ORIGINAL ARTICLE

Isolation and susceptibility profile of bacteria in diabetic foot and venous stasis ulcer of patients admitted to the emergency room of the main university hospital in the state of Goiás, Brazil

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ABSTRACT

Background: Infected lower limb injuries (diabetic ulcers and venous stasis ulcers) cause great suffering and functional disability with social and economic impact and increase in risk of severe complications.

Objective: To characterize the microbiota and determine the antimicrobial susceptibility profile of isolated bacteria in lower limb injuries secondary to the venous stasis ulcer and diabetic foot.

Methods: Patients with lower limb lesions were included in the study, both diabetics and patients with venous stasis ulcer, receiving care at the emergency service of a university hospital in Goiânia (Brazil) from February 2005 to August 2006. Samples were collected with cotton swab to perform culture and antimicrobial sensitivity test applying standardized techniques.

Results: Presence of bacteria was detected in 88.46% of the samples. Gram-positive cocci were characterized as *Staphylococcus aureus* and *Staphylococcus epidermidis*. Among Gram-negative rods, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis* and *Enterobacter* sp. were detected.

Conclusions: Isolated microorganisms of lower limb injuries (diabetic foot and venous stasis ulcer) included Gram-positive and Gram-negative bacteria, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*, which werethe most frequent and highly resistant to several kinds of antimicrobial agents.

Keywords: Microbiota, venous stasis ulcer, diabetic foot, infection.

RESUMO

Contexto: Lesões infectadas de membros inferiores (úlceras diabéticas e úlceras de estase venosa) são causa de grande sofrimento e incapacitação funcional com impacto social, econômico e aumento do risco de complicações severas.

Objetivo: Caracterizar a microbiota e determinar o perfil de suscetibilidade antimicrobiana das bactérias isoladas de lesões de membros inferiores secundárias a úlcera de estase venosa e pé diabético.

Métodos: Foram incluídos no estudo pacientes portadores de lesões de membros inferiores, sendo diabéticos, e pacientes com úlcera de estase venosa, atendidos em um serviço de urgência de um hospital universitário de Goiânia (GO), no período de fevereiro de 2005 a agosto de 2006. A coleta de material foi realizada com *swab* de algodão para realização de cultura e teste de sensibilidade antimicrobiana, empregando-se técnicas preconizadas.

Resultados: Das amostras analisadas, foi detectada a presença de bactérias em 88,46%. Os cocos gram-positivos foram caracterizados como *Staphylococcus aureus* e *Staphylococcus epidermidis*. Dentre os bastonetes gram-negativos, detectou-se *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis* e *Enterobacter* sp.

Conclusões: Os microrganismos isolados das lesões de membros inferiores (pé diabético e úlcera de estase venosa) incluíram bactérias gram-positivas e negativas, sendo *Staphylococcus aureus*, *Pseudomonas aeruginosa* e *Escherichia coli* as mais freqüentes, com elevada resistência a diversos antimicrobianos.

Palavras-chave: Microbiota, úlcera de estase venosa, pé diabético, infecção.

Introduction

Among the most common lower limb lesions are diabetic and venous stasis ulcers.[1] Plantar lesions known as diabetic foot, a chronic and frequent complication of diabetes mellitus,[1] result especially from neuropathy and degenerative microangiopathy characterized by alteration in capillary structure and protective endothelial function.[2] Increased plantar pressure, skin changes such as dryness, fissures, mycosis, osteoarticular deformities, muscle atrophy and bone prominences, formation of callus[3] and repetition traumas can result in skin and subcutaneous tissue infection, abscesses and deep layer phlegms,[4] significantly increasing risk of amputation,[5] which is also associated with early arteriosclerosis.[6]

Venous stasis ulcers are also frequent lesions[7] and are related to physiopathological mechanisms of chronic venous insufficiency.[7] They generate social and economic impact, work disability and expenses associated with treatment.[7,8]

Microorganisms associated with lower limb lesions mentioned above are part of skin microbiota, and associations of anaerobic and facultative aerobic bacteria are common, resulting in mixed infections.[9]

