Coagulase-negative staphylococci strains resistant to oxacillin isolated from neonatal blood cultures

Valéria Cataneli Pereira/+, Maria de Lourdes Ribeiro de Souza da Cunha

Departamento de Microbiologia e Imunologia, Instituto de Biociências, Universidade Estadual Paulista, Botucatu, SP, Brasil

Coagulase-negative staphylococci (CoNS) are the microorganisms most frequently isolated from clinical samples and are commonly found in neonatal blood cultures. Oxacillin is an alternative treatment of choice for CoNS infections; however, resistance to oxacillin can have a substantial impact on healthcare by adversely affecting morbidity and mortality. The objective of this study was to detect and characterise oxacillin-resistant CoNS strains in blood cultures of newborns hospitalised at the neonatal ward of the University Hospital of the Faculty of Medicine of Botucatu. One hundred CoNS strains were isolated and the mecA *gene was detected in 69 of the CoNS strains, including 73.2% of* Staphylococcus epidermidis *strains, 85.7% of* Staphylococcus haemolyticus *strains, 28.6% of* Staphylococcus hominis *strains and 50% of* Staphylococcus lugdunensis *strains. Among these oxacillin-resistant CoNS strains, staphylococcal cassette chromosome mec (SCC*mec*) type I was identified in 24.6%, type II in 4.3%, type III in 56.5% and type IV in 14.5% of the strains. The data revealed an increase in the percentage of CoNS strains isolated from blood cultures from 1991-2009. Furthermore, a predominant SCC*mec *profile of the oxacillin-resistant CoNS strains isolated from neonatal intensive care units was identified with a prevalence of SCC*mec *types found in hospital-acquired strains.*

Key words: coagulase - negative staphylococci - *mecA* - SCC*mec*

Coagulase-negative staphylococci (CoNS) are members of the normal human skin microflora and are the microorganisms most frequently isolated from clinical samples (Kloos & Bannerman 1994). CoNS are also the most common microorganisms identified in blood cultures of newborns in neonatal intensive care units (NICUs), causing 40-60% of the cases of bacteraemia (Maayan-Metzger et al. 2000).

The continued emergence of antimicrobial drug resistance is a serious problem for the antibiotic treatment of patients with staphylococcal infections in the clinic. Studies have reported that 60-85% of *Staphylococcus* strains isolated from clinical samples are resistant to methicillin (Kuehnert et al. 2006). The major problem lies in the fact that infections caused by methicillin-resistant *Staphylococcus* strains are difficult to treat. In some cases, the isolates are only susceptible to glycopeptides and new drugs, such as linezolid, tigecycline, daptomycin and quinupristin/dalfopristin (Critchley et al. 2003, Otto 2009).

The resistance of *Staphylococcus* species to oxacillin is mediated by the production of a supplemental penicillin-binding protein (PBP 2' or PBP 2a) that has a low affinity for semi-synthetic penicillins. The protein is encoded by the *mecA* gene and this gene is identical in all *Staphylococcus* strains. Thus, *mecA* is a useful molecular marker of oxacillin resistance (Archer & Niemeyer 1994). The *mecA* gene is carried on a specific mobile genetic element, called the staphylococcal cassette chromosome *mec* (SCC*mec*). So far, 11 SCC*mec* types have been described in *Staphylococcus aureus* (IWG-SCC 2009). SCC*mec* types I, II and III are the most common in nosocomial isolates of oxacillin-resistant *Staphylococcus* strains, whereas the other types are characteristic of community-acquired strains. *SCCmec* type III encodes the largest number of resistance genes and is associated with serious hospital infections (Ito et al. 1999). In contrast, SCC*mec* type IV is small, more mobile and carries only genes that regulate the expression of the *mecA* gene (Ito et al. 2001).

As phenotypic methods for the detection of oxacillin-resistant - CoNS [or methicillin-resistant CoNS (MRCoNS)] strains may sometimes yield questionable results, molecular tests for the detection of the *mecA* gene have been proposed. Detection of the *mecA* gene by polymerase chain reaction (PCR) is the gold standard for the identification of oxacillin-resistant *Staphylococcus* and SCC*mec* typing by multiplex PCR permits the characterisation of the *Staphylococcus* species and the determination of susceptibility to different non-beta-lactam antibiotics. In this respect, the objective of the present study was to detect and characterise MRCoNS strains in blood cultures of newborns hospitalised in the NICU of the University Hospital, Faculty of Medicine (HC-FMB), São Paulo State University (UNESP), Botucatu, state of São Paulo, Brazil.

