DOI: https://doi.org/10.30795/jfootankle.2021.v15.1225

Original Article

Neuropathic foot ulcer: microbiological assessment in chronic osteomyelitis

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Abstract

Objective: To determine the microbiological profile and antimicrobial susceptibility patterns of organisms isolated from chronic osteomyelitis secondary to neuropathic foot ulcers; secondarily, to describe the clinical outcomes of 52 patients admitted to a neuropathic foot referral center.

Methods: Retrospectively chart review of 52 patients with clinically infected neuropathic foot ulcers admitted to our service for treatment between 2005 and 2013. Tissue samples were collected for culture at the operating room after extensive debridement in order to determine the infectious agents and their resistance profile using the disk-diffusion technique, following CLSI criteria.

Results: A total of 52 patients were analyzed (40 males and 12 females). The mean age was 58 (37-72) years. Each patient presented with an average of 2.13 microorganisms, distributed as follows: 51% Gram-positive cocci, 43% Gram-negative bacilli. Among *Staphylococcus aureus* isolates, the prevalence of methicillin resistance was almost 50%, and the prevalence of coagulase-negative staphylococci (CoNS) was more than 75%.

Conclusion: *S. aureus*, *E. faecalis*, and CoNS were the most frequently isolated pathogens. Methicillin resistance was highly prevalent. A combination of extensive surgical debridement and prolonged antimicrobial therapy led to remission of infection in 77% of patients.

Level of Evidence IV; Therapeutic Studies; Case Series.

Keywords: Antibiotics; Arthropathy, neurogenic/diagnosis; Foot/microbiology; Osteomyelitis; Ulcer.

Introduction

Neuropathic inflammatory osteoarthropathy of the foot, or simply neuropathic foot, is a syndrome first reported more than 100 years ago by Herbert William Page[1]. It was initially described as a complication of tabes dorsalis, but metabolic causes, such as diabetes and alcohol abuse, are now most frequently observed[2].

Symptoms vary from slight loss of sensitivity to total numbness and burning pain. Patients with this syndrome are at high risk of ulcers and infections of the foot, which may culminate in osteomyelitis and amputation[2].

Worldwide, diabetic foot ulcers are a major medical, social, and economic problem. They are the leading cause of hospitalization in diabetic patients. If not promptly treated, amputation of the infected foot is required[3]. It is estimated that 25% of patients with diabetes will present with a foot ulcer at some point in their lives[4].

Diabetic neuropathy causes damage to the peripheral nerves throughout the body, in particular the feet. In patients with neuropathic feet, injuries frequently go unnoticed, leading to severe infections and amputations, with a risk of amputation 25 times greater than in healthy individuals; indeed, diabetic neuropathy is the leading cause of non-traumatic amputations (57,000 per year)[4]. Even when amputation cannot be avoided, good quality of life may be obtained by good follow-up care from a multidisciplinary foot team[5].

Study performed at the Instituto de Ortopedia e Traumatologia, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, SP, Brazil.

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Conflicts of interest: none. **Source of funding:** none. **Date received:** February 25, 2021. **Date accepted:** March 09, 2021. **Online:** April 30, 2021

How to cite this article: Lima, ALLM, Rosemberg DL, Oliveira PRD, Saito GH, Macedo RS, Sposeto RB, Godoy-Santos AL, Fernandes TD. Neuropathic foot ulcer: microbiological assessment in chronic osteomyelitis. J Foot Ankle. 2021;15(1):14-8.

Proper management of infections in the neuropathic foot requires appropriate antibiotic selection based on culture and antimicrobial susceptibility. Several studies have found methicillin-resistant *Staphylococcus aureus* (MRSA) and polymicrobial infection present in as many as 15–30% of diabetic patients with chronic osteomyelitis secondary to neuropathic ulcers. Infection with multidrug-resistant organisms (MDROs) may increase the length of hospital stay, cost of management, and number of surgical procedures, and may cause additional morbidity and mortality[6-13]. Although increasing antimicrobial resistance is a concerning problem in the BRICS countries, there is a paucity of data regarding the frequency of MDRO infections and the outcome of such infections among diabetic foot ulcers in these regions, especially Brazil[14].

Our hypothesis is that, since our service assists patients with diagnoses other than diabetes, and our population differs from the majority of others in which insensitive foot infections were studied, the microbiological profile and antimicrobial susceptibility patterns of organisms isolated from chronic osteomyelitis (CO) secondary to neuropathic foot ulcers will be different from the standard profile related in the literature[15].

Thus, the objective of this study is to determine the microbiological profile and antimicrobial susceptibility patterns of organisms isolated from CO secondary to neuropathic foot ulcers in patients treated at our tertiary neuropathic foot center.