Staphylococcus aureus and *Streptococcus* sp are present in moderate lower limb infections without systemic toxicity, in superficial lesions with cellulitis, moderate ulceration and mild ischemia.[10] In severe infections with extensive cellulitis, ulcer, lymphangitis and ischemia, gram-positive cocci are present (*Staphylococcus* sp, *Streptococcus* spand *Enterococcus* sp), anaerobic bacteria, such as bacteroids and facultative gram-negative (*Escherichia coli*, *Enterobacter* sp, etc.), and nonfermenting gram-negative rods (*Pseudomonas* and *Acinetobacter)* .[10] Our aim was to isolate and characterize the microorganisms of lower limb lesions (diabetic foot and venous stasis ulcer), as well as to determine susceptibility profile of isolated bacteria.

Method

The study population was comprised of patients with lower limb lesions (diabetic foot and venous stasis ulcer), who were admitted to a university hospital in Goiânia, Brazil. The study was carried out after approval by the Ethics Committee and signing of a consent form by the patient or responsible. Collection was performed in deep layers using cotton *swab* after skin disinfection with physiologic solution and Povidine®, local anesthesia with 2% lidocaine without vasoconstrictor and surgical debridement of devitalized tissues. The samples were conditioned in Stuart medium and sent to the laboratory for culture and antimicrobial sensitivity test (antibiogram).

The samples were sowed in sheep blood agar (5%) and incubated at 37 ºC for 24-48 hours. Colonies were initially identified by gram staining, based on their development in selective and nonselective culture mediums, biochemical/enzymatic tests[11] and techniques automated by the MicroScan® system (Dade Behring – West Sacramento, California, USA). Susceptibility of isolated bacteria was determined by the automated system, and the results were interpreted according to recommendations by the Clinical and Laboratory Standards Institute.[12]

Results

In this study, 79 cases of lower limb lesions were assessed: 50 diabetic foot and 29 stasis ulcers. A total of 104 cultures were performed, 92 (88.46%) of them being positive. In 65 cultures, gram-negative bacteria were isolated; of these, 42 (45.66%) were enterobacteria, 23 (25%) were nonfermenting rods and 27 (29.34%) were staphylococcus.

The 12 (11.54%) negative cultures corresponded to samples of the first collection from nine individuals with diabetic feet and three with stasis ulcers. In 10 patients with diabetic foot more than one sample was collected for each case, due to unfavorable evolution of the lesion, corresponding to 25 cultures. Figure 1 illustrates a diabetic foot lesion, and Figure 2 shows a stasis ulcer.

Figure 1 - Diabetic foot with plantar abscess and elimination of secretion

Figure 2 - Venous stasis ulcer with signs of chronic venous insufficiency

Prevalent bacteria in lesions (Figure 3) were: *Staphylococcus aureus* , *Staphylococcus epidermidis* and gram-negative rods: *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis* and *Enterobacter* sp(Table 1). There was prevalence of 70.66% in gram-negative rods isolated from lower limb lesions. In diabetic foot, the most frequent species was *Staphylococcus aureus* , followed by *E. coli* and *P. aeruginosa*;however, in venous stasis ulcer, *P. aeruginosa*, followed by *S. aureus* and *Enterobacter* sp were prevalent (Figure 3).

