MDRO), with positive extended spectrum beta-lactamase (ESBL), as well as 12 strains of methicillin-resistant S. aureus (MRSA) (Tables 4 and 5).

**DISCUSSION**

We found a sociodemographic characteristic that was mainly characterized by men, with a predominant age group between 50 and 70 years old, retired, married, with low level of education, and living in the city of Manaus. Bona et al.\textsuperscript{12}, in an epidemiological study conducted in Fortaleza, capital of Ceara State, retrospectively analyzed 67 medical records of patients hospitalized for infected diabetic foot, and found a higher frequency of female patients (52%), with an age range similar to that found in the present study, and also observed a higher frequency of patients from the state capital. Pedras et al.\textsuperscript{13}, studying 206 diabetic patients with indication for amputation, observed a majority of male patients, with an average age of 66 years, married and with low education, characteristics similar to those found in the present study. Such aspects indicate that the sociodemographic variables can exert a significant role both in the prevalence and in the evolution of the diabetic foot. Low socioeconomic level, low education and lack of family support may be predisposing factors to the complications of DM and that can contribute to increased risk of lower extremity amputations\textsuperscript{14}.

**Table 4. Distribution of bacterial resistance profile of the most prevalent Gram-positive germs.**

| Antibiotics            | Staphylococcus aureus n - resistant / total isolated | % | Enterococcus faecalis n - resistant / total isolated | % |
|------------------------|------------------------------------------------------|---|----------------------------------------------------|---|
| Amoxicillin            | -- / 19                                             | 0 | -- / 17                                            | 0 |
| Amoxicillin / Sulbactam| -- / 19                                             | 0 | -- / 17                                            | 0 |
| Fusidic Acid           | 0 / 19                                              | 0 | -- / 17                                            | 0 |
| Benzylpenicillin       | 19 / 19                                             | 100 | 0 / 17                                            | 0 |
| Ciprofloxacin          | 10 / 19                                             | 55.5 | -- / 17                                            | 0 |
| Clindamycin            | 12 / 19                                             | 63.15 | -- / 17                                            | 0 |
| Daptomycin             | 0 / 19                                              | 0 | 0 / 17                                            | 0 |
| Erythromycin           | 14 / 19                                             | 78.94 | -- / 17                                            | 0 |
| Gentamycin             | 1 / 19                                              | 5.26 | 2 / 17                                             | 11.76 |
| Linezolid              | 0 / 19                                              | 0 | 0 / 17                                            | 0 |
| Oxacillin              | 12 / 19                                             | 63.15 | -- / 17                                            | 0 |
| Rifampicin             | 15 / 19                                             | 47.36 | -- / 17                                            | 0 |
| Teicoplanin            | 0 / 19                                              | 0 | 0 / 17                                            | 0 |
| Trimetropim Sulfamethoxazole | 0 / 19 | -- | -- | -- |
| Vancomycin             | 0 / 19                                              | 0 | 1 / 17                                             | 5.88 |

\textit{n - resistant} = number of resistant germs; total isolated = total number of isolated germs; \% = percentage.

**Table 5. Distribution of bacterial resistance profile of the most prevalent Gram-negative germs.**

| Antibiotics              | Proteus mirabilis R*/N** % | Klebsiella pneumoniae Escherichia coli % | Escherichia coli % | Morganella morganii R / N % | Pseudomonas aeruginosa R / N % |
|--------------------------|---------------------------|-----------------------------------------|-------------------|-----------------------------|--------------------------------|
| Amikacin                 | 0 / 12 0                 | 0 / 6 0                                 | 0 / 5 0           | 1 / 4 25                    |
| Amikacin/ Sulbactam      | 6 / 12 50                | 5 / 10 50                               | 6 / 10 100        | -- --                       |
| Ceftriaxone              | 4 / 12 33,3              | 4 / 10 40                               | 2 / 6 33,3        | 1 / 5 20                    | -- -- |
| Cefepime                 | 3 / 12 25                | 3 / 10 30                               | 1 / 6 16,6        | 0 / 5 0                     | 1 / 4 25 |
| Cefoxitin                | 0 / 12 0                 | 0 / 10 0                                | 1 / 6 16,6        | 3 / 5 60                    | -- -- |

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According to the 7th Brazilian Guideline for Hypertension, the prevalence of hypertension is estimated between 50 and 75% in patients with DM. In the present study, hypertension was the most prevalent comorbidity (56.2%), within the range estimated by that guideline.

