

Prevalence of resistance phenotypes in *Staphylococcus aureus* and coagulase-negative isolates of venous ulcers of primary healthcare patients

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ABSTRACT

Introduction: In venous ulcers, the presence of *Staphylococcus aureus* and *coagulase-negative staphylococcus* resistance phenotypes can aggravate and limit the choices for treatment. **Methods:** *Staphylococcus* isolated from 69 patients (98 ulcers) between October of 2009 and October of 2010 were tested. The macrolide, lincosamide, streptogramin B (MLS_B) group resistance phenotype detection was performed using the *D-test*. Isolates resistant to ceftaxime and/or oxacillin (disk-diffusion) were subjected to the confirmatory test to detect minimum inhibitory concentration (MIC), using oxacillin strips (*E-test*[®]). **Results:** The prevalence of *S. aureus* was 83%, and 15% of *coagulase-negative staphylococcus* (CoNS). In addition were detected 28% of *methicillin-resistant Staphylococcus aureus* (MRSA) and 47% of *methicillin-resistant coagulase-negative staphylococcus* (MRCoNS). Among the *S. aureus*, 69.6% were resistant to erythromycin, 69.6% to clindamycin, 69.6% to gentamicin, and 100% to ciprofloxacin. Considering the MRSA, 74% were highly resistant to oxacillin, MIC $\geq 256\mu\text{g/mL}$, and the MLS_C constitutive resistance predominated in 65.2%. Among the 20 isolates sensitive to clindamycin, 12 presented an inducible MLS_B phenotype. Of the MRCoNS, 71.4% were resistant to erythromycin, ciprofloxacin and gentamicin. Considering the isolates positive for β -lactamases, the MIC breakpoint was between 0.5 and $2\mu\text{g/mL}$. **Conclusions:** The results point to a high occurrence of multi-drug resistant bacteria in venous ulcers in primary healthcare patients, thus evidencing the need for preventive measures to avoid outbreaks caused by multi-drug resistant pathogens, and the importance of healthcare professionals being able to identify colonized versus infected venous ulcers as an essential criteria to implementing systemic antibacterial therapy.

Keywords: Methicillin-resistant *Staphylococcus aureus*. Drug resistance bacterial. Varicose ulcer. Primary health care.

INTRODUCTION

Venous ulcers are the most common type of wound affecting the lower limbs. The ulcers have multi-factor causes, are recurrent, and do not settle easily¹. The chronic exposure of these ulcers to the environment, associated with the intrinsic factors of the patient, predisposes the onset of infectious processes that worsen the prognosis for cure². Within this context, the healthcare professionals treating these wounds must have the necessary clinical judgment to evaluate the possibility of infection.

Among the many potential infectious agents present in these wounds, from an epidemiological perspective, *Staphylococcus aureus* and *Staphylococcus coagulase-negative* are of significant clinical interest as these pathogens are commonly found in skin and soft tissue infections³. The resistance phenotypes in these microorganisms, when present in venous ulcers, is an important health condition due to the pathogenicity involved and also the many virulence factors affecting the therapeutic process⁴.

The possibility of multi-drug resistant agents (MDROs) is also a problem. Studies in different countries or regions, point to a growing prevalence of these pathogens⁵⁻⁸.

Within this context, particular emphasis is given to methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant coagulase-negative *Staphylococcus* (MRCoNS), as well as those susceptible to methicillin: methicillin-sensitive *Staphylococcus aureus* (MSSA) and methicillin-sensitive coagulase-negative *Staphylococcus* (MSCoNS). These microorganisms can present other resistance phenotypes of clinical interest, such as constitutive or inducible resistance to the *macrolide, lincosamide, streptogramin B* (MLS_B) group⁹⁻¹¹.

In vascular ulcers, these microorganisms integrate the lesion environment with a significant bioburden^{12,13}. Therefore, continuous monitoring of the infectious agents and their susceptibility to antimicrobials is recommended as an important infection control strategy¹⁴, besides contributing to an effective therapy approach in primary healthcare.

The objectives of the present study were: to verify the prevalence and minimum inhibitory concentration of the isolated (MRSA, MSSA, MRCoNS and MSCoNS) of venous ulcers of primary healthcare patients; evaluate the susceptibility profile of these isolates to antimicrobials; and detect constitutive and inducible MLS_B resistance phenotypes.