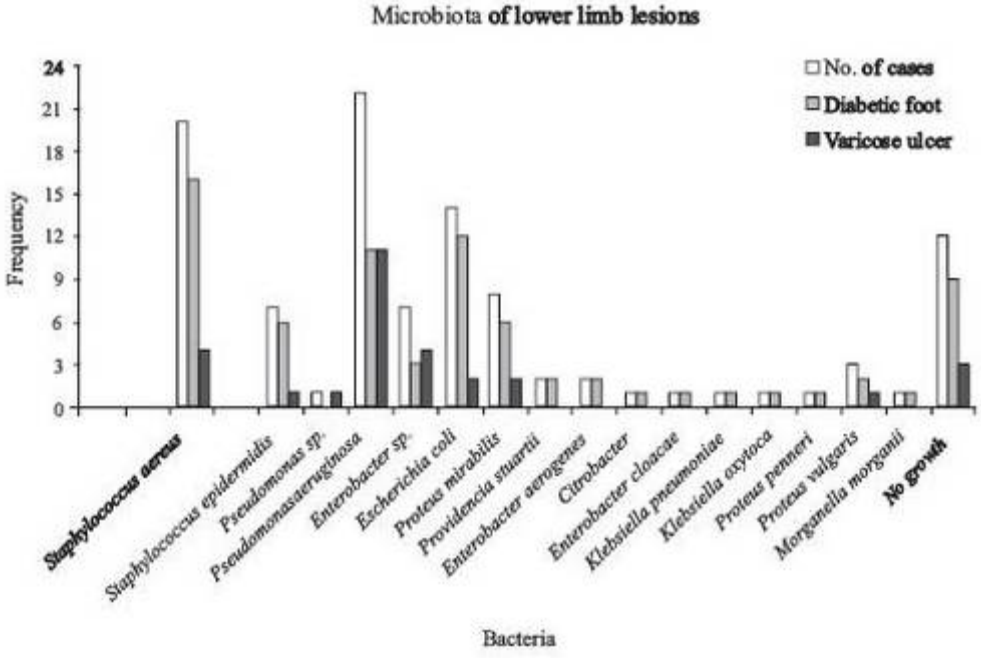

Figure 3 - Distribution of isolated bacteria of patients with diabetic foot and/or venous stasis ulcers admitted to a university hospital in Goiânia, Brazil

Table 1 - Distribution of isolated bacteria of patients with diabetic foot and/or venous stasis ulcers admitted to a university hospital in Goiânia, Brazil

Bacteria	No. of cultures	%	Diabetic foot	%	Venous stasis ulcer	%
Staphylococcus aureus	20	19.23	16	15.38	4	3.84
Staphylococcus epidermidis	7	6.73	6	5.76	1	0.96
Pseudomonas aeruginosa	22	21.15	11	10.57	11	10.57
Pseudomonas sp	1	0.96	0	-	1	0.96
Escherichia coli	14	13.46	12	11.53	2	1.92
Proteus mirabilis	8	7.69	6	5.76	2	1.92
Proteus vulgaris	3	2.88	2	1.92	1	0.96
Proteus penneri	1	0.96	1	0.96	0	-
Enterobacter aerogenes	2	1.92	2	1.92	0	-
Enterobacter cloacae	1	0.96	1	0.96	0	-
Enterobacter sp	7	6.73	3	2.88	4	3.84
Providencia stuartii	2	1.92	2	1.92	0	-
Klebsiella pneumoniae	1	0.96	1	0.96	0	-
Klebsiella oxytoca	1	0.96	1	0.96	0	-
Citrobacter sp	1	0.96	1	0.96	0	-
Morganella morganii	1	0.96	1	0.96	0	-
Negative culture	12	11.54	9	8.65	3	28.84
Total	104	100	75	72.12	29	27.88

Table 2 shows the results of cultures obtained in 10 patients with diabetic foot with unfavorable evolution. In the first culture, only one bacterium was isolated, and in 20% there was no microbial development. In subsequent cultures, there was prevalence of *S. aureus* and *P. aeruginosa*.In only two cases (A and I), the bacterium detected in the first collection was recovered in subsequent cultures (A and I). Cases A, B, C, D and E progressed requiring lower limb amputation, in which an association of bacteria was isolated in patients A and C, with highlight to *P. aeruginosa*and *S. aureus*. In case B, *S. epidermidis* and *P. aeruginosa* were isolated, whereas in case E *P. aeruginosa and S. aureus were isolated*.

Table 2 - Bacteria isolated from different cultures of 10 patients with diabetic foot and unfavorable evolution

Patient	No. of cultures	First culture	Subsequent cultures
A	7	*Staphylococcus aureus*	*Enterobacter cloacae, P. aeruginosa, S. aureus, Proteus mirabilis*
B	4	*S. epidermidis*	*P. aeruginosa*
C	8	*Citrobacter* sp	*S. epidermidis, P. aeruginosa, S. aureus*
D	2	*S. epidermidis*	*E. coli*
E	4	*P. aeruginosa*	*S. aureus*
F	2	*Klebsiella oxytoca*	*P. aeruginosa*
G	2	No growth	*Escherichia coli*
H	2	No growth	*Proteus mirabilis*
I	2	*Enterobacter* sp	*Enterobacter* sp
J	2	*Proteus mirabilis*	*Staphylococcus aureus*