This research was approved by the Research Ethical Committee (CEP) of the FMB (395/2005-CEP). The study was exempted from written informed consent from the study participants and/or their legal guardians because the samples of *Staphylococcus* included in the

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+ Corresponding author: cataneli@ibb.unesp.br
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study were already isolated and stored in the Collection of Cultures of the Department of Microbiology and Immunology, UNESP.

One hundred CoNS strains isolated from blood cultures of newborns hospitalised in the NICU of HC-FMB/UNESP were studied in the period from 1990-2009. Samples representing the number of hospitalisations each year were randomly selected for the study. Phenotypic identification of the CoNS species was performed using biochemical tests according to the simplified method of Cunha et al. (2004). PCR-based determination of internal transcribed spacer (ITS) regions was used for genotypic identification as according to Couto et al. (2001) to confirm the phenotypic identification.

Susceptibility to oxacillin was tested by the agar diffusion method using impregnated disks based on the criteria of the Clinical and Laboratory Standards Institute (CLSI 2009). Oxacillin (1 µg) and cefoxitin (30 µg) disks were used. The international reference strains, *S. aureus* ATCC 25923 (oxacillin susceptible) and ATCC 33591 (oxacillin resistant), were used in all experiments. Screening medium consisting of Mueller-Hinton agar supplemented with 4 µg/mL oxacillin and 4% NaCl was used for the identification of oxacillin-resistant CoNS strains.

Oxacillin resistance was determined by detection of the *mecA* gene in the CoNS isolates. The primers and PCR conditions described by Murakami et al. (1991) were used for the amplification procedure. *SCCmec* typing of the MRCoNS strains was performed by multiplex PCR. The primers and PCR conditions described by Oliveira and Lencastre (2002) and modified by Machado et al. (2007) were used for the amplification procedure.

One hundred CoNS strains were isolated from neonatal blood cultures. The phenotypic method identified 82% of the strains as *Staphylococcus epidermidis*, 7% as *Staphylococcus hominis*, 6% as *Staphylococcus haemolyticus*, 2% as *Staphylococcus lugdunensis*, 2% as *Staphylococcus warneri* and 1% as *Staphylococcus saprophyticus*. The genotypic method (PCR-ITS) identified 82% of the strains as *S. epidermidis*, 7% as *S. hominis*, 7% as *S. haemolyticus*, 2% as *S. lugdunensis*, 1% as *S. warneri* and 1% as *S. saprophyticus*.

The oxacillin (1 µg) disk diffusion method detected oxacillin resistance in 64% of the 100 CoNS strains studied. In contrast, 63% of the isolates were resistant when the cefoxitin (30 µg) disk test was used. Screening on Mueller-Hinton agar supplemented with 4% oxacillin and 4% NaCl demonstrated resistance to oxacillin in 64% of the CoNS isolates. The *mecA* gene was detected by PCR in 69 of the CoNS isolates, including 73.2% of *S. epidermidis* strains, 85.7% of *S. haemolyticus* strains, 28.6% of *S. hominis* strains and 50% of *S. lugdunensis* strains (Table). The oxacillin (1 µg) and cefoxitin (30 µg) disk diffusion methods had 92.8% and 91.3% sensitivity, respectively, and 100% specificity for the CoNS isolates studied. Sensitivity was 92.8% and specificity was 100% for the screening method.

SCC*mec* typing was performed on all isolates carrying the *mecA* gene. Among these oxacillin-resistant CoNS strains, SCC*mec* type I was identified in 24.6%, type II in 4.3%, type III in 56.5% and type IV in 14.5% of the strains. Among the *S. epidermidis* strains resistant to oxacillin, 16.7% were characterised as SCC*mec* type I, 3.3% as type II, 63.3% as type III and 16.7% as type IV. Among the *S. haemolyticus* strains carrying the *mecA* gene, 83.3% were characterised as SCC*mec* type I and 16.7% as type II. The two oxacillin-resistant *S. hominis* isolates were characterised as SCC*mec* type I and the only *mecA*-positive *S. lugdunensis* isolate was found to be SCC*mec* type III (Table).