METHODS

Setting and ethical considerations

This sectional, clinical and observational study was performed using a quantitative approach and is part of a broader project named: *Avaliação de úlceras venosas de estase no contexto do atendimento ambulatorial na rede municipal de saúde de Goiânia: ampliando as perspectivas* (The evaluation of venous stasis ulcers in the context

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of outpatient care in the municipal health network of Goiânia: improving the perspectives), integrated to Rede Goiana de Pesquisa em Avaliação e Tratamento de Feridas (*Goiânia Network for Research in Wound Evaluation and Treatment*), approved by the Research Ethics Committee of Universidade Federal de Goiás (protocol 041/2009). In this city, primary healthcare comprises a low- and medium-complexity service network, distributed into seven health districts or administrative regions, which include outpatient clinics and health centers, which provide treatment to individuals with leg ulcers.

The data were obtained between October of 2009 and October of 2010. The following inclusion criteria were established: age equal to or more than 18 years; have at least one venous ulcer for at least six weeks; present at least three clinical signs of infection on the lesion, according to the suggestions of the European Wound Management Association². The exclusion criteria were: having used antimicrobials within 30 days before the specimens were collected.

A total of 69 patients were included in the study, all of which signed the Free and Informed Consent Form. The participants had between one and five lesions, on one or both legs, totaling 98 venous ulcers.

The data were obtained by administering a standardized instrument that includes the list of clinical signs and symptoms for infection proposed by the European Wound Management Association², in addition to a clinical exam and collection of a specimen from the ulcer for microbiological analysis.

Collection of the clinical specimens

After fully cleaning the ulcers using sodium chloride at 0.9%, the clinical specimens of the lesions were collected using a sterile swab (*COPAN*®), following the technique proposed by Levine et al¹⁵, referenced by Angel et al¹⁶.

The microbiological analyses were performed following the recommended techniques¹⁷⁻¹⁸. The quality of the procedures was controlled using standard strains of the American Type Culture Collection (*Staphylococcus aureus* ATCC® 25923).

Bacterial strains and screening to detect MRSA and MRCONS isolates

The samples were inoculated in a selective culture medium for *Staphylococcus* sp., Mannitol salt agar (*Himedia*®). To increase the probability of isolating MRSA and/or MRCoNS, the samples were also cultivated on tryptic soy agar (TSA) *Himedia*® and tryptic soy broth (TSB) *Himedia*® supplemented with 4% NaCl and 6mcg/mL oxacillin¹⁹⁻²⁰. The culture media were incubated at 35°C for 24 to 48 hours.

The identified microorganisms were tested for 12 antimicrobials using the disk-diffusion technique²¹. The results were interpreted simultaneously to the analysis, according to the criteria established by the Clinical and Laboratory Standards Institute²⁰ and the British Society for Antimicrobial Chemotherapy²².

Determining the resistance phenotypes

Specimens that presented resistance to oxacillin or ceftioxin were subjected to the confirmatory test to detect the minimal inhibitory concentration, E-test®.

The detection of the inducible MLS_B phenotype was performed using disk approximation, D-test²⁰. In this test, the 2µg-clindamycin disk was placed 15mm apart from the 15µg-erythromycin disk. The

flattened inhibition halo around the clindamycin (halo D) classified the result as positive for the inducible phenotype, and, therefore, resistance to clindamycin.

The databank was created using SPSS 17.0 (*Statistical Package for the Social Sciences for Windows*®), and descriptive statistics was performed.

RESULTS

Characteristics of the population

Of the 69 evaluated patients, 40 (58%) were male, and 29 (42%) were female. Of these, 35 (50.7%) were ≤ 60 years old, and 34 (49.3%) > 60 years old. Regarding their social class, 32 (46.4%) were in social class D according to Brazilian socio-economic classification criterion, from the Brazilian Association of Research Companies (*Associação Brasileira de Empresas de Pesquisa* - ABEP). Fifty-two (75.4%) patients has only one lesion, while 17 (24.6%) had multiple lesions.

Prevalence and susceptibility of the isolated specimens

Considering the total 98 investigated lesions, 80 (81.7%) tested positive for *Staphylococcus* sp. Among these, 83 *S. aureus* isolates were obtained, 23 (28%) of which were MRSA and 60 (72%) MSSA. Regarding the CoNS, 15 isolates were identified, 7 (47%) of which were resistant (MRCoNS) and 8 (53%) were sensitive (MSCoNS) to methicillin.

