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Original

Bone infections in the diabetic foot: microbiology report

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Abstract

Aim: To describe and characterize the microbiology of diabetic foot osteomyelitis (DFO) in hospitalized patients of the San Juan de Dios Hospital (HSJD) in 2018.

Methods: The registry of patients seen at the Diabetic Foot Unit was consulted to obtain the demographic data of hospitalized patients with a diagnosis of osteomyelitis in addition to the information system of the Microbiology division of the Clinical Laboratory to obtain data on species of bacteria and their antimicrobial susceptibility profiles isolated from the respective bone samples. With these data, a database for the project was built and the descriptive statistical analysis was carried out in Excel spreadsheets.

Results: *Enterococcus faecalis* was the most frequently isolated agent (21%), followed by *Staphylococcus aureus (*14%) of which 70% presented a methicillin resistance phenotype and *Escherichia coli* as third place (11%). Other Gram-negative bacilli occupied the fourth place, being *Enterobacter cloacae* (8%) and *Klebsiella pneumoniae* (8%) the main isolated species; it should be noted the presence of 24% of strains carrying extended spectrum ß-lactamases, with *K. pneumoniae* as the main carrier species of this resistance.

***Conclusions**: S. faecalis* was reported for the first time as the most isolated bacterium in DFO, which allows us to conclude that the microbiology of these conditions in Costa Rica differs from that reported in the international literature and it is necessary to update the Costa Rican medical community. It is also important to note that even though the percentage obtained from Gram-negative rods carrying ESBL is lower than that reported in the literature, 100% of the *K. pneumoniae* strains were carriers of this resistance phenotype, therefore it is possible to consider the local level that the preliminary finding of this species should modify the therapeutic spectrum to be used pending final reports.

Keywords: Osteomyelitis, Diabetic Foot, Microbiology

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Abbreviations: AMP, Ampicillin; BLEE, Beta-lactamase Extended Spectrum; CAZ, Ceftazidime; CTX, Cefotaxime; HSJD, Hospital San Juan de Dios; IDF, Infection of Diabetic Foot; IDSA Infectious Diseases Society of America; MRSA, Methicillin-Resistant Staphylococcus aureus; DFO, Diabetic Foot Osteomyelitis; AST, Antimicrobial Susceptibility Test; TZP, Piperacillin-tazobactam; VAN, Vancomycin; VISA, Vancomycin intermediate Staphylococcus aureus

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Diabetic foot infection (DFI) is one of the main infectious complications in patients with diabetes mellitus, with a reported incidence of up to 50% of the total diabetic foot ulcerations, resulting in an increased rate of hospitalization and the risk of lower extremity amputation.[1] The main risk factors for this condition are vasculopathy, peripheral neuropathies, and immunopathologies, the latter two having a greater weight on the pathophysiology of these infections.[2]

The microbiology of IDF is varied and depends on whether the infection is acute or chronic. In the case of acute cases, Gram-positive skin-colonizing cocci are the most frequently isolated microorganisms; usually, these are cases whose onset was a sharp trauma to the skin with the most frequently isolated bacteria *S. aureus* and hemolytic streptococci of groups A, B, C and G.[3] In the case of chronic conditions these tend to be polymicrobial in nature and there may be a mixture of aerobic and anaerobic microorganisms, with a great variety of Gram-negative bacilli, such as the enterobacteria *Escherichia coli*, *Proteus* sp. and *Klebsiella* species*; in* addition to non-fermenting species of the genus *Pseudomonas*; in the case of anaerobic microorganisms, the presence of the genera *Peptococcus*, *Peptostreptococcus*, *Clostridium*, *Fusobacterium* and *Bacteroides* has been reported, usually are found in cases of ischemia and gangrene called "fetid foot".[4]

The most important infectious complication of IDF is osteomyelitis, which is defined as a bone infection present in approximately 20% of cases of patients with IDF;[5] In addition, it significantly increases the risk of limb amputation, which is one of the most difficult complications to manage.[6] Diabetic foot osteomyelitis (DFO) usually occurs through continuity dissemination from a previous IDF affecting the soft tissue surrounding the bone; because of this, the microbiology of DFO is very similar to that seen in IDF, which is shown in Table 1.

Table 1. Description of microbiology reported internationally in DFO tables

Gram-positive cocci	Gram-negative bacilli
*Staphylococcus aureus**	*Escherichia coli**
*Staphylococcus epidermidis**	*Klebsiella pneumoniae**
	Proteus s.p.
	*Pseudomonas aeruginosa**

*Increased antimicrobial multidrug resistance has been detected in these species[8].

