The Brazilian Journal of
INFECTIOUS DISEASES

www.elsevier.com/locate/bjid

Original Article

Gram-negative osteomyelitis: clinical and microbiological profile

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ARTICLE INFO

Article history:
Received 21 July 2011
Accepted 18 August 2011

Keywords:
Osteomyelitis
Fractures, open
Gram-negative bacterial infections
Accidents, traffic

A B S T R A C T

Introduction: Despite the growing interest in the study of Gram-negative bacilli (GNB) infections, very little information on osteomyelitis caused by GNB is available in the medical literature.
Objectives and methods: To assess clinical and microbiological features of 101 cases of osteomyelitis caused by GNB alone, between January 2007 and January 2009, in a reference center for the treatment of high complexity traumas in the city of São Paulo.
Results: Most patients were men (63%), with median age of 42 years, affected by chronic osteomyelitis (43%) or acute osteomyelitis associated to open fractures (32%), the majority on the lower limbs (71%). The patients were treated with antibiotics as inpatients for 40 days (median) and for 99 days (median) in outpatient settings. After 6 months follow-up, the clinical remission rate was around 60%, relapse 19%, amputation 7%, and death 5%. Nine percent of cases were lost to follow-up. A total of 121 GNB was isolated from 101 clinical samples. The most frequently isolated pathogens were *Enterobacter* sp. (25%), *Acinetobacter baumannii* (21%) e *Pseudomonas aeruginosa* (20%). Susceptibility to carbapenems was about 100% for *Enterobacter* sp., 75% for *Pseudomonas aeruginosa* and 60% for *Acinetobacter baumannii*.
Conclusion: Osteomyelitis caused by GNB remains a serious therapeutic challenge, especially when associated to nonfermenting bacteria. We emphasize the need to consider these agents in diagnosed cases of osteomyelitis, so that an ideal antimicrobial treatment can be administered since the very beginning of the therapy.

Introduction

Osteomyelitis is even today one of the most challenging bone and joint infections, although the disease first descriptions go back to the Hippocrates era.[1] Gram-positive (GP) bacteria, especially *Staphylococcus*, classically cause this disease, but Gram-negative bacteria have grown in importance as causative agents.[2,3] Such importance can be explained by the increasing number of orthopedic surgeries with the use of implants and, especially, the rising number of high-energy traumas associated with open fractures, as a consequence of traffic accidents and war injuries.[4-6]

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Although Gram-negative bacilli (GNB) represent a minor portion of all the pathological agents isolated in osteomyelitis cases, they are of major clinical importance due to the peculiarities of their antimicrobials susceptibility pattern and due to the co-morbidities generally affecting those patients.[4,7] The importance of multidrug resistant GNB was described more recently, as being causative agents of acute post-traumatic osteomyelitis among US military personnel with open fractures of the lower limbs, especially nonfermenting GNB.[5]

Currently there is insufficient information in the literature to draw any conclusion with regard to the variables involving osteomyelitis caused by these agents. The objective of this study was to describe clinical and microbiologic characteristics of acute and chronic osteomyelitis episodes, treated in a university hospital providing care for high complexity orthopedics cases in the city of São Paulo.

Methods

Study design

For this retrospective analysis, our study included all patients with a diagnosis of osteomyelitis who were treated at our institution from January 2007 to January 2009. Cases whose bone or bone marrow aspirate cultures grew GNB were included. Only the first positive culture for each identified patient was considered. Patients exhibiting GP growth together with GNB and patients with contaminated cultures were excluded.

A total of 173 bone samples yielding positive culture growth for any GNB were identified. After checking the culture results, 51 patients were excluded due to a concomitant growth of at least one GP agent. Out of the 122 remaining patients, 12 were excluded as the first positive culture occurred outside the study period and 9 were excluded for exhibiting positive cultures considered as contamination. Data of 101 patients who fulfilled the inclusion criteria were reviewed.

Five clinical outcomes were analyzed six months after hospital discharge: disease remission, amputation of the affected limb, infection relapse, death and loss to follow-up.

Definitions

A culture sample was considered contamination when collected in the first debridement of an open fracture. Remission was defined as the absence of clinical signs and symptoms of infection, X-rays without signs of osteomyelitis, and, whenever possible, normal inflammatory markers. Infection relapse was defined as the return of signs or symptoms within six months from hospital discharge. Those cases with no observations reported in the patient chart after six months were considered lost to follow-up.

Symptoms of infection at presentation were divided into four groups: local pain, hyperemia, sinus tract formation, or a radiograph showing loosening of the prosthesis.

We classified osteomyelitis as hematogenous or contiguous, depending on the bone infection mechanism, and as acute or chronic, according to the onset of symptoms.[8-10] In this study, contiguous osteomyelitis has been divided into post-operative osteomyelitis or open-fracture associated osteomyelitis.