Regarding the MRSA, the analysis of the susceptibility profile revealed resistance to erythromycin (69.6%), clindamycin (69.6%), gentamicin (69.6%), and ciprofloxacin (100%) compared to the MSSA. The latter presented the following values: erythromycin (38%), clindamycin (18%), tetracycline (28%), and rifampicin (15%). MRCoNS showed resistance to erythromycin, ciprofloxacin and gentamicin, with 71.4% (**Table 1**).

The drugs with better activity for both isolates, MRSA and MSSA, were mupirocin, linezolid, sulfamethoxazole-trimethoprim, tetracycline and quinupristin/dalfopristin, with sensitivity above 98%. The antimicrobials with the best activity for MRCoNS and MSCoNS were linezolid, mupirocin, quinupristin/dalfopristin and sulfamethoxazole-trimethoprim, with sensitivity above 87.5%.

MRCoNS and MSCoNS isolates with intermediate sensitivity were not identified. On the other hand, among the MRSA, it was found that 4.3% were sensitive to erythromycin and 17.4% to oxacillin. Among the MSSA, 25% were sensitive to erythromycin and ciprofloxacin, 2% to rifampicin and 4% to gentamicin.

Characterization of the resistance phenotypes

All the MRSA and MRCoNS were resistant to ceftioxin (marker), while 95.6% showed reduced sensitivity to oxacillin. The E-test results evidenced that 17/23 (74%) MRSA has a high level of resistance to oxacillin (≥ 256µg/mL). The minimum inhibitory concentration (MIC) for the remaining specimens was between 32 and 96µg/mL. It was found that 3/7 (43%) of MRCoNS had a MIC of 1.5µg/mL (**Table 2**).

Regarding the MLS_B phenotype, the MRSA were highlighted because of their constitutive resistance, in which 15 (65.2%) isolates presented natural resistance to clindamycin. Between the two clindamycin-sensitive MRSA, only one tested positive in the D-test, and, therefore, also for the inducible phenotype.

TABLE 1 - Susceptibility of methicillin-resistant *Staphylococcus aureus* and methicillin-sensitive *Staphylococcus aureus* and methicillin-resistant coagulase-negative staphylococcus and methicillin-sensitive coagulase-negative *Staphylococcus* (f=98), isolated from venous ulcers of patients treated in dressing rooms of municipal health centers in Goiânia, to the tested antimicrobials, October of 2009 to October of 2010.

Antimicrobials	MRSA (n= 23/28%)		MSSA (n= 60/72%)		MRCoNS (n= 7/47%)		MScONS (n= 8/53%)	
	R	S	R	S	R	S	R	S
	%		%		%		%	
Erythromycin	69.6	26.1	38.0	37.0	71.4	28.6	25.0	75.0
Clindamycin	69.6	30.4	18.0	70.0	42.9	57.1	12.5	87.5
Ciprofloxacin	100.0	-	12.0	63.0	71.4	14.3	25.0	75.0
Linezolid	-	100.0	-	100.0	-	100.0	-	100.0
Mupirocin	-	100.0	-	100.0	-	100.0	-	100.0
Oxacillin	78.3	4.3	-	100.0	-	100.0	-	100.0
Cefoxitin	100.0	-	-	100.0	100.0	-	-	100.0
Tetracycline	-	100.0	28.0	72.0	28.6	71.4	50.0	50.0
Trimethoprim-sulfamethoxazole	-	100.0	2.0	98.0	14.3	85.7	12.5	87.5
Rifampicin	34.8	65.2	15.0	83.0	28.6	71.4	37.5	62.5
Gentamicin	69.6	30.4	-	96.0	71.4	28.6	25.0	75.0
Quinupristin-dalfopristin	-	100.0	-	100.0	-	100.0	-	100.0

MRSA: methicillin-resistant *Staphylococcus aureus*; MSSA: methicillin-sensitive *Staphylococcus aureus*; MRCoNS: methicillin-resistant coagulase-negative staphylococcus; MScONS: methicillin-sensitive coagulase-negative *Staphylococcus*; R: resistant; S: sensitive.

TABLE 2 - Distribution of the minimum inhibitory concentration (µg/mL) of methicillin-resistant *Staphylococcus aureus* and methicillin-resistant coagulase-negative staphylococcus isolated from the venous ulcers of patients treated in dressing rooms of municipal health centers in Goiânia, October of 2009 to October of 2010.

