

Antimicrobial resistance profile of *Staphylococcus aureus* isolates obtained from skin and soft tissue infections of outpatients from a university hospital in Recife -PE, Brazil *

Perfil de resistência antimicrobiana de isolados de *Staphylococcus aureus* provenientes de infecções de pele e tecidos moles de pacientes ambulatoriais de um hospital universitário em Recife - PE, Brasil

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Abstract: BACKGROUND: *Staphylococcus aureus* has a notable ability to acquire resistance to antibiotics, and methicillin resistance represents a growing public health problem. Methicillin-resistant *S. aureus* (MRSA) has also become important outside the hospital environment, particularly in the United States. In Brazil, since 2005, cases of community skin infections caused by MRSA have been reported, but resistance studies involving outpatients are scarce.

OBJECTIVE: To know the resistance profile of *S. aureus* involved in skin and soft tissue infections of patients seen at the Dermatology outpatient clinic of a university hospital in Recife, Pernambuco State, northeastern Brazil.

METHODS: Prospective study involving 30 patients with skin and soft tissue infections, seen at the Dermatology outpatient clinic from May until November 2011. To evaluate the susceptibility of *S. aureus* to antibiotics, the disk diffusion method and oxacillin screening agar were used.

RESULTS: From a total of 30 samples of skin lesions, 19 (63%) had positive culture for *S. aureus*. The following resistance patterns of *S. aureus* were observed: penicillin, 95%; tetracycline, 32%; erythromycin, 21%; gentamicin, 16%; cefoxitin, 11%; oxacillin, 11%; trimethoprim-sulfamethoxazole, 11%; chloramphenicol, 11%; clindamycin, 5%; and ciprofloxacin, 0%. One of the identified MRSA was obtained from a patient without risk factors for its acquisition, and was resistant, beyond to the beta-lactams, only to tetracycline.

CONCLUSIONS: With regard to the resistance patterns of *S. aureus*, resistances to tetracycline, erythromycin and gentamicin were the highest. It was documented, for the first time in Pernambuco, a case of skin infection caused by community-associated MRSA.

Keywords: Community-acquired infections; Drug resistance, microbial; Skin; *Staphylococcus aureus*

Resumo: FUNDAMENTOS: O *Staphylococcus aureus* possui uma notável habilidade de adquirir resistência antimicrobiana, sendo a resistência à meticilina um problema de saúde pública crescente. O *S. aureus* resistente à meticilina (MRSA) vem se tornando importante também fora do ambiente hospitalar, particularmente nos Estados Unidos. No Brasil, desde 2005, têm sido relatados casos de infecções cutâneas comunitárias causadas por MRSA, porém estudos de resistência envolvendo pacientes ambulatoriais são escassos.

OBJETIVO: Conhecer o perfil de resistência de *S. aureus* envolvidos em infecções de pele e partes moles de pacientes atendidos no ambulatório de Dermatologia de um hospital universitário de Recife, Pernambuco.

MÉTODO: Estudo prospectivo envolvendo 30 pacientes com infecções de pele e tecidos moles atendidos no ambulatório de Dermatologia de maio a novembro de 2011. Para avaliação da suscetibilidade dos *S. aureus* aos antibióticos foram utilizados teste de disco-difusão e placa de screening de oxacilina.

RESULTADOS: Das 30 amostras analisadas, 19 (63%) tiveram cultura positiva para *S. aureus*. Os seguintes padrões de resistência dos *S. aureus* foram observados: penicilina, 95%; tetraciclina, 32%; eritromicina, 21%; gentamicina, 16%; cefoxitina, 11%; oxacilina, 11%; sulfametoxazol-trimetoprima, 11%; clorafenicol, 11%; clindamicina, 5%; e ciprofloxacina, 0%. Um dos MRSA identificados foi obtido de paciente sem fatores de risco para sua aquisição, e além de aos betalactâmicos, mostrou-se resistente apenas à tetraciclina.

CONCLUSÕES: Em relação aos padrões de resistência dos *S. aureus*, destacaram-se as resistências à tetraciclina, eritromicina e gentamicina. Documentou-se, pela primeira vez em Pernambuco, um caso de infecção cutânea causada por MRSA associado à comunidade.