The CoNS strains studied were isolated from blood cultures of newborns hospitalised in the NICU of HC-FMB between 1990-2009. Thirty-one of these strains were isolated between 1990-1994 and 16 of them (51.6%) were resistant to oxacillin. Twenty-two strains were isolated between 1995-2002 and 14 (63.6%) were MR-CoNS. Eighteen (78.3%) of the 23 strains isolated between 2003-2006 were resistant to oxacillin. During the

TABLE

Determination of oxacillin-resistant coagulase-negative staphylococci by disk diffusion method, screening method, detection of the *mecA* gene and typing of staphylococcal cassette chromosome *mec* (SCC*mec*)

	n	Oxacillin[a] n (%)	Cefoxitin[b] n (%)	Screening[c] n (%)	*mecA* n (%)	SCC*mec* (%)			
						I	II	III	IV
Staphylococcus epidermidis	82	56 (68.3)	55 (67)	56 (68.3)	60 (73.2)	16.7	3.3	63.3	16.7
Staphylococcus haemolyticus	7	6 (85.7)	6 (85.7)	6 (85.7)	6 (85.7)	83.3	16.7	0	0
Staphylococcus hominis	7	1 (14.3)	1 (14.3)	1 (14.3)	2 (28.6)	100	0	0	0
Staphylococcus lugdunensis	2	1 (50)	1 (50)	1 (50)	1 (50)	0	0	100	0
Staphylococcus warneri	1	0	0	0	0	0	0	0	0
Staphylococcus saprophyticus	1	0	0	0	0	0	0	0	0

a: 1µg disk diffusion method; *b*: 30µg disk diffusion method; *c*: on Mueller-Hinton agar supplemented with 4% oxacillin and 4% NaCl.

last period studied (2007-2009), 24 strains were isolated and 21 (87.5%) were MRCoNS (Fig. 1).

Among the oxacillin-resistant CoNS strains isolated between 1990-1994, five (31.2%) were characterised as SCC*mec* type I, six (37.5%) were type III and five (31.2%) were type IV. Between 1995-2002, there were three (21.4%) SCC*mec* type I strains, 10 (71.4%) type III strains and one (7.2%) type IV strain. Among the strains isolated between 2003-2006, one (5.5%) was type I, three (16.7%) were type II, 11 (61.1%) were type III and three (16.7%) were type IV strains. During the last period (2007-2009), eight (38.1%) type I strains, 12 (57.1%) type III strains and one (4.8%) type IV strain were identified (Fig. 2).

The present study evaluated oxacillin resistance among the 100 CoNS strains isolated from blood cultures of newborns in the NICU of HC-FMB. The rate of oxacillin resistance among the CoNS strains isolated between 1990-2009 was 69%. High rates of oxacillin resistance among neonatal CoNS isolates have been reported by Hira et al. (2007), who observed that 87% of strains were MRCoNS. In a study conducted between 2005-2006 at a hospital in Porto Alegre, Brazil, 27 of the 57 strains identified as MRCoNS were isolated from the neonatal ward (Barreto & Picoli 2008).

In the present study, 73.2% of the *S. epidermidis* isolates were resistant to oxacillin. Similar results have been reported by Krediet et al. (2004), who found that 97% of *S. epidermidis* isolates from an NICU between 1999-2001 were oxacillin resistant. Although *S. epidermidis* was the most prevalent CoNS member and had the highest rate of resistance among the MRCoNS species studied, other resistant species were also identified. Oxacillin resistance was detected in 85.7% of *S. haemolyticus* strains. According to Ferreira et al. (2003), 96% of *S. haemolyticus* strains isolated in Brazil are resistant to oxacillin and *S. haemolyticus* species are the second most prevalent nosocomial MRCoNS. The *mecA* gene was detected in 28.6% of the *S. hominis* strains studied here. This species has been associated with high rates of methicillin resistance, as 100% of isolates from an NICU in Spain were methicillin resistant **(**Chaves et al. 2005). The first report of the *mecA* gene in *S. lugdunensis* was published by Kawaguchi et al. (1996), who detected the gene in one of two isolates obtained from a neonatal ward. In the present study, *S. lugdunensis* was also isolated from two samples and one of the strains was resistant to oxacillin. According to Tee et al. (2003), the identification of *S. lugdunensis* is important not only because of the clinical implications of this species as the most aggressive CoNS strain, but also for determination of oxacillin susceptibility so that early treatment with adequate antibiotics can be initiated and good clinical outcome can be guaranteed.