MRSA	Number	Percentage
MIC µg/mL		
256	17	74.0
32	3	13.1
96	1	4.3
64	1	4.3
48	1	4.3
total	23	28.0
MRCoNS		
1.5	3	43.0
2	2	28.5
0.5	1	14.2
0.75	1	14.2
total	7	47.0

MRSA: methicillin-resistant *Staphylococcus aureus*; MRCoNS: methicillin-resistant coagulase-negative staphylococcus; MIC: minimum inhibitory concentration.

TABLE 3 - Distribution of the macrolide, lincosamide, streptogramin B phenotype detected for methicillin-sensitive *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* and methicillin-sensitive coagulase-negative *Staphylococcus* and methicillin-resistant coagulase-negative *Staphylococcus* isolated from venous ulcers of patients treated in dressing rooms of municipal health centers in Goiânia, October of 2009 to October of 2010.

MLS _B resistance phenotype	MSSA		MRSA		MRCoNS		MScONS	
	n	%	n	%	n	%	n	%
Erythromycin-resistant and clindamycin-sensitive, D-test (+) MLS _B i inducible resistance	12	20.0	1	4.4	1	14.2	-	-
Erythromycin- and clindamycin-resistant- MLS _B c constitutive resistance	1	1.7	15	65.2	2	28.6	1	12.5
Erythromycin-resistant and clindamycin-sensitive, D-test (-) MSB	8	13.3	1	4.4	2	28.6	1	12.5
Erythromycin-sensitive and clindamycin-sensitive	39	65.0	6	26.0	2	28.6	6	75.0

MLS_B: macrolide-lincosamide-streptogramin B; MSSA: methicillin-sensitive *Staphylococcus aureus*; MRSA: methicillin-resistant *Staphylococcus aureus*; MRCoNS: methicillin-resistant coagulase-negative staphylococcus; MScONS: methicillin-sensitive coagulase-negative *Staphylococcus*; MSB: macrolide-streptogramin B.

Among the MRCoNS, only one isolate was positive for the MLS_Bi phenotype and another for the MLS_Bc phenotype (Table 3).

The highlight regarding the MSSA was their inducible resistance. Considering the 20 clindamycin-sensitive isolates, 12 (20%) presented inducible MLS_B phenotype.

Constitutive resistance was found in only one MSSA and one MScONS isolate. No inducible resistance was observed among the MScONS, and 75% of these bacteria were sensitive to both erythromycin and clindamycin.

DISCUSSION

This is the first study that analyzes the resistance phenotypes of microorganisms isolated from venous ulcers of individuals treated at primary healthcare services in Goiânia, State of Goiás, Brazil.

The present study results show high prevalence of *S. aureus* (83%) in venous ulcers. In a study performed in Goiânia, State of Goiás, Brazil, involving patients with leg ulcers (including venous, diabetic and traumatic ulcers) treated in dressing rooms, *S. aureus* was the most frequent bacteria (65%)²³. A high bioburden of this pathogen has been reported for chronic lesions, and this is consensual in literature. *Staphylococcus aureus* is part of the normal skin microbiota, and, therefore, they are frequently found in wounds^{12,23}. However, the association of the patient's intrinsic factors and the increase in the microbial load predispose infection¹.

Among the isolated *S. aureus*, there was a 28% prevalence of MRSA, whereas for MSSA the prevalence was 72%. We found several studies regarding the occurrence of microorganisms on chronic wounds or samples from skin and soft tissue lesions; however, the frequency of pathogens on venous ulcers is not specified, which makes any comparison impossible^{13,23-24}.

In a reference outpatient clinic in Manaus and in reference dressing rooms in Goiânia, Brazil, the MRSA prevalence is of, respectively, 15.5% and 20.5%²³⁻²⁴. Some factors that may contribute to increasing bacterial resistance are the common use of antimicrobial therapy among patients with chronic wounds and the possibility of environment contamination⁷.

Bacteria producing this multi-drug resistance phenotype can spread rapidly in the environment. A continuous monitoring and preventive measures should be implemented^{14,25}.

Monitoring with specimens from hospitals is more common. A study in the United States²⁶ found that the MIC of 439 hospital MRSA ranged between 4 and 256 µg/mL, and only 18 isolates presented MIC ≤ 64 µg/mL.