Palavras-chave: Infecções comunitárias adquiridas; Pele; Resistência microbiana a medicamentos; *Staphylococcus aureus*

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INTRODUCTION

In conditions such as the loss of integrity of the cutaneous barrier or reduction of immunity, *Staphylococcus aureus* stops behaving as a commensal to become the most common etiological agent of skin infections.¹ This microorganism has a notable ability to acquire resistance to antibiotics. Penicillin was introduced in the clinical practice in 1941 and ten years after it was no longer effective in most staphylococcal infections.² This resistance is mediated by beta-lactamases denominated penicillinases, which are enzymes that hydrolyze the beta-lactam ring of the antibiotics that together with penicillin form the beta-lactam group, inactivating them.^{3,4} In 1959 the production of penicillinase-resistant beta-lactams was initiated.^{2,5,6} Methicillin and oxacillin were the first of these medications to be used.⁶

The first methicillin-resistant *S. aureus* (MRSA) strain was reported in England in 1961, after being isolated from a hospitalized patient.^{5,7} Immediately after, similar cases were reported in other European countries, and later in Japan, Australia and the United States, with dissemination of this microorganism, transforming it into the main cause of hospital-acquired infections.⁵ *S. aureus* strains denominated as MRSA are resistant to all beta-lactams (except for cephalosporins with anti-MRSA activity) and to combinations of these with beta-lactamase inhibitors.^{6,8}

The first genuine cases of community-associated MRSA (CA-MRSA) infection were described in 1993 in Australia.² The isolates had been identified in the end of the 1980's in an indigenous population on the West of the country.⁹ Aside from the absence of risk factors, what the cases had in common was the fact that the infections had been caused by non-multiresistant MRSA. In the second half of the 1990's, MRSA infections were also reported in the United States, occurring in children of the community and without risk factors. Since then, CA-MRSA has emerged worldwide, principally in the United States, where in many metropolitan areas it is the most common identifiable cause of skin and soft tissue infections.^{2,10,11}

Due to the constant changes in the epidemiology and the resistant patterns of *S. aureus*, their vigilance in different regions is important. The objective of the present study was to evaluate the antimicrobial resistance profile of *S. aureus* isolates obtained from outpatients with skin and soft tissue infections.

MATERIALS AND METHODS

The study was prospective, observational, descriptive, case series type, involving patients with signs of skin and soft tissue infections who sought treatment at the Dermatology outpatient clinic of a university hospital in Recife, Pernambuco State, northeast

Brazil, between May and November 2011. The inclusion criteria were: presence of clinical signs of primary impetigo, secondary impetigo, ecthyma, folliculitis, furunculosis, carbuncle, acute abscess of subcutaneous cellular tissue, necrotizing fasciitis, periporitis or sycosis; and beginning of the infection in the community. Hospitalization at the time of study entry was an exclusion criterion. The research project was approved by the Ethical Committee in Research under registration number 34/2011. To participate in the study, the patients or their legal representative signed a free informed consent.

Data collection

At the time of the consultation, the following data were collected from each patient participating in the study: age; gender; hospitalization, residency in a long-term care facility or dialysis in the previous 12 months; presence of permanent indwelling catheter or percutaneous medical device; domiciliary contact in the previous 12 months with individuals with some of the risk factors for acquisition of MRSA previously cited; prior positive culture for MRSA; use of antibiotics in the previous 12 months and at the time of study entry. The type of skin infection presented by patient was also noted in the data collection form.

Sample collection

The samples of purulent material were obtained by rubbing a sterile cotton swab in the most exuberant lesion of each patient, after previous decontamination of the margins and surface of the lesions with 70% alcohol. In the cases of furuncles or abscesses with indication of drainage, the samples were collected from the deepest part of the lesion. After collection, the swabs were immediately placed in Stuart's transport medium and transported to the Bacteriology sector of the laboratory of the hospital, where conventional methods were carried out for isolating and identifying the bacteria, as well as the susceptibility tests to the antimicrobials.

Phenotypic characterization

The gram-positive cocci that presented positive tests for catalase, desoxyribonuclease and mannitol fermentation were considered *S. aureus*.

Susceptibility tests

The resistance profile of the *S. aureus* isolates was evaluated using the disk diffusion method, according to the recommendations of the Clinical and Laboratory Standards Institute, using the following antimicrobial disks: penicillin (10 µg), oxacillin (1 µg), clindamycin (2 µg), trimethoprim-sulfamethoxazole

(1.25/23.75 µg), chloramphenicol (30 µg), gentamicin (10 µg), ceftiofloxacin (30 µg), erythromycin (15 µg), ciprofloxacin (5 µg) and tetracycline (30 µg).⁸ The isolates that appeared, in the disk diffusion method, as sensitive to clindamycin and resistant to erythromycin were submitted to the D test for detection of the MLS_B phenotype (inducible macrolide-lincosamide-streptogramin B resistance), in which clindamycin resistance is only apparent after induction by erythromycin.