Methods

This study was approved by the Institutional Review Board and registered on the Plataforma Brasil database under CAAE (Ethics Evaluation Submission Certificate) number: 44731621.3.0000.0068.

Medical charts of 52 patients with clinically infected neuropathic foot ulcers admitted to our university-affiliated Diabetic Foot Center between January 2005 and December 2013 were reviewed.

Osteomyelitis was diagnosed on the basis of suggestive changes on plain radiographs and magnetic resonance imaging (MRI), and confirmed by microbiological examination (Figure 1). All cases were analyzed at 6 months of follow-up. Only results of bone culture obtained at the time of surgical debridement following antisepsis were considered for microbiological characterization.

Inclusion criteria

1. Patients admitted for treatment of chronic osteomyelitis secondary to neuropathic foot infection;
2. Surgical debridement performed between January 2005 and December 2013.

Exclusion criteria

Previous surgical manipulation of the affected foot.

Specimen acquisition and susceptibility testing

All bone specimens were collected from the clean surgical site, in the operating room, after extensive debridement or amputation (Figure 2). Samples were sent to the microbiology laboratory in bottles containing thioglycolate growth medium. Susceptibility tests were performed in all cases using the disk-diffusion technique; when required, minimum inhibitory concentrations were obtained using automatic methods or "e-test", and reported in accordance with the CLSI criteria[16].

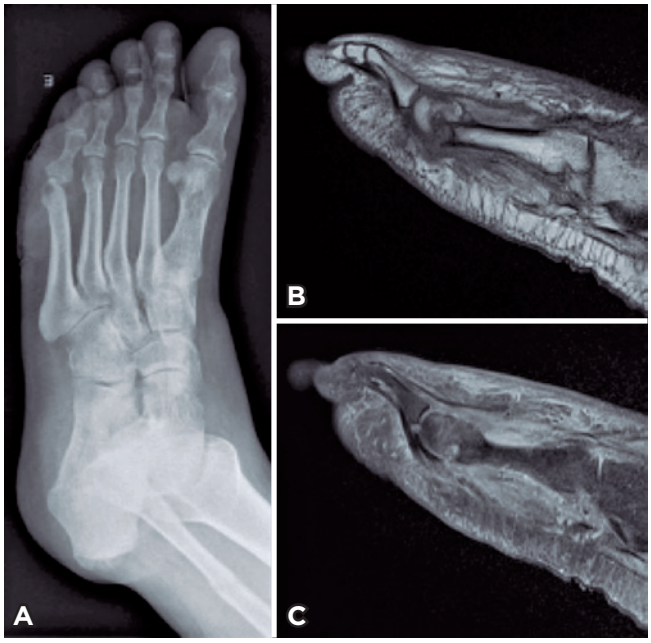

Figure 1. A) Radiography showing signal abnormality in the head of the 5th metatarsus, B) T1-weighted MRI showing a lesion in the head of the 5th metatarsus. C) T2-weighted MRI of the same foot.

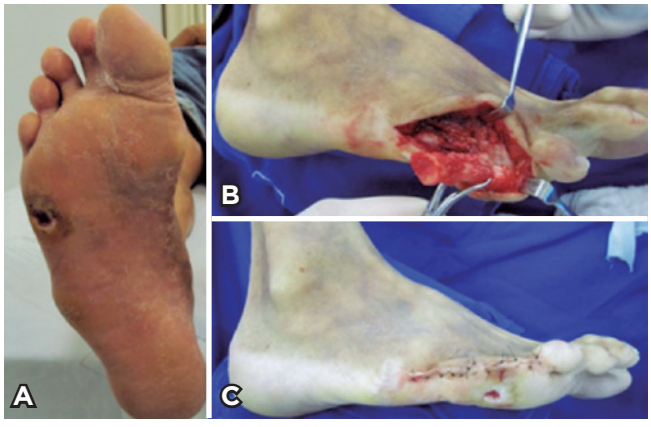

Figure 2. A) Ulcer on foot. B) Surgical debridement. C) Clinical outcome after surgery.

Statistical analysis

Quantitative variables were expressed as means ± standard deviation, while qualitative variables were expressed as percentages.

The association of study variables with MDRO and non-MDRO infections was tested using Student's *t* test or Fisher's exact test as appropriate.

Odds ratios (ORs) (with 95% CIs) for having MDRO-associated ulcers were calculated. Multiple logistic regressions were employed to identify independent predictors of MDRO infections and predictors of glycemic control.