MRSA is known to be a pathogen of healthcare-associated infections (HCAIs) and has been associated with the resistance to methicillin and other drugs. The multi-drug resistance of the hospital MRSA isolates has also been verified in other studies, however in strains obtained from hospitals²⁵⁻²⁷.

All the MRSA isolated in this study were resistant to cefoxitin on the antibiogram (marker) and confirmed by the E-test®. Most isolates (74%) showed a high resistance level to methicillin, with an MIC ≥ 256 µg/mL. The MIC of the remaining isolated ranged between 32 and 96 µg/mL.

Methicillin is a penicillin resistant to the action of penicillinases and the resistance mechanism of *S. aureus* to this drug can be verified by the production of low-affinity penicillin binding proteins (PBP) or by the hyperproduction of β-lactamases. Classic MRSA strains are associated with the production of PBP2a or mecA gene mediated PBP2'^{20,28}.

There is a scarcity of studies on *Staphylococcus* sp. isolated from venous ulcers of patients treated in outpatient clinics in Brazil as well as in other countries. On the other hand, the prevalence of the identified pathogens ranges significantly in the health centers of different countries and in the same region²⁶⁻³¹. Different services have different conditions, and, for this reason, comparisons are not pertinent. In addition to this difficulty, a variety of methods used are used in the studies¹²⁻¹³. However, it should be observed that most studies regarding these microorganisms are performed in a hospital setting²⁶⁻²⁷.

Few studies investigate the presence of CoNS in the venous ulcers of primary healthcare patients. The approach to this group of microorganisms is another original contribution made by the present study. A survey in the databases found many studies with CoNS from blood samples and many oxacillin-resistance strains could present the *mecA* gene³¹.

Despite the identification of low frequency of *coagulase-negative Staphylococcus* among these microorganisms, it was observed there was an increased in MRCoNS isolates. MDROs microorganisms were identified in the study. The co-existence of β-lactams-resistant strains as well as the MLS_B group is evidence of this result. This fact is of great concern, and requires rigor from healthcare professionals in the implementation of contact precaution measures, with the purpose to avoid the dissemination of pathogens with multiple resistance phenotypes.

MSSA and MSCoNS were both 100% sensitive to this marker. Cefoxitin is recommended as a phenotypic marker because it predicts *mecA* gene mediated methicillin resistance^{20,32}.

Multi-drug resistant microorganisms have been identified in people with skin and soft tissue infections, in the hospital and other health care service environments¹⁴. MRSA is referred to as an endemic nosocomial pathogen, with an observed increasing prevalence rate^{5,27}. A study conducted in outpatient clinics in the United States indicated an increase in MRSA isolates obtained from the community for this type of infection³.

The isolates recovered from hospitals sets, present an increase in MRCoNS³³. The present study found high prevalence rates for MRCoNS (47%) in people with venous ulcers treated in the community. This finding, in addition to being pioneer, suggests that the phenomenon of resistance to β-lactams is not restricted to the hospital setting.

Regarding the susceptibility profile of the MRSA isolated in this study, the results confirmed the resistance to the group of β-lactams, in addition to the crossed resistance to clindamycin, erythromycin, ciprofloxacin, rifampicin and gentamicin. These results agree with those reported in other countries, however, in studies performed with patients treated at the hospital^{28,34}.

It was observed that oxacillin-resistant *S. aureus* has an associated resistance to ciprofloxacin (53.1%), sulfamethoxazole-trimethoprim (53.5%) and rifampicin (24.8%)²⁵. Clindamycin, sulfamethoxazole-trimethoprim, tetracycline and linezolid are choices of oral therapy in cases of mild to moderate infections, due to community MRSA³⁵.

The isolates showed a resistance above 69.6% to the described drugs. In this sense, considering the local population, these agents are not recommended. However, all MRSA isolates (100%) were sensitive to mupirocin, linezolid, sulfamethoxazole-trimethoprim, tetracycline and quinupristin/dalfopristin. These are important alternatives for the treatment of infections by MRSA. Though, considering the infection site (skin and soft tissues), it is necessary to evaluate the bioavailability (pharmacokinetic and pharmacodynamics) of the drugs (sulfamethoxazole-trimethoprim and tetracycline), in the treatment for leg ulcers³⁶⁻³⁷.

