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Antimicrobial resistance and persistence of *Staphylococcus epidermidis* clones in a Brazilian university hospital

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ABSTRACT

Oxacillin is an alternative for the treatment of *Staphylococcus* spp. infections; however, resistance to this drug has become a major problem over recent decades. The main objective of this study was to epidemiologically characterize coagulase-negative staphylococci (CoNS) strains recovered from blood of patients hospitalized in a Brazilian teaching hospital. Oxacillin resistance was analyzed in 160 strains isolated from blood culture samples by phenotypic methods, detection of the *mecA* gene, and determination of intermediate sensitivity to vancomycin on brain heart infusion agar supplemented with 4 and 6 μg/mL vancomycin. In addition, characterization of the epidemiological profile by staphylococcal cassette chromosome *mec* (SCC*mec*) typing and clonal analysis by pulsed-field gel electrophoresis (PFGE) were performed. The *mecA* gene was detected in 72.5% of the isolates. Methicillin-resistant CoNS isolates exhibited the highest minimum inhibitory concentrations and multiresistance when compared to methicillin-susceptible CoNS strains. Typing classified 32.8% of the isolates as SCC*mec* I and 50% as SCC*mec* III. PFGE typing of the SCC*mec* III *Staphylococcus epidermidis* isolates identified 6 clones disseminated in different wards that persisted from 2002 to 2009. The high oxacillin resistance rates found in this study and clonal dissemination in different wards highlight the importance of good practices in nosocomial infection control and of the rational use of antibiotic therapy in order to prevent the dissemination of these clones.

Coagulase-negative staphylococci (CoNS) isolates are usually etiologic agents of catheter-related bloodstream infections. Oxacillin resistance rates are currently high among CoNS, a fact that has become a major problem in the treatment of these infections since multiresistance is frequent and few therapeutic alternatives are available. High resistance rates ranging from 66 to 95% are reported for blood culture isolates (Cuevas et al., 2008; Natoli et al., 2009, Bouchami et al., 2011).

The most frequent oxacillin resistance mechanism is the presence of the *mecA* gene. This gene is carried by a mobile genetic element, known as the staphylococcal cassette chromosome *mec* (SCC*mec*). Eleven SCCmec types are known so far, which differ in their genetic composition. SCC*mec* typing is a useful epidemiological tool (Machado et al., 2007) since the prevalence of the different types varies between hospital and community environments. Some of these types carry genes that confer resistance to multiple antibiotics such as beta-lactams, macrolides, lincosamines, streptogramins, aminoglycosides, and tetracycline. Hence, when a bacterial cell acquires this SCC*mec*, it also acquires a multidrug resistance phenotype (Ito et al., 2003).

In view of the high rates of oxacillin resistance and the emergence of resistance to other antimicrobials such as vancomycin, knowledge of the epidemiology of infections caused by *Staphylococcus* spp. is of the utmost importance. Over the past few years, molecular tools have been used in epidemiological studies with good results. Therefore, the aim of the present study was to epidemiologically characterize CoNS isolated from patients with bacteremia who were hospitalized in a Brazilian teaching hospital.

One hundred sixty CoNS isolates stored in the culture collection of the Department of Microbiology and Immunology, Botucatu School of Medicine (BSM), Universidade Estadual Paulista (UNESP), were studied. The strains were isolated between 2002 and 2009 from blood cultures of patients hospitalized in different wards of BSM-UNESP, Brazil.

The simplified scheme proposed by Cunha et al. (2004) was used for the identification of CoNS. Isolates that were not identified as *Staphylococcus epidermidis* were submitted to genotypic identification using primers comprising conserved sequences adjacent to the 16S and 23S genes according to the protocols of Barry et al (1991) and Couto et al (2001). The following international reference strains were used as

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Table 1
Determination of oxacillin susceptibility in coagulase-negative staphylococci isolates by phenotypic and genotypic methods

PCR	Phenotypic methods															
	Disk diffusion															
	Oxacillin				Cefoxitin				Screening				E-test			
	S		R		S		R		S		R		S		R	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
mecA+ (n = 116)	1	0.9	115	99.1	0	0	116	100	6	5.2	110	94.8	1	0.9	115	99.1
mecA− (n = 44)	40	90.9	4	9.1	40	90.9	4	9.1	41	93.2	3	6.8	40	90.9	4	9.1

S = oxacillin-sensitive isolate; R = oxacillin-resistant isolate.

quality control strains: *Staphylococcus capitis* subsp. *urealyticus* (ATCC 49325), *Staphylococcus caprae* (ATCC 35538), *S. epidermidis* (ATCC 12228), *Staphylococcus haemolyticus* (ATCC 29970), *Staphylococcus hominis* subsp. *novobiosepticus* (ATCC 700237), *Staphylococcus saprophyticus* (ATCC 15305), *Staphylococcus schleiferi* subsp. *schleiferi* (ATCC 43808), *Staphylococcus xylosus* (ATCC 29979), and *Staphylococcus warneri* (ATCC 10209).

Susceptibility to oxacillin was tested by the agar disk diffusion method using oxacillin (1 μg) and cefoxitin (30 μg) disks and by screening on Mueller-Hinton agar supplemented with 4 μg/mL oxacillin and 4% NaCl (CLSI, 2011). *Staphylococcus aureus* ATCC 25923 (oxacillin sensitive, *mecA* gene negative) and ATCC 33591 (oxacillin resistant, *mecA* gene positive) were used as controls in all experiments.