Results of the susceptibility profile in the most frequent four bacteria are presented in Figures 4 to 7. *S. aureus* and *P. aeruginosa* were prevalent both in diabetic foot lesions and in venous stasis ulcers, whereas the third most isolated bacteria was *E. coli* in diabetic foot and *Enterobacter* sp in venous stasis ulcer. All *Staphylococcus aureus* were sensitive to vancomycin, tobramycin, Synercid (quinupristin-dalfopristin) and linezolid. Sensitivity of *S. aureus* to gatifloxacin, ampicillin/sulbactam and cefazolin was 80%, whereas it was 77% for rifampicin (Figure 4), with resistance to ampicillin, penicillin, amikacin, cephalothin, amoxicillin/clavulanate and oxacillin. *P. aeruginosa* was sensitive to meropenem, imipenem e polymyxin B (Figure 5). *E. coli* was sensitive to imipenem and meropenem, ceftazidime, cefepime, aztreonam, gentamicine and amikacin, and less sensitive to ciprofloxacin (Figure 6). It was resistant to ampicillin, amoxicillin/clavulanate, chloramphenicol and cephalothin. *Enterobacter* sp was sensitive to amikacin, gentamicine, cefepime, piperacillin/tazocin, ceftriaxone, ceftazidime, meropenem, ciprofloxacin and aztreonam (Figure 7).

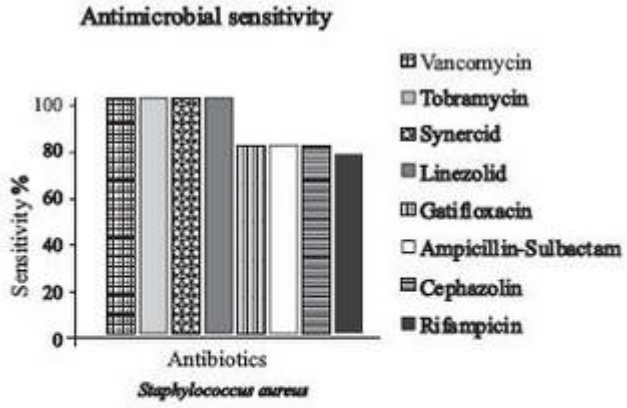

Figure 4 - Antimicrobial sensitivity of Staphylococcus aureus isolated from lower limb lesions in patients admitted to the emergency room of a university hospital in Goiânia, Brazil

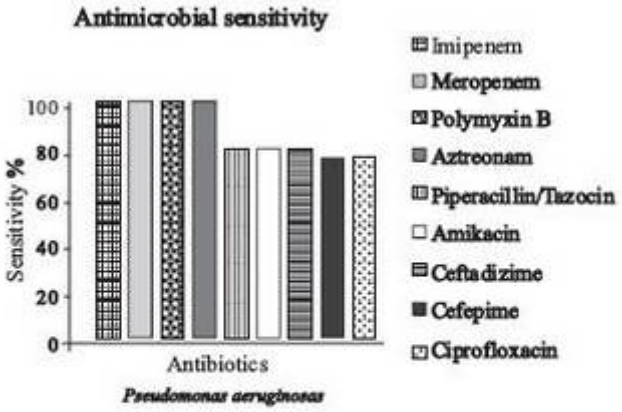

Figure 5 - Antimicrobial sensitivity of Pseudomonas aeruginosa isolated from lower limb lesions in patients admitted to the emergency room of a university hospital in Goiânia, Brazil

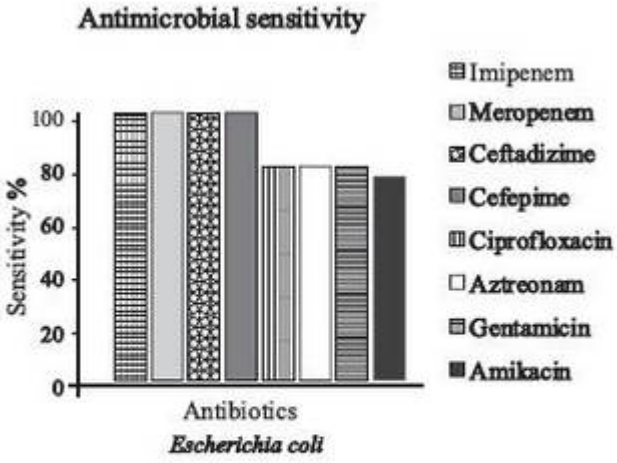

Figure 6 - Antimicrobial sensitivity of *Escherichia* coli isolated from lower limb lesions in patients admitted to the emergency room of a university hospital in Goiânia, Brazil