Results

Fifty-two patients were treated during the period of analysis, 40 men (77%) and 12 women (23%). Mean age was 58 years (range, 37-72). Diabetes was the major cause of neuropathic foot (46 patients, 88%), followed by Hansen's disease (4 patients, 8%) and spinal cord injury and alcoholic neuropathy (2 cases each, 2%). Most ulcers (39, 75%) were located in the forefoot; the rest were located in the transition of the midfoot and hindfoot. Regarding surgical approach, 21 patients (41%) underwent debridement only, 27 (51%) underwent partial foot amputation, and 4 (8%) underwent below-knee amputation.

One-hundred and nine bacterial isolates were identified as causative agents of infection (mean, 2.13 isolates per patient). There was a predominance of Gram-positive cocci (51%), followed by Gram-negative bacilli (GNB) (43%). The most prevalent agents were *Staphylococcus aureus* (18%), *Enterococcus faecalis* (18%), and coagulase-negative staphylococci (CoNS) (14%). The profile of the isolated organisms is detailed in table 1.

Among *S. aureus* isolates, the prevalence of methicillin resistance (MRSA) was 48%, with 100% susceptibility to sulfamethoxazole/trimethoprim (SMX/TMP). Among CoNS, 77% were methicillin-resistant (MRCoNS), again with 100% susceptibility to SMX/TMP. All isolates of *E. faecalis* were susceptible to ampicillin and vancomycin. Among GNB, the Enterobacteriaceae predominated (77%), with 82% susceptibility to ciprofloxacin and piperacillin/tazobactam and 100% susceptibility to carbapenems. The results of susceptibility studies are summarized in table 2.

All patients received antimicrobial treatment guided by susceptibility tests for 6 months after debridement. Ciprofloxacin was the most frequently used drug (33%), followed by amoxicillin (21%) and SMX/TMP (19%) (Table 3).

At 6-month follow-up, 75% of patients were in remission, without signs of infection; 23% of patients presented recurrence of infection; and 2% had been lost to follow-up.

Discussion

In the present study, we described the microbiological profile and antimicrobial susceptibility patterns of organisms isolated from patients with neuropathic foot ulcers at a diabetic foot referral center. The organisms were isolated from bone cultures of patients with confirmed diagnosis of osteomyelitis, collected intraoperatively. As we hypothesized, the microbiological profile observed in this study differs from the standard profile described in the literature. This is consistent with the fact that our patients differed from those of most previous studies, as we also included non-diabetic neuropathic feet. Michalek et al.[15] postulated in their paper that different populations in different countries have different infections.

Previous reports have used swab cultures to describe the microbiological profile of patients with neuropathic ulcers[14,17-19]. However, this method is susceptible to contamination, possibly detecting organisms that are not actually sources of infection. Senneville et al.[20] compared superficial swab cultures with percutaneous bone biopsy cultures and found that swab cultures do not reliably identify bone organisms[8]. Peri-wound bone cultures have also been performed to identify osteomyelitis in neuropathic foot with ulcers. However, the reliability of this method and whether it avoids potential contaminants is unclear. By collecting intraoperative bone cultures, we could reliably describe the microbiological profile and antimicrobial susceptibility patterns of organisms in the diabetic foot center of a tertiary hospital[8,9]. Interestingly, we observed a high rate of isolates per specimen, despite thorough debridement and irrigation prior to collection of bone cultures. This finding suggests that osteomyelitis caused by multiple organisms may be an increasing issue that physicians should be prepared to deal when treating neuropathic foot infections[21].

Table 1. Microorganisms Isolated from the 52 patients with neuropathic foot osteomyelitis

Staphylococcus aureus	19 (17.43%)
Enterococcus faecalis	19 (17.43%)
Coagulase-negative *Staphylococcus* spp.	15 (13.76%)
Morganella morganii	10 (9.17%)
Proteus spp.	8 (7.34%)
Acinetobacter spp.	6 (5.50%)
Escherichia coli	5 (4.59%)
Serratia marcescens	5 (4.59%)
Pseudomonas aeruginosa	4 (3.67%)
Enterobacter cloacae	3 (2.75%)
Bacteroides spp.	3 (2.75%)
Finegoldia magna	2 (1.83%)
Klebsiella spp.	2 (1.83%)
Streptococcus spp.	2 (1.83%)
Sphingomonas paucimobilis	1 (0.92%)
Stenotrophomonas maltophilia	1 (0.92%)
Prevotella melaninogenica	1 (0.92%)
Eikenella corrodens	1 (0.92%)
Citrobacter koseri	1 (0.92%)
Clostridium spp.	1 (0.92%)
TOTAL	**109**