Culture samples

All bones samples were collected in the surgical center after extensive debridement. Samples were sent to the microbiology laboratory in bottles containing thioglycolate growth medium. Susceptibility tests were performed in most 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 in force at that time.[11]

Statistical analysis

An essentially descriptive statistical analysis was performed. Frequency and percentage were used for the categorical and ordinal variables. Mean, median, range (minimum and maximum values) and standard deviation (SD) were used for the continuous variables. All the analyses were performed using software SAS® 9.1.3 and Microsoft Excel® 2007.

Results

Population data

Collected data are summarized in Table 1. Patients were mostly men (63.4%), median age of 42 years and with infection in the lower limbs (71%). Osteomyelitis classification was mainly chronic (43%) and associated to open-fractures (32%). The median days of hospital stay was 41 days and the major presentation of infection was a discharging sinus.

Outcomes and surgical procedures

After hospital discharge, outcomes were analyzed after six months follow-up, as shown in Table 2. The extension of antimicrobial treatment and the number of surgical procedures required for infection treatment are reported in Table 3.

Microbiology

All infectious agents found in bone cultures are shown in Table 4. A total of 121 agents were found in 101 bone samples, with a high proportion of nonfermenting GNB. Figs. 1, 2, and 3 illustrate the susceptibility profile to antimicrobials for the 3 most frequently isolated pathogens.

We found a low prevalence of co-morbidities and risk factors for infections in this population. Seventeen patients (17%) were smokers, eight (8%) had diabetes mellitus, three (3%) were illicit drug users, and one (1%) was diagnosed with neoplasia. No patient was diagnosed with HIV infection.

Table 1 - Characteristics of 101 patients with GNB osteomyelitis

	n (%)
Age	
Mean (SD)	44.34 (19.37)
Median	42
Age group	
0 to 20 years	8 (7.9%)
21 to 40 years	40 (39.6%)
41 to 60 years	29 (28.7%)
Over 60 years	24 (23.8%)
Gender	
Male	64 (63.4%)
Female	37 (36.6%)
Affected segment	
Leg	31 (30.7%)
Thigh	28 (27.7%)
Hip	14 (13.9%)
Foot and ankle	13 (12.9%)
Spine	7 (6.9%)
Hand and wrist	6 (5.9%)
Arm	2 (2%)
Forearm	0 (0%)
Time with symptoms – days	
Median	65
Length of hospital stay – days	
Median	41
Acute hematogenous	0 (0%)
Acute open fracture related	33 (32.7%)
Chronic	44 (43.6%)
Acute post-operative	24 (23.8%)
Presentation of infection	
Discharging sinus	70 (69.3%)
Local pain	20 (19.8%)
Hyperemia	7 (6.9%)
Functional disability	4 (4%)

Table 2 - Outcomes after 6 months of follow-up

Outcomes after 6 months follow-up	n (%)
Disease clinical remission	61 (60.4%)
Relapse	19 (18.8%)
Loss to follow-up	9 (8.9%)
Amputation	7 (6.9%)
Death	5 (5%)
Total	101 (100%)

Table 3 - Extent of antimicrobial use and number of surgical procedures

	n (%)
Days of inpatient antimicrobial treatment	
Mean (SD)	44.69 (35.52)
Median	40
Range	1-176
Days of outpatient antimicrobial treatment	
Mean (SD)	82.97 (61.81)
Median	99
Range	4-180
Number of surgical procedures	
Mean (SD)	3.41 (3.03)
Median	2
Range	1-13

Table 4 – Pathogens isolated from bone samples

Pathogens isolated	n (%)
Enterobacter sp.	30 (24.7%)
Acinetobacter baumannii	26 (21.4%)
Pseudomonas aeruginosa	24 (19.8%)
Klebsiella pneumoniae	10 (8.2%)
Serratia marcescens	8 (6.6%)
Proteus mirabilis	7 (5.7%)
Escherichia coli	6 (4.9%)
Providencia stuarti	3 (2.4%)
Morganella morganii	3 (2.4%)
Stenotrophomonas maltophilia	2 (1.6%)
Leclercia adecarboxylata	1 (0.8%)
Pantoea agglomerans	1 (0.8%)
Total	121

Discussion

There has been an increasing amount of literature on GNB infection in recent years, mainly due to the difficulties of treating such infections. Such difficulties results from expression and transmission of genes tied to antimicrobial resistance, which reduces global efficacy of antibiotics. GNB, which are especially important in nosocomial infections, frequently exhibit resistance to multiple antibiotic classes.[3]

Very few follow-up studies of patients with specific GNB osteomyelitis diagnostic are available in the literature. Although some studies have assessed risk factors for the