The oral therapies commonly used to treat community MRSA are tetracycline, rifampicin, clindamycin, linezolid and sulfamethoxazole-trimethoprim. It is recommended that a combined therapy be used when rifampicin is chosen, in order to avoid mutant strains^{28,35}. In this study, 34.8% of the MRSA and 28.6% of the MRCoNS isolates were resistant to rifampicin. Among the MSSA isolates, the resistance was 15%, whereas MSCoNS showed a higher resistance rate (37.5%).

This fact is concerning, considering that there is a limited choice of drugs that are administered orally. Similar results were found in strains obtained in hospitals located in the same city, with 40.7% resistance to rifampicin²⁵. It is presumed that the prevalence of rifampicin-resistant strains has increased in the outpatient health service settings.

Further studies are needed to effectively support the use of antimicrobials at some time during the treatment. However, if necessary, the indication must consider the signs and symptoms of infections, and not only the colonization. This recommendation can avoid the dissemination of multi-drug resistant bacteria^{14,38}.

The MSSA were sensitive to most of the evaluated antimicrobials, except for erythromycin. All isolates (100%) were sensitive to mupirocin, linezolid, and quinupristin/dalfopristin. High sensitivity (98%) to sulfamethoxazole-trimethoprim was also observed.

In Brazil, topic mupirocin is used, in the form of a cream at 2%. It is indicated in the treatment for infected wounds and ulcers, impetigo

and to eradicate MRSA in the nostrils³⁶. The topic use of mupirocin for the decontamination of venous ulcers has not been described^{35,39} nor is it recommended according to a systematic review about the topical indication of this antimicrobial³⁸. On the venous ulcer treatment guidelines, the use of topical antibacterial agents can be necessary to reduce the microbial load, but this document does not indicate the class type of the agent³⁹.

A study conducted in Brazil found 38% of MRCoNS isolates, and multi-drug resistance was detected in 86%; however, the MLS_Bi phenotype was not investigated³³.

Erythromycin (macrolides) and clindamycin (lincosamides) are antimicrobials belonging to the MLS_B group, and are widely recommended to treat staphylococcal infections of the skin and soft tissues, in addition to being an alternative for patients allergic to penicillin¹⁹. Some of the drugs on this group are of oral use³⁶, which facilitates their indication in primary healthcare services. The exception is quinupristin (streptogramin), which in association with dalfopristin should be administered intravenously³⁶.

In the present study, 100% of the *S. aureus* and CoNS were sensitive to these two streptogramins. However, their administration can cause arthralgia, nausea, vomiting, diarrhea, and exanthema; and they are no longer available in Brazil³⁶.

The resistance to macrolides and derivatives in Brazil, along with the group of lincosamides and streptogramins B comprise the MLS_B phenotype¹⁹. These antimicrobials are chemically different but with a similar action mechanism, affecting the ribosomal binding and inhibiting protein synthesis. For the laboratory point of view, this resistance phenotype can be identified on the antibiogram through disk diffusion.

The MLS_Bc constitutive resistance is coded by the *erm* gene and appears on the antibiogram as the resistance to erythromycin and clindamycin. The MLS_Bi inducible resistance, on the other hand, appears on the antibiogram as the resistance to erythromycin and sensitivity to clindamycin. To detect the MLS_Bi phenotype, it is necessary to perform a complementary test by disk-approximation (*D-test*), in which the clindamycin halo is flattened by induction. In this case, this drug is not recommended¹⁹.

MRSA isolates in this study presented higher prevalence of MLS_Bc resistance (65.2%) compared to MLS_Bi. Two isolates, initially sensitive to clindamycin, tested positive on the *D-test*, thus, presenting inducible resistance. Among the MRCoNS, the three MLS_B phenotypes were identified, including one isolate with a positive *D-test*. These data have great clinical relevance, because when the MLS_Bi phenotype is not detected the clindamycin treatment can fail¹⁹.

The MSSA isolates were predominantly resistant to MLS_Bi (20%), which reinforces the need to investigate the induced resistance to clindamycin in *S. aureus*.

Multi-drug resistant bacteria in venous ulcers limit the options for treatment, and can thus predispose to complications in the clinical outcomes. Skin and soft tissue infections, alone, are risk factors for the transmission of MDROs *S. aureus* and *coagulase-negative Staphylococcus* in the population⁶. These infections account for most infections by MRSA in the community²⁸, which are usually treated in outpatient clinic settings.