Table 2. Susceptibility of each microorganism

	Clinda	Levo	Sulfa	Teico	Vanco	Ampi	Cipro	Ceftri	Erta	Pip/Tazo	MR	MS
S. Aureus	12.50%	12.50%	100%	100%	100%	/	/	/	/	/	47.37%	52.63%
Coag Neg S.	0%	10%	100%	90%	100%	/	/	/	/	/	76.92%	23.08%
E. Faecalis	/	/	/	/	100%	100%	/	/	/	/	/	/
Enterobactéria	/	/	/	/	/	/	82.35%	88.24%	100.00%	88.24%	/	/

Clinda: Clindamycin; Levo: Levofloxacin; Sulfa: Sulfamethoxazole; Teico: Teicoplanin; Vanco: Vancomycin; Ampi: Ampicillin; Cipro: Ciprofloxacin; Ceftri: Ceftriaxone; Erta: Ertapenem; Pip/Tazo: Piperacillin/Tazobactam; MR: Multidrug resistant; MS: Multidrug sensitive.

Table 3. Antimicrobial agents used for treatment of neuropathic foot osteomyelitis

Antimicrobial agent	
Ciprofloxacin	17 (25.37%)
Sulfamethoxazole/Trimethoprim	10 (14.93%)
Ertapenem	8 (11.94%)
Teicoplanin	7 (10.45%)
Amoxicillin	6 (8.96%)
Amoxicillin/Clavulanic acid	5 (7.46%)
Levofloxacin	4 (5.97%)
Cefalexin	3 (4.48%)
Clindamycin	2 (2.99%)
Metronidazole	2 (2.99%)
Cefepime	1 (1.49%)
Moxifloxacin	1 (1.49%)
Vancomycin	1 (1.49%)

The distribution of isolates according to type of microorganism is highly variable in the literature[15]. This difference may be explained either by the intrinsic variations of microbiological profile among different centers or by the different methods used for collection of samples. *S. aureus* is considered the most prevalent pathogen in diabetic foot infections. In the present study, even though *S. aureus* was one of the most commonly observed gram-positive microorganism, *E. faecalis* was equally prevalent. In agreement with previous reports, we observed a 48% rate of MRSA, confirming the concern of increasing antimicrobial resistance in neuropathic foot infections[14,18,22]. On the other hand, vancomycin-resistant strains were not observed in this study, unlike in previous reports[23-25]. We also noted a high prevalence of CoNS, which may be attributed to the impaired host defenses observed in diabetic patients[26,27]. A previous study showed that the distribution of CoNS in bone and swab cultures were significantly different[8]. These findings indicate that bone cultures may be necessary to accurately identify this low-virulence organism. Therefore, this microorganism may be more prevalent than previously reported. Among GNB, Enterobacteriaceae were highly prevalent, representing 77% of these cultures.

The strength of the present study lies on the fact that all bone cultures were collected intra-operatively, using a relatively large population with confirmed diagnosis of osteomyelitis. This allowed us to collect bone samples precisely from the affected tissue by direct visualization. Collecting bone samples after debridement and antisepsis also decreased the risk of possible contaminant organisms.

However, this study was not without limitations. It was performed in a single tertiary care center, to which the most severe cases are referred, often after treatment failure and prior antimicrobial treatment. Therefore, the microorganism profile and resistance patterns described herein cannot be extrapolated to the general population or to other specialist diabetes centers.

Conclusion

In diabetic patients with chronic osteomyelitis secondary to neuropathic ulcers, *S. aureus*, *E. faecalis*, and CoNS were the most frequent pathogens isolated. Occurrence of MRSA and MRCoNS was high, but 100% susceptibility to SMX/TMP was preserved. A combination of extensive surgical debridement and prolonged antimicrobial therapy led to remission of infection in 77% of patients at 6 months of follow-up.

Authors' contributions: Each author contributed individually and significantly to the development of this article: ANLML*(https://orcid.org/0000-0002-2396-9880) Interpreted the results of the study, participated in the reviewing process; DLR *(https://orcid.org/0000-0003-0183-8641) Wrote the paper, interpreted the results of the study; PRDO *(https://orcid.org/0000-0003-1377-6556) Conceived and planned the activities that led to the study, interpreted the results of the study, participated in the reviewing process; GHS *(https://orcid.org/0000-0002-1211-9258) Interpreted the results of the study, participated in the reviewing process; RSM *(https://orcid.org/0000-0002-5025-4338) Participated in the reviewing process, approved the final version; RBS *(https://orcid.org/0000-0003-1085-0917) Participated in the reviewing process, approved the final version; ALGS *(https://orcid.org/0000-0002-6672-1869) Interpreted the results of the study, participated in the reviewing process; TDF *(https://orcid.org/0000-0002-9687-7143) Participated in the reviewing process, approved the final version. All authors read and approved the final manuscript. *ORCID (Open Researcher and Contributor ID).

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