Regarding the antimicrobial treatment of DFOs, the Infectious Diseases Society of America (IDSA) recommends designing empirical therapies based on the local microbiology of each center to optimize the use of antimicrobials and the therapeutic success of DFOs[7] because it has been shown that this practice leads to therapeutic success in follow-ups of up to 12 months.[5] Specifically for Costa Rica, there are no local studies describing the microbiology of DFOs in Diabetic Foot Units, so the empirical therapies used are usually based on international literature reports regarding the most probable etiological agents. The current empirical therapy applied to severe DFO cases in this HSJD Diabetic Foot Unit consists of the combination of vancomycin with a third-generation cephalosporin such as cefotaxime or ceftazidime, which is not supported by local microbiological data.

Methods

This retrospective observational study was conducted at the HSJD between July and August 2019, collecting the information obtained from the databases of the Diabetic Foot Unit and the Microbiology division of the HSJD during 2018. Data was collected and analyzed, to initially describe the patient population in terms of gender and age. The object of the study was to characterize the bacteria isolates and their antimicrobial resistance profiles from bone samples of patients diagnosed with osteomyelitis seen at the Diabetic Foot Unit of the HSJD during 2018. Regarding the antimicrobial resistance profile, a classification of the antibiogram between Gram-positive and Gram-negative was performed, using the classification based on the automatized PSA cards used for its determination. The inclusion criteria was hospitalized patients with a diagnosed osteomyelitis and a bone sample with positive bacteriological culture during 2018 and the sample analyzed corresponded to all patients who met the inclusion criteria. Being a descriptive observational study, measures of central tendency such as means and percentages were used to describe the variables studied using Microsoft Excel 2019 software; in addition, the study protocol complied with the scientific and bioethical requirements of the Scientific Ethical Committee (CEC) of the HSJD.

Reults

The description at the species level and antimicrobial resistance profile of bacteria isolated from preventive bone samples of 48 patients with a diagnosis of osteomyelitis was performed; as for the demographic characteristics of the study population, 69% were male and 31% female; 40% with an age range between 61 and 70 years (Figure 1).

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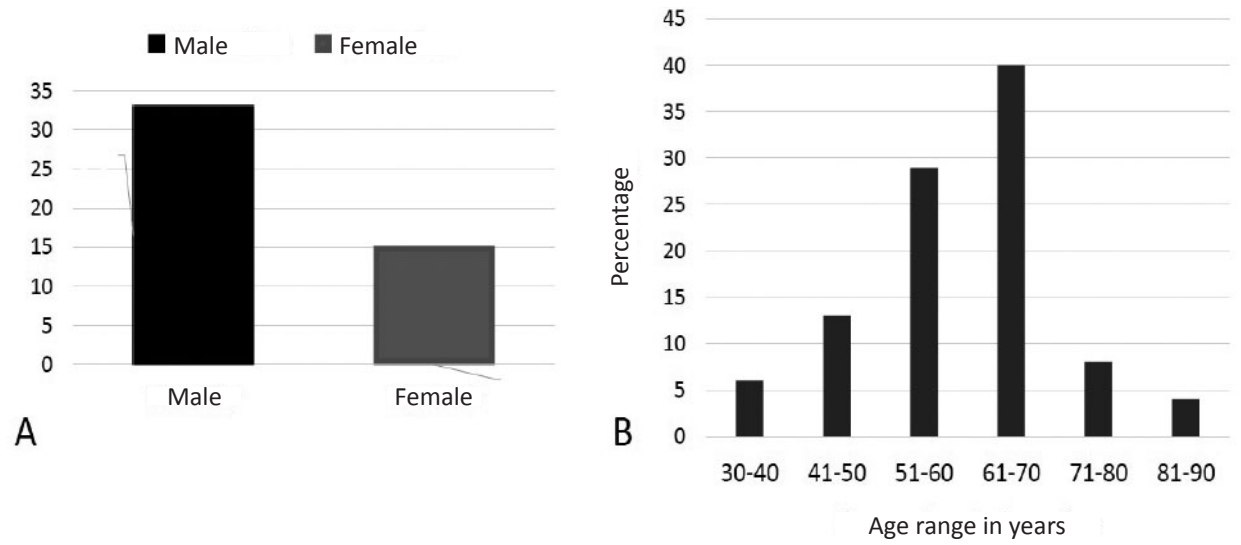

Figure 1. Distribution of patients with DFO in 2018 according to sex and age range. In A) the percentages are expressed according to sex and in B) the percentages are according to age range.

A total of 73 isolates were identified; 57% corresponded to Gram-negative bacilli and 43% to Gram-positive cocci. The most frequent microorganism was *Enterococcus faecalis (*21%), followed by *Staphylococcus aureus* (14%), *Escherichia coli* (11%), *Enterobacter cloacae* (8%), *Streptococcus agalactiae* (8%), and *Klebsiella pneumoniae* (8%) (Figure 2).