The long treatment period, due to factors that affect on the healing process of the ulcers, could predispose a more frequent use of antimicrobial agents, and, thus, contribute to an increase in resistance rates. There is a strong association between the occurrence of a chronic wound and the duration of the empirical antimicrobial therapy⁷. Infection treatment failures within the community have been associated with complication of the infection, as well as the increase in the consumption of antimicrobials in primary healthcare⁴⁰.

The present study findings have clinical and epidemiological relevance, because they evidence the need for contact precaution measures; greater control in cleaning and disinfecting equipment between patients; appropriate hand washing, as well as the use of the recommended individual protection equipment, besides providing a physical appropriate for the procedures, i.e., adequate dressing rooms.

The correct treatment for venous ulcers includes the microbiological testing to identify pathogens³⁸⁻⁴⁰. A careful indication of antimicrobials should be associated to other therapies that, together, could improve the healing process²³.

The existence of MDROs pathogens and failures during the treatment are important factors for the patient¹⁴. The healthcare professional can help prevent infection through specialist healthcare procedures, such as thorough cleaning of the ulcer, applying appropriate coverage, evaluating the ulcer for dressing changes, forwarding to other professionals, and assessing the antimicrobial treatment outcome by evaluating the clinical signs and symptoms.

The identification of multi-drug resistant bacteria, such as *S. aureus* and *coagulase-negative Staphylococcus* resistant to methicillin and to the MLS_B group in outpatients with venous ulcers reveals a panorama about the susceptibility profile of the pathogens that affect this population with chronic wounds. The epidemiological data can contribute with the surveillance systems, in the sense of reviewing the measures that could avoid outbreaks and severe infections on individuals with venous ulcers, besides providing safe healthcare.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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ABSTRACT IN PORTUGUESE

Prevalência de fenótipos de resistência em *Staphylococcus aureus* e *coagulase negativos* isolados de úlceras venosas de pessoas atendidas na atenção primária

Introdução: Em úlceras venosas, a presença de *Staphylococcus aureus* e *coagulase negativo* com fenótipos de resistência pode constituir fator agravante e limita as opções terapêuticas. **Métodos:** Foram avaliados estafilococos isolados de 69 pacientes, representando 98 úlceras no período de outubro de 2009 a outubro de 2010. A detecção fenotípica da resistência ao grupo *macrolide*, *lincosamide*, *streptogramin B* (MLS_B) foi realizada pelo *D-test*. Isolados resistentes a cefoxitina e/ou oxacilina (disco-difusão) foram submetidos ao teste confirmatório para detecção da *minimum inhibitory concentration* (MIC), empregando fitas de oxacilina (*E-test*[®]). **Resultados:** A prevalência de *S. aureus* foi de 83% e de 15% de *coagulase-negative staphylococcus* (CoNS). Identificou-se 28% de *methicillin-resistant*

Staphylococcus aureus (MRSA) e 47% de *methicillin-resistant coagulase-negative staphylococcus* (MRCoNS). Entre o *S. aureus*, 69,6% apresentaram resistência a eritromicina, 69,6% a clindamicina, 69,6% a gentamicina e 100% a ciprofloxacina. Setenta e quatro por cento dos MRSA apresentaram elevado nível de resistência a oxacilina, MIC \geq 256 μ g/mL, e em 65,2% predominou a resistência constitutiva MLS_Sc. Dos 20 isolados sensíveis a clindamicina, 12 apresentaram fenótipo MLS_Sb induzível. Um total de 71,4% dos MRCoNS apresentaram resistência a eritromicina, ciprofloxacina e gentamicina. Dos isolados positivos para a enzima β -lactamases, as MIC tiveram *breakpoint* entre 0,5 a 2 μ g/mL. **Conclusões:** Os resultados sinalizam elevada ocorrência de bactérias multirresistentes em úlceras venosas de pacientes recebendo atenção primária, evidenciando a necessidade de medidas preventivas que evitem surtos causados por patógenos resistentes a múltiplas drogas e a importância dos profissionais em discernir infecção de colonização em úlcera venosa, critério fundamental na indicação antibioticoterapia sistêmica.

Palavras-chaves: *Staphylococcus aureus* resistente à metilina. Resistência bacteriana a fármacos. Úlcera varicosa. Atenção primária à saúde.

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