PCR for detection of the *mecA* gene was performed as described by Murakami et al. (1991). The SCC*mec* type of CoNS was determined using the multiplex PCR method modified by Machado et al. (2007). *S. epidermidis* and *S. haemolyticus* isolates were typed by pulsed-field gel electrophoresis (PFGE) according to the protocol modified by McDougal et al. (2003). International clones kindly provided by Dr Antonio Carlos Campos Pignatari, Laboratório Especial de Microbiologia Clínica, Disciplina de Infectologia, Universidade Federal de São Paulo/Escola Paulista de Medicina, and Dr Agnes Marie Sá Figueiredo, Universidade Federal do Rio de Janeiro, Instituto de Microbiologia Prof. Paulo de Góes, Brazil, were used as controls.

The E-test was used to determine the *in vitro* susceptibility of the CoNS isolates to the following drugs: oxacillin, netilmicin, erythromycin, sulfamethoxazole-trimethoprim, and vancomycin. The isolates were classified as sensitive or resistant according to breakpoints (μg/mL) recommended by the CLSI (2011). In the Results section, isolates presenting intermediate levels were considered to be resistant. Isolates exhibiting minimum inhibitory concentrations (MIC) for vancomycin ≥2 μg/mL by the E-test were evaluated regarding vancomycin heteroresistance. This heteroresistance was analyzed by the modified E-test method (macromethod) according to the protocol described by Walsh et al (2001) and by screening on BHI agar supplemented with 4 and 6 μg/mL vancomycin (CLSI, 2011).

In this study, the strains isolated from the wards of BSM-UNESP were divided into 7 complexes according to specialty: Internal Medicine Wards, Surgery Wards, Pediatric Wards, Gynecology and Obstetrics, Emergency Room, Intensive Care Units, and other wards.

Of the 160 isolates analyzed, 111 were identified as *S. epidermidis*, followed by *S. haemolyticus* (n = 16), *S. hominis* subsp. *novobiosepticus* (n = 13), *S. saprophyticus* (n = 9), *S. capitis* subsp. *urealyticus* (n = 5), *S. schleiferi* subsp. *schleiferi* (n = 3), and *S. xylosus*, *S. warneri*, and *S. caprae* with 1 isolate each. Oxacillin resistance was detected by the oxacillin disk diffusion method in 119 (73.8%) isolates and by the cefoxitin disk method in 120 (75%) (Table 1). Therefore, 1 isolate was identified as oxacillin resistant by the cefoxitin disk method but not by the oxacillin disk method. The test was repeated since the E-test corroborated the cefoxitin disk result, and this result was confirmed.

The *mecA* gene was detected in 116 isolates (72.5%). The highest frequency of the gene was observed among *S. haemolyticus* strains, with 12 (75%) positive isolates, followed by *S. epidermidis* with 82 (70.7%) positive isolates, *S. hominis* subsp. *novobiosepticus* with 9 (7.8%) isolates, *S. saprophyticus* with 7 (6%) isolates, and *S. schleiferi* and *S. capitis* subsp. *urealyticus* with 3 (2.6%) isolates each. Four CoNS isolates were *mecA* negative and oxacillin resistant by the phenotypic methods. These isolates were tested by the disk diffusion method using amoxicillin-clavulanic acid disks and were found to be sensitive, a fact suggesting resistance due to hyperproduction of β-lactamase.

Analysis of the CoNS strains by the E-test method revealed 119 (74.4%) isolates that were resistant to oxacillin, 104 (65%) resistant to erythromycin, only 16 (10%) resistant to netilmicin, and 73 (45.6%) resistant to trimethoprim-sulfamethoxazole. When the isolates were divided into methicillin-resistant (MR) CoNS and methicillin-sensitive (MS) CoNS, a large difference was observed between the 2 groups, with a predominance of drug-resistant isolates among those resistant to oxacillin. The highest resistance rates among MRCoNS were observed to erythromycin, with 90 (77.6%) isolates, followed by resistance to trimethoprim-sulfamethoxazole, with 66 (56.9%) isolates, and to netilmicin, with only 15 (12.9%) isolates. High rates of resistance to erythromycin and trimethoprim-sulfamethoxazole were observed among *S. epidermidis*, *S. haemolyticus*, and *S. hominis* subsp. *novobiosepticus*. Table 2 shows the MIC for oxacillin and vancomycin according to CoNS species studied.

Nine of 67 isolates with MIC ≥2 μg/mL grown on BHI agar supplemented with 4 μg/mL vancomycin (*S. epidermidis*, n = 5; *S. haemolyticus*, n = 3; *S. capitis*, n = 1) and 1 *S. epidermidis* isolate were able to grow on BHI agar containing 6 μg/mL vancomycin. All isolates with MIC ≥2 μg/mL showed no heteroresistance to vancomycin when analyzed by the macromethod.

Table 2
MIC for oxacillin and vancomycin in coagulase-negative staphylococci

CoNS	Oxacillin				Vancomycin			
	No. of isolates according to MIC range μg/mL				No. of isolates according to MIC range μg/mL			
	0.023–0.094	0.125–0.75	0.94–32	>32–>256	0.38–0.5	0.75–1	1.5–2	3–4
S. epidermidis	14	20	38	39	1	9	97	4
S. haemolyticus	4	0	4	7	0	6	8	2
S. hominis subsp. *novobiosepticus*	1	3	9	0	2	6	5	0
S. saprophyticus	1	3	3	2	1	3	5	0
S. capitis subsp. *urealyticus*	0	2	3	0	0	2	3	0