Oxacillin screening agar

S. aureus isolates that were identified as oxacillin-resistant and/or ceftiofloxacin-resistant by the disk diffusion method were taken to the laboratory of the Microbiology and Immunology Department of the university, where they were submitted to the oxacillin screening agar, being made, initially, subcultures on nutrient agar plates. After the colonies grew, direct suspension of the colonies was carried out to obtain a turbidity equivalent to a 0.5 McFarland standard, and proceeding with the inoculum in an area at 10 to 15 mm in diameter in plates containing Mueller Hinton agar medium with NaCl (4% w/v; 0.68 mol/L) and 6 µg/ml of oxacillin. These plates were incubated at 35°C for 24 hours, and the lineage in which plates was observed growth of more than one colony was considered oxacillin resistant.⁸ As quality control, standard strains for MRSA and MSSA were used: *S. aureus* ATCC 29213 and *S. aureus* ATCC 33591, respectively.¹²

RESULTS

A total of 30 patients were evaluated: 18 females (60%) and 12 males. In relation to age, 1 (3.3%) patient was younger than 2 years of age, 3 (10%) were between the ages of 2 and 17 years, 22 (73.3%) were between the ages of 18 and 59 years, and 4 (13.3%) were 60 years of age or older. From the 30 collected samples, 29 (97%) had positive cultures for bacteria; 19 (66%) of which had *S. aureus* isolated.

The highest resistance rates of the *S. aureus* found were to penicillin, tetracycline, gentamicin and erythromycin (Table 1).

From the 19 *S. aureus* isolates, 1 was resistant to ceftiofloxacin and another was resistant to ceftiofloxacin and to oxacillin in the disk diffusion method. These two *S. aureus* isolates were submitted to the oxacillin screening agar and both of them were resistant to oxacillin by this test, being characterized as MRSA. These isolates were then classified as resistant to all researched beta-lactams researched. One of the MRSA isolates was obtained from a patient without risk factors for its acquisition (Table 2). The patients from whom MRSA were isolated were not using antibiotics at the time of study entry.

TABLE 1: Antimicrobial resistance profile of *Staphylococcus aureus* obtained from skin and soft tissue infections of patients attending the Dermatology outpatient clinic of a university hospital in Recife, Pernambuco State, northeastern Brazil between May and November 2011

	<i>Staphylococcus aureus</i>			
	Sensitive		Resistant	
	N	%	N	%
Antimicrobials				
Penicillin	1	5%	18	95%
Tetracycline	13	68%	6	32%
Erythromycin	15	79%	4	21%
Gentamicin	16	84%	3	16%
Ceftiofloxacin	17	89%	2	11%
Oxacillin	17	89%	2	11%
Trimethoprim-sulfamethoxazole	17	89%	2	11%
Chloramphenicol	17	89%	2	11%
Clindamycin	18	95%	1	5%
Ciprofloxacin	19	100%	0	0%

DISCUSSION

In the present study, *S. aureus* was the most frequent pathogen isolated from skin and soft tissue pyogenic lesions. This result was already expected and agrees with the results of other studies.¹³⁻¹⁶

With regard to the resistance patterns of the *S. aureus* isolates, the resistances to tetracycline, erythromycin and to gentamicin were the highest. In 2001, Zavadinack et al. observed, in an study of antimicrobial resistance of *S. aureus* obtained from cutaneous abscesses of community origin in the state of Paraná, a lower level of resistance to tetracycline (9.86%), erythromycin (11.49%) and gentamicin (2.86%).¹ The highest resistance rates found to these antibiotics in our study can be justified, in part, by the indiscriminate and inadequate use of these drugs along the years, favored by their low cost. The use of antimicrobials selects the lineages of resistant bacteria and it is likely the main cause of antimicrobial resistance.³

The resistance rate to gentamicin, in the present study, was also higher than that reported by Diamantis et al., who found that only 0.7% of the *S. aureus* strains were resistant to this antibiotic. In this American study, the resistances to erythromycin (51.7%), methicillin (27.3%) and clindamycin (23.8%) were the highest.¹⁷

Ribeiro et al., in 2005, reported the first cases of skin and soft tissue infections caused by CA-MRSA in Brazil.¹⁸ Since then, various other similar studies have been carried out, especially in the southern region of the country. In 2009, for example, two cases of skin and soft tissue infection caused by CA-MRSA were

TABLE 2: Characteristics of the patients with methicillin-resistant *S. aureus* attending the Dermatology outpatient clinic of a university hospital in Recife, Pernambuco State, northeastern Brazil between May and November 2011