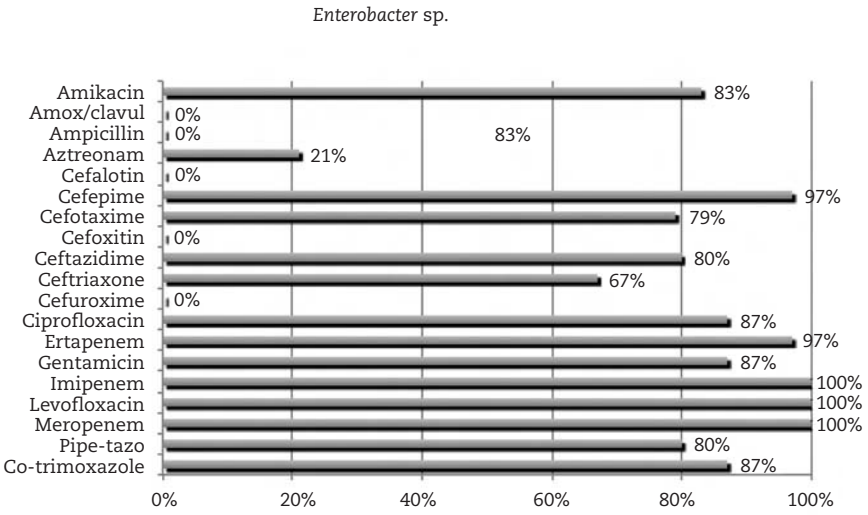

Fig. 1 - Susceptibility profile of *Enterobacter* sp.

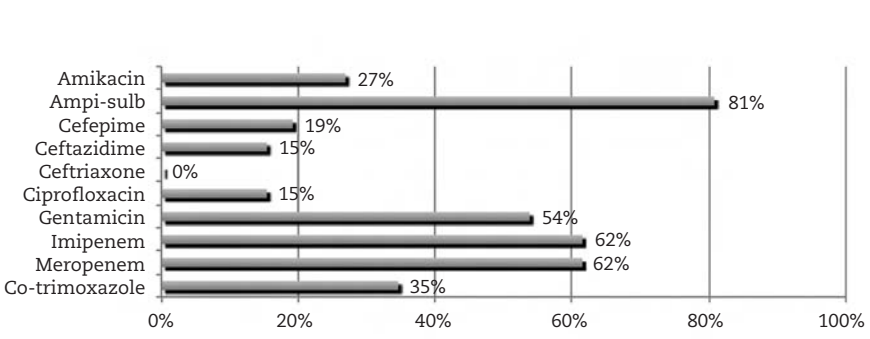

Fig. 2 - Susceptibility profile of *Acinetobacter baumannii*.

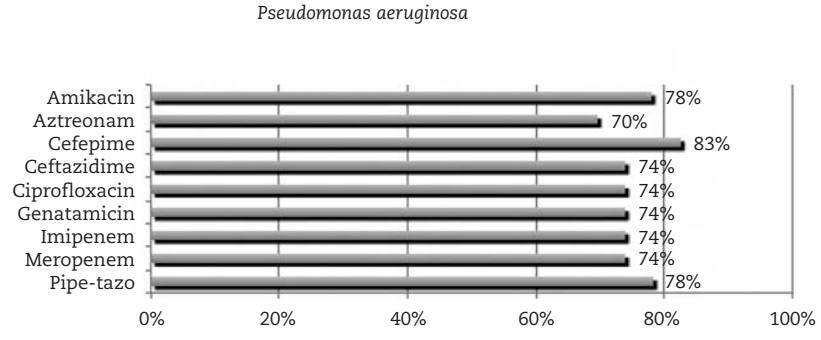

Fig. 3 - Susceptibility profile of *Pseudomonas aeruginosa*.

development of prosthetic joint infections,[12,13] there is no available data that allows for an appropriate comparison with the information reported in this study.

The Orthopedics and Traumatology Institute is a reference center for the treatment of high complexity trauma in the city of São Paulo, generally caused by traffic accidents. Most cases seen at our center are young patients with few co-morbidities. These characteristics differ substantially from those reported in other studies in the medical literature. This study shows a predominance of male patients in their third and fourth decade of life, with acute post-traumatic osteomyelitis of the lower limbs. The absence of hematogenic dissemination enhances the importance of the hospital setting and of high-energy traumas with open fractures as potential factors for the development of GNB osteomyelitis.

The extended hospitalization time and the prolonged use of antimicrobials, both in inpatient and outpatient settings, show the high complexity associated with the treatment of these cases. Our results indicate a disease remission rate of about 60%, and a relapse rate of about 19%, although six months follow-up may be considered too short to evaluate the outcome of osteomyelitis treatment. No data from other studies are available to allow for an appropriate comparison of these outcomes, but a few authors report remission rates around 80% for Gram-positive infections and up to 20% relapse rate.[2]

A total of 121 agents have been isolated in cultures from 101 patients. The most frequently isolated agents were *Enterobacter* sp., *Acinetobacter baumannii* and *Pseudomonas aeruginosa* representing about 70% of all the isolates. The susceptibility profile shows carbapenems with preserved activity against *Enterobacter* sp., yet with progressively reduced susceptibility to *P. aeruginosa* and *A. baumannii*.

Conclusion

GNB osteomyelitis constitutes an important therapeutic challenge, especially when associated to nonfermenting bacteria. These pathogens should be taken into account for the initial selection of antibiotics in osteomyelitis-confirmed cases, with view at optimizing antimicrobial regimen from the very beginning of the treatment.

Conflict of interest

The authors declare that this work received financial support from Merck Sharp and Dohme as part of the Investigator Initiated Study Protocol.

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