Hinchliffe et al. found that peripheral arterial disease in diabetic patients with foot ulcers reaches 50% of frequency, which negatively impacts the capacity for tissue regeneration of these patients’ extremities, resulting in high rates of amputations. In the present study, peripheral arterial disease was present in 27.6% of patients. We believe that peripheral arterial disease, associated with infection, was responsible for the high amputation rate.

In our series, there was a non-negligible mortality, close to 5%. Assuming that these deaths were due to systemic infection whose primary focus was the foot injury, since the reason for hospitalization was infected foot injury, we can infer that the prevention of initial foot injuries is of utmost importance. This is fully reachable with simple prophylactic measures, through adequate orientation, made in primary care level, already well established in clinical guidelines. In addition, compliance to such guidelines could ultimately have avoided these deaths, as well as the amputations that left sequelae in the survivors.

The most frequent surgical procedures performed were amputations (60%), with a predominance of minor amputations (53.3%). Schaper et al., in a prospective European multicenter study with 1,232 patients, hospitalized and outpatient ones, found an amputation rate of 23% and a death rate of 6%. We believe that the highest amputation rate we found was because we only analyzed hospitalized patients, therefore with more severe injuries.

We used the Wagner and PEDIS classifications as parameters to classify wounds’ severity. Wagner’s classification, despite of not covering crucial clinical scores for both the presence of ischemia and neuropathy, remains the most widely used in research and clinical practice, being elementary and easy to apply. The PEDIS classification was originally developed by the International Working Group on the Diabetic Foot (IWGDF) for research purposes, being more complex than the Wagner’s one, and incorporating objective criteria of severity from a local and systemic infectious point of view. In this way, this becomes a useful tool to guide the empirical use of antimicrobials before obtaining the results of culture and antibiogram.

In this study, there no patients with Wagner or PEDIS grades 0 and 1, since we only included patients...
with infected diabetic foot that required hospitalization for surgery. There was predominance of grade 2 Wagner and grade 3 PEDIS injuries, slightly different from that found by Bona et al.\textsuperscript{12}, in Ceara, and by Oliveira et al.\textsuperscript{19}, in Goiania, who mostly observed more severe injuries (Wagner grade 4). A possible explanation for this difference is the easy access to urgent services in vascular surgery in the city of Manaus. As expected, we observed higher rates of amputations in the most severe degrees. Jeon et al.\textsuperscript{20}, studying 158 patients with infected diabetic foot, also found a positive correlation between the degrees of severity and the rate of amputations.

In this study, we performed 105 culture tests of infected tissue fragments from diabetic feet, and in 95 (90.5\%) samples there was bacterial growth. The fact that there are negative results from tissue fragment cultures of lesions in which there was, clinically, the existence of infection, can be explained by technical failure in the collection of the material or in its culture seeding. The use of antibiotics before the collection of biological material can also be a less likely cause of negative cultures, since the culture came from a fragment of biological tissue. Unlike the studies by Mendes et al.\textsuperscript{21}, Oliveira and Oliveira Filho\textsuperscript{9} and Xie et al.\textsuperscript{22}, among the positive results, we found the growth of only one microorganism in each culture. This finding was probably due to the methodology adopted for culture. In this sense, we obtained only deep tissue biological material, the biopsy being performed after debridement and proper wound cleaning, thus reducing the possibility of contamination by superficial microorganisms and skin colonizers. We should note that this is a confirmation of the correct execution of the material collection method used.

As Oliveira and Oliveira Filho\textsuperscript{9}, we found a higher prevalence of gram-negative bacteria (60.0\%), with a predominance of the Enterobacteriaceae family (51.5\%). Among these, we can highlight: Proteus mirabilis, Morganella morganii, Escherichia coli and Klesbisiella pneumoniae; there was a low frequency of Pseudomonas aeruginosa (4.2\%). These bacteria are often found in areas of hot climate\textsuperscript{23} and may be related both to climatic and to environmental and socio-cultural factors, as well as hygiene, among others\textsuperscript{24,25}.

The predominant isolated strains in the wounds were S. aureus (20\%), followed by E. faecalis (17.9\%), both Gram-positive cocci. Xie et al.\textsuperscript{22}, studying 117 patients with infected diabetic foot, also predominantly found S. aureus and E. faecalis.