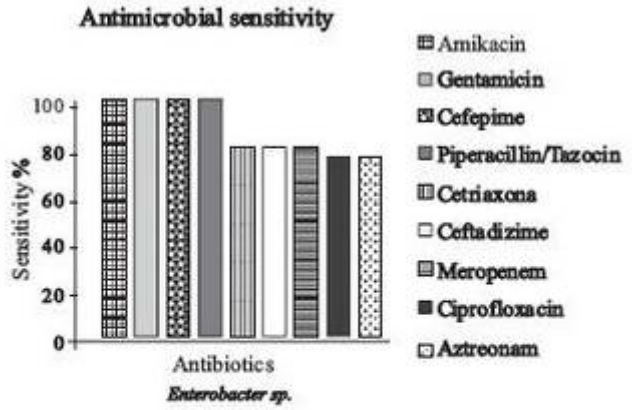

Figure 7 - Antimicrobial sensitivity of *Enterobacter* sp isolated from lower limb lesions in patients admitted to the emergency room of a university hospital in Goiânia, Brazil

Discussion

A high frequency of *S. aureus*, *P. aeruginosa* and enterobacteria was detected in assessed lesions, similar to that reported by Assis et al.[13] and Jorge et al.[14] Regarding gram-positive cocci, thee was prevalence of *S. aureus* and *S. epidermidis,* in agreement with the reports by Goldstein et al.[15], Routh et al.[16] and Slovenkai et al.[17]

Selection and dissemination of multiresistant microorganisms have been occurring both in hospitals and in the community and represent a great challenge in therapy.[15,18-20] In this study, methicillin-resistant *S. aureus* (MRSA) had high prevalence (69%), different from the results found by Goldstein et al.[15] and Carvalho et al.[18], who found rates lower than 20%. Isolation rate of gram-negative bacteria in this study was similar to that found by Carvalho et al.,[18] when assessing patients with diabetic foot and mainly isolating enterobacteria.

Rocha et al.[21] reported the problem associated with multiresistance of gram-positive and gram-negative bacteria, especially *Escherichia coli*, in more severe cases. Most staphylococci detected in this study were resistant to amoxicillin/clavulanate, cephalothin, oxacillin and clindamycin, similar to what was described by Unachukwu et al.[20] and Rocha et al.[21]

Due to unfavorable evolution of 10 patients with diabetic foot, subsequent collections were performed (Table 2). There was prevalence of *Staphylococcus aureus* and *Pseudomonas aeruginosa,* and the bacteria isolated in the first collection were recovered in only two cases. In four cases that progressed to lower limb amputation, there was presence of *P.aeruginosa* and/or *S. aureus,* in agreement with Rocha et al.,[21] who considered diabetic foot as the main cause of nontraumatic limb amputation.

The prevalence of gram-negative rods and resistant staphylococci observed in this study makes choice of antimicrobials difficult for empirical treatment. Therefore, culture and antibiogram should be performed; however, if this procedure is not feasible, use of ampicillin/sulbactam in association with piperacillin/tazobactam and ciprofloxacin is recommended when there is no suspicion of infection by *Pseudomonas*.

Conclusion: A mixed microbiota of lower limb lesions was detected, with gram-positive and gram-negative bacteria, *Staphylococcus aureus* , *Pseudomonas aeruginosa* and *Escherichia coli* being the most frequent, with high resistance to many antimicrobials and a high rate of MRSA (69%). According to the *in vitro results*, ampicillin/sulbactam in association with piperacillin/tazobactam could be an option for the *in vitro* treatment of most cases of lower limb lesions, as well as ciprofloxacin when there is no suspicion of infection by *Pseudomonas*.

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