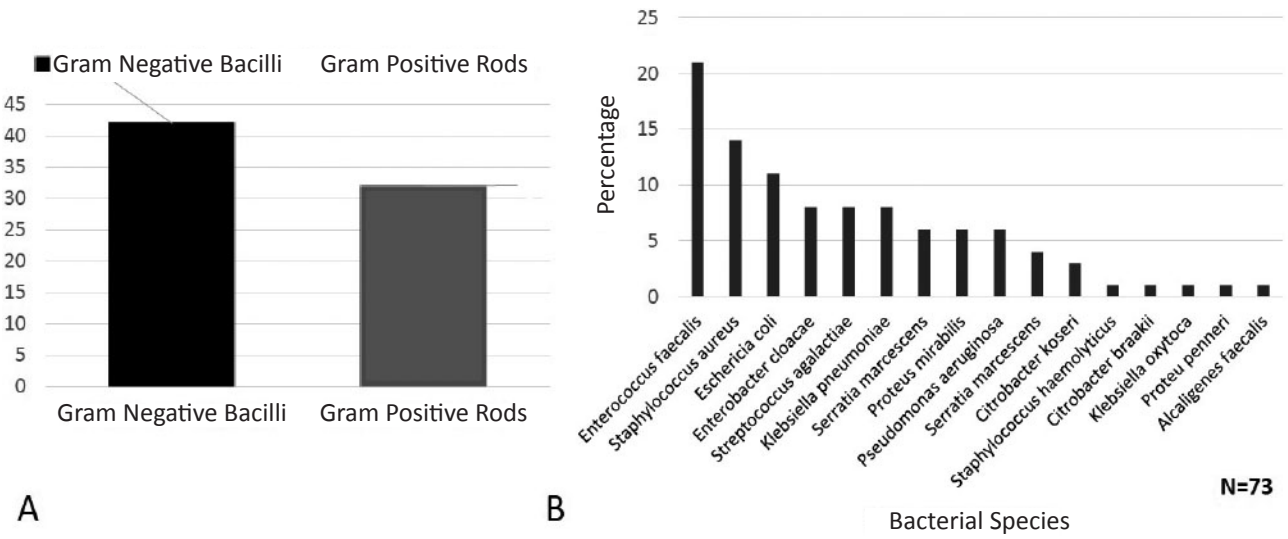

Figure 2. Frequency distribution of bacteria isolated from bone samples of DFO patients in 2018. A) depicts the frequencies according to Gram stain morphology and B) breaks down the total distribution of the 75 bacterial species isolated.

In Enterococcus faecalis, 100% of the isolates were sensitive to AMP and VAN and in Staphylococcus aureus where the same percentage of sensibility for VAN and 70% of samples were methicillin-resistant (MRSA) (Figure 3). Regarding the resistance profile of Klebsiella pneumoniae, 100% of the isolates were BLEE positive (Figure 4).

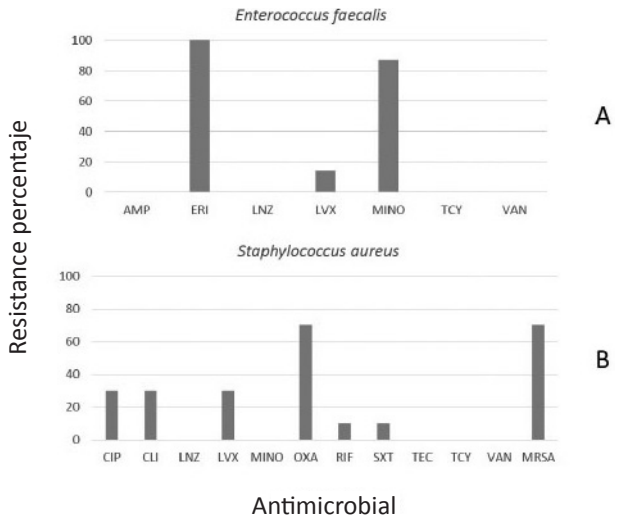

Figura 3. Resistance profile of the main Gram-positive cocci isolated from DFO bone samples in 2018. In A) is shown in the profile for 15 *Enterococcus faecalis* isolates and in B) the profile for 10 *Staphylococcus aureus* isolates along with strains with MRSA phenotype.