SCC*mec* typing permitted the differentiation of types I-IV among the oxacillin-resistant CoNS strains studied. SCC*mec* type I was detected in 24.6% of the oxacillin-resistant CoNS strains and type II was found in 4.3% of the strains. SCC*mec* type III was found in 56.5% of the MRCoNS isolates and was the most prevalent among the species studied. Similar results have been reported in a Brazilian study conducted by Machado et al. (2007), who characterised MRCoNS species and revealed the presence of SCC*mec* type I in 28%, type II in 3% and type III in 52% of the strains. Whereas SCC*mec* type I contains only the *mecA* resistance gene, type II carries multiple resistance genes and has been associated with strains that began to be prevalent in the 1980s (Ito et al. 2001). Type III is the largest of the SCC*mec* types (66,896 bp) and encodes the largest number of resistance genes. This type is found in many hospital pathogens that can cause serious infections (Ito et al. 1999).

SCC*mec* type IV was identified in 14.5% of the MRCoNS strains studied. In contrast, Machado et al. (2007) found that only 0.8% of MRCoNS strains carried SCC*mec* type IV. The smaller number of strains carrying *SCCmec* type IV, compared with the hospital types, most likely means an increase in its mobility and capacity of

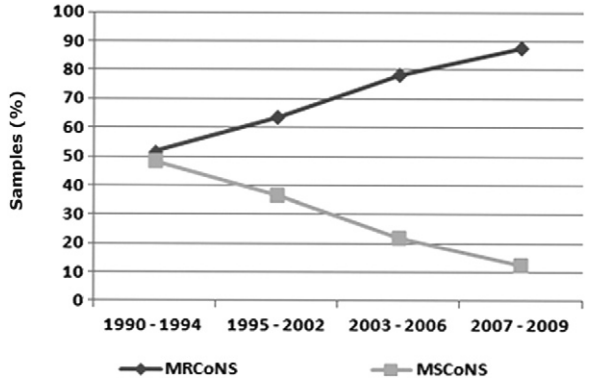

Fig. 1: evolution of oxacillin resistance in coagulase-negative staphylococci strains in blood cultures of newborns hospitalised at the neonatal ward of the University Hospital, Faculty of Medicine, São Paulo State University, Botucatu, state of São Paulo, Brazil, between 1991-2009. MRCoNS: methicillin-resistant coagulase-negative staphylococci; MSCoNS: methicillin-susceptible coagulase-negative staphylococci.

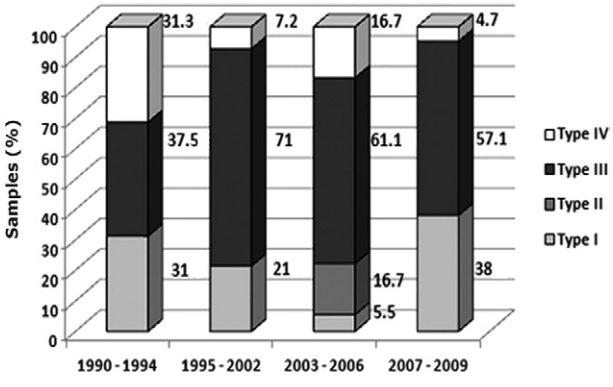

Fig. 2: staphylococcal cassette chromosome *mec* type staphylococci strains in blood cultures of newborns hospitalised at the neonatal ward of the University Hospital, Faculty of Medicine, São Paulo State University, Botucatu, state of São Paulo, Brazil, between 1991-2009. MRCoNS: methicillin-resistant coagulase-negative staphylococci; MSCoNS: methicillin-susceptible coagulase-negative staphylococci.