Patient N°	Age	Gender	Risk Factor for MRSA	Type of infection	Resistance pattern of the MRSA isolates*
1	47 years	M	none	furuncle	PEN, TET
2	3 months	F	hospitalization in the previous 12 months	secondary impetigo	PEN, ERY, CHL, CLI

*Antimicrobials: penicillin (PEN), tetracycline (TET), erythromycin (ERY), chloramphenicol (CHL), clindamycin (CLI).

reported in the city of Porto Alegre.^{19,20} In the same year, Rozenbaum et al. reported the case of 10-year-old non-immunocompromised girl, who had been admitted to a hospital in the city of Rio de Janeiro in 2007, presenting clinical manifestations of septic shock, and from whom MRSA was isolated. Seven days before admission, the patient had developed furunculosis involving the buttocks and cellulite in the left thigh; and she had no history of previous hospitalization or healthcare-associated procedures in the last year.²¹

Gelatti, in a prospective study carried out in the city of Porto Alegre, between September of 2007 and March of 2008, found that 8.6% of the *S. aureus* isolated from patients with skin infections attended in a Dermatology outpatient clinic or with up to 48 hours of hospital admission were CA-MRSA. Patients with recent hospital admission or surgical interventions, presence of intravenous catheter or long-term indwelling intravascular or cutaneous devices, and admission to nursing homes were excluded from this study.¹⁵

CA-MRSA infection had already been reported in northeastern Brazil by Nascimento-Carvalho et al., who studied, retrospectively, *S. aureus* obtained from patients younger than 20 years of age with infections caused by this microorganism and that were treated between 1995 and 2005 in a pediatric teaching hospital in Salvador, Bahia State. Out of the 122 *S. aureus* isolated from outpatients or within 48 hours after admission to a hospital and without the risk factors researched for MRSA, six (4.9%) were resistant to oxacillin in the disk diffusion method. This study evaluated a higher number of *S. aureus* isolates than the present study; however, it was a retrospective study of infections caused by *S. aureus* in general. Only two of the isolates characterized as CA-MRSA in the study were obtained from patients with skin infections.²²

There were already reports of MRSA identified in studies of infections and colonizations by *S. aureus* in hospitalized patients in Pernambuco^{23,24,25} standing out the Cavalcanti et al.'s study, which studied *S. aureus* of patients in the first 48 hours of intensive care unit (ICU) admission in a university hospital in

Recife, and found three MRSA isolates colonizing patients considered as being of community origin (patients admitted from their residences or hospitalized for less than 48 hours before ICU admission).²⁵ This study, however, differently from the present study, evaluated only colonization.

The first sample of MRSA identified in our study was obtained during the drainage of a furuncle presented by a 40-year-old man. Skin and soft tissue infections represent approximately 90% of cases of CA-MRSA infection,² and furunculosis is the most frequently observed disease.²⁶

The patient, from whom the first MRSA isolate was obtained in the present study, had no risk factors for acquisition of MRSA, characterizing the MRSA isolate, according to the Centers for Disease Control and Prevention (CDC) criteria, as a CA-MRSA. The CDC defines CA-MRSA as MRSA that has been isolated from patients who have no history of positive culture for MRSA from any body site obtained more than 48 hours after admission to a hospital (if hospitalized); prior MRSA infection or colonization; hospitalization, surgery, residence in health institution, hemodialysis or peritoneal dialysis in the last year; and presence of percutaneous devices or catheters.²⁷ Moreover, the first MRSA isolate was resistant, beyond to the beta-lactams, only to tetracycline, being characterized phenotypically as a CA-MRSA. In contrast to HA-MRSA, which is characterized by resistance to various classes of antimicrobials, CA-MRSA is generally resistant only to beta-lactams and to one or two other classes of antibiotics.²⁸

The second MRSA isolate of the study was obtained from a three-month-old girl who although was born by normal delivery, remained hospitalized for a little longer than 48 hours at the time of birth. Moreover, the MRSA isolate obtained from this patient was multiresistant, being characterized epidemiologically and phenotypically as a HA-MRSA.^{27,28}

CONCLUSION

The present study showed that the antimicrobial resistance patterns of *S. aureus* vary according to

geographic region, being more evident in the study population the resistance to tetracycline, erythromycin and gentamicin. These results suggest that the beta-lactams, with the exception of penicillin, ampicillin and amoxicillin, continue to represent a good option of empirical therapy for patients with pyodermitis who seek treatment at the Dermatology outpatient clinic of this university hospital.

Until then, there was no evidence in the literature of cases of skin and soft tissue infections caused by CA-MRSA in Pernambuco State. The study then permitted to document, for the first time in the state, a case of skin infection caused by this microorganism, contributing with new information about its the epidemiology in northeastern Brazil. □

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