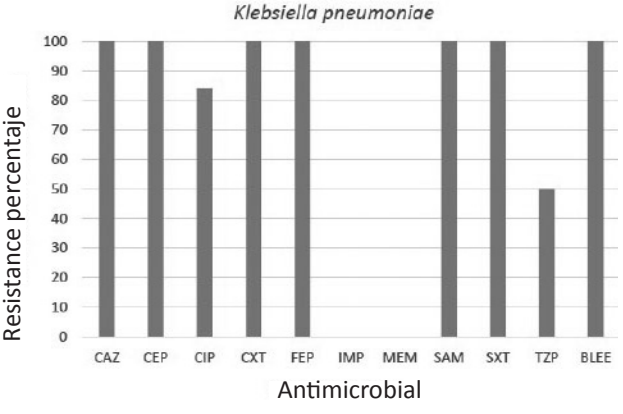

Figura 4. Resistance profile of 6 *Klebsiella pneumoniae* isolates and percentage of strains carrying BLEE-type resistance mechanism obtained from bone samples of DFO patients in 2018.

The non-lactose fermenting agent isolated was Pseudomonas aeruginosa, which showed a resistance profile of 50% for piperacillin/tazobactam but for the rest of the antimicrobials, including ceftazidime, 100% sensitivity was detected.

Discussion

The characteristics of the patient population studied coincided with those described in the international literature in terms of sex and age, where DFO has typically occurred in many cases in older male patients with an average age of around 62 years.[10] In terms of the distribution of the bacterial species isolated, the prevalence of *Enterococcus faecalis* differed from that reported in the literature, which places this species in fourth place in terms of frequency behind *Staphylococcus aureus*, coagulase-negative *Staphylococcus,* and *Streptococcus* sp.[11] As for the enterobacteria isolated in this study, their distribution in terms of frequency coincided with that reported in the literature, with *Escherichia coli* as the most frequently isolated species.[11]

The percentage of MRSA reported in the literature is close to 31%,[13] in the sample analyzed, a much higher percentage was identified, consistent with the local and national behavior of methicillin resistance in *S. aureus,* the reasons for which have not been fully studied in the country but which are presumably due to greater consumption (previous exposure) of these patients and the general population to short- and broad-spectrum antimicrobial guidelines. Furthermore, according to the IDSA, the fact that a patient is a carrier of a methicillin-resistant strain is an independent risk factor that increases the risk of therapeutic failure.[7]

The enzymatic mechanism of resistance found in these patients was BLEE-mediated, present in 24% of the isolates. The worldwide figure reported in the literature is around 38% of BLEE for these cases.[13] In the specific case of *Klebsiella pneumoniae* isolates, all isolates showed resistance to cephalosporins (BLEE) and this species is a risk factor associated with a higher number of fatalities.[13] Thus, it is possible to consider locally that the preliminary finding of *K. pneumoniae* should lead to modify the antimicrobial spectrum to one with coverage against BLEE (piperacillin/tazobactam, ertapenem or meropenem). As for the isolates of *Pseudomonas aeruginosa,* given that there was a 100% sensitivity to ceftazidime, which is a frequently used anti-pseudomonal antimicrobial in the Diabetic Foot unit, its use was supported by this local microbiology report. The use of vancomycin is consistent with the phenotypes of resistance

of Gram-positive cocci; therefore, the local data shown here falls in line with its use in current empirical therapy.

Conclusions

E. faecalis was reported for the first time as the most frequent bacterium in DFO, which allows us to conclude that the microbiology of these conditions in Costa Rica differs from that reported in the international literature and it is necessary to inform of these findings to the Costa Rican medical community. In addition, it is important to highlight that although the percentage of Gram-negative bacilli carrying BLEE is lower than that reported in the literature, 100% of the strains of K. pneumoniae were carriers of this resistance phenotype; therefore, the preliminary finding of this species makes it necessary to consider the use of an antimicrobial agent with a spectrum against BLEE.

Taking all this data into consideration, while waiting for the cultures with species and PSA report that will allow then the modification of the treatment to monotherapy or a combination of agents with the lowest possible spectrum, the following empirical antimicrobial therapy scheme is suggested: a) without risk factors for BLEE or Pseudomonas aeruginosa: monotherapy with ceftaroline 600 mg IV every 12 hours or combination therapy with vancomycin 20-30 mg/kg IV loading dose followed by 15 mg/kg IV every 12 hours, plus cefotaxime 2 g IV every 8 hours; b) with risk factors for BLEE and/or Pseudomonas aeruginosa: combination therapy with vancomycin 20-30 mg/kg IV loading dose followed by 15 mg/kg IV every 12 hours, plus piperacillin/tazobactam 4.5 g IV every 6 hours. Alternatively, depending on renal functionality and contraindications for its use, vancomycin could be substituted by linezolid 600 mg IV every 12 hours.

Risk factors for BLEE and pseudomonas aeruginosa are listed in Table 2.

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