transfer between strains (Healy et al. 2004), suggesting that diseases caused by clones that carry SCC*mec* type IV will tend to increase (Machado et al. 2007). All strains carrying SCC*mec* type IV were identified as *S. epidermidis* and this type was detected in 15% of oxacillin-resistant strains. Types I-III were also found in *S. epidermidis* at frequencies of 16.7%, 3.3% and 63.3%, respectively. Similar results regarding the distribution of SCC*mec* among *S. epidermidis* strains have been reported by Machado et al. (2007) with types I and IV found in 19% and 0.8%, respectively, whereas the percentage of type III was lower (41.8%). These authors did not detect type II in the *S. epidermidis* strains studied. The oxacillin-resistant *S. haemolyticus* strains carried SCC*mec* type I (83.3%) and type II (16.7%). SCC*mec* type I was detected in *S. hominis* strains and SCC*mec* type III was found in *S. lugdunensis*. These types are characteristic of nosocomial MRCoNS.

Analysis of the present data revealed an increase in the percentage of CoNS strains isolated from blood cultures during the period from 1991-2009. Furthermore, the results identified a predominant SCC*mec* profile of the oxacillin-resistant CoNS strains isolated from the NICU. There was a prevalence of SCC*mec* types found in hospital-acquired strains. SCC*mec* types I, III and IV were detected in MRCoNS isolates throughout the period studied and their proportion showed little variation, whereas type II was only detected in strains isolated between 2003-2006. The main limiting factor of the study is the uncertainty as to whether the isolates represent true infections, as no clinical data are presented. A certain percentage of isolates most likely represent skin contamination. Nevertheless, the percentage of MRCoNS strains is elevated and represents a matter of concern in terms of infection control practices in the neonatal ward of the HC-FMB.

REFERENCES

Archer G, Niemeyer DM 1994. Origin and evolution of DNA associated with resistance to methicillin in staphylococci. *Trends Microbiol 2*: 343-347.

Barreto MF, Picoli SU 2008. *Staphylococcus* em um hospital de Porto Alegre (RS). *Rev Bras Anal Clin 40*: 285-287.

Chaves F, Alvarez MG, Sanz F, Alba C, Otero JR 2005. Nosocomial spread of a *Staphylococcus hominis* subsp. *novobiosepticus* strain causing sepsis in a neonatal intensive care unit. *J Clin Microbiol 43*: 4877-4879.

CLSI - Clinical and Laboratory Standards Institute 2009. *Performance standards for antimicrobial susceptibility testing*, 19th informational supplement, CLSI, Wayne, 10 pp.

Couto I, Pereira S, Miragaia M, Sanches IS, Lencastre H 2001. Identification of clinical staphylococcal isolates from humans by internal transcribed spacer PCR. *Clin Microbiol 39*: 3099-3103.

Critchley IA, Blosser-Middleton RS, Jones ME 2003. Baseline study to determine in vitro activities of daptomycin against Gram-positive pathogens isolated in the United States in 2000-2001. *Antimicrob Agents Chemother 47*: 1689-1693.

Cunha MLRS, Sinzato YK, Silveira LVA 2004. Comparison of methods for the identification of coagulase-negative staphylococci. *Mem Inst Oswaldo Cruz 99*: 855-860.

Ferreira RBR, Iorio NLP, Malvar KL, Nunes APF, Fonseca LS, Bastos CCR, Santos KNR 2003. Coagulase-negative staphylococci: comparison of phenotypic and genotypic oxacillin susceptibility tests and evaluation of the agar screening test by using different concentrations of oxacillin. *J Clin Microbiol 41*: 3609-3614.

Healy CM, Hulten KG, Palazzi DL, Cambell JR, Baker CJ 2004. Emergence of new strains of methicillin-resistant *Staphylococcus aureus* in a neonatal intensive care unit. *Clin Infect Dis 39*: 1460-1466.

Hira V, Sluijter M, Estevao S, Horst-Kreft D, Ott A, Groot R, Hermans PW, Kornelisse RF 2007. Clinical and molecular epidemiologic characteristics of coagulase-negative staphylococcal bloodstream infections in intensive care neonates. *Pediatr Infect Dis