All E. faecalis isolated showed high levels of sensitivity to the different antibiotic classes. Shettigar et al.\textsuperscript{26}, in a prospective epidemiological study with 100 patients, found E. faecalis with high rates of resistance to antibiotics such as erythromycin (94\%), tetracyclines (91\%) and ciprofloxacin (89\%).

In the reference hospital in which we carried out the present study, the scheme of empirical antibiotics widely available and often used by the vascular surgeons is the association of ciprofloxacin and clindamycin, based on national clinical guidelines\textsuperscript{27}. Vries et al.\textsuperscript{28} reported resistance for this combination by only 15\% of S. aureus and 22\% of Gram-negative germs. In our series, we observed a high rate of S. aureus resistant to clindamycin (63.2\%) and ciprofloxacin (55.5\%), only 56.5\% of Gram-negative germs being sensitive to ciprofloxacin. Although these percentages refer to bacterial resistance demonstrated in vitro, these data point to the need to adapt the empirical antibiotic therapy initially used for the treatment of patients with infected diabetic foot at the studied Hospital. Provably, this is the most important evidence found in the present study.

There has been a constant concern about the increase in multidrug-resistant bacteria since the 90s, with great attention focused on the beta-lactamases or carbapenemases producing Gram-negative germs of the Enterobacteriaceae family, known as MDRO, emerging as one of the major problems in the treatment of infections in diabetic patients\textsuperscript{24,29}. We isolated four strains of K. pneumoniae and four of E. coli that were multiresistant, with positive results for ESBL, corresponding to 14\% of the isolated Gram-negative bacteria. All positive ESBL were sensitive to carbapenems, but resistant to the empirical scheme initially used. Sekhar et al.\textsuperscript{30}, in a study with 108 patients hospitalized for the treatment of infected diabetic foot, observed a higher prevalence of MDRO in diabetic patients admitted with chronic foot injuries. These authors reported that the cultures of microorganisms such as E. coli, P. mirabilis and K. oxytoca, which that produce ESBL, also displayed high sensitivity to carbapenems.
Among the 19 (20.0%) cases of S. aureus, we found 12 (63.1%) MRSA strains, thus observing a high frequency compared with Mendes et al.\textsuperscript{21}, who found only 24.5% of MRSA. However, Mendes et al.\textsuperscript{21} studied only 49 patients, most of whom were treated on an outpatient basis, therefore with less severe injuries when compared with the injuries in the present study. We believe that the high frequency of MRSA we found may be due to frequent previous hospitalizations (51.4%) and to previous use of antimicrobials (59.0%).

Lauf et al.\textsuperscript{31} compared the use of ertapenem and tigecycline for the treatment of MRSA, with cure in 66% of patients treated with tigecycline of in 77% in those treated with ertapenem. In the present study, although ertapenem and tigecycline were not tested in MRSA, there was a 100% in vitro response to vancomycin, as well as to daptomycin, linezolid, teicoplanin and sulfamethoxazole-trimethoprim.

Our study’s limitations were the lack of information on the time since the last hospitalization (in the cases of patients with previous in-hospital treatment) and not having explored which antibiotic regimens had been previously used (in those patients who had used them prior to hospitalization).

We conclude that, in the studied reference hospital, the sociodemographic characteristics of patients for the treatment of infected diabetic foot was mainly composed of men, aged 50 to 70 years, married, retired, with low level of education, and from the city of Manaus. There was a predominance of Gram-negative bacteria from the Enterobacteriaceae family. However, when analyzing the frequency of individual germs isolated, we observed that Staphylococcus aureus and E. faecalis, both Gram-positive cocci, were the most frequently isolated. Regarding the bacterial resistance, there were high rates of resistance of Gram-negative germs to ciprofloxacin and of methicillin-resistant S. aureus, therefore also resistant to clindamycin. In view of this finding, we point out the need to adapt the initial empirical antibiotic therapy for patients with infected diabetic foot admitted to the Hospital 28 de Agosto. We therefore propose the association of vancomycin with a carbapenem or piperacillin-tazobactam as an initial empirical antibiotic therapy. Based upon our data, we further highlight the importance of cultures of tissue fragments of the foot lesions of these patients, associated with respective antibiograms, for the adequacy of antibiotic therapy during the treatment of this group of patients, always seeking to reduce the rates of amputations.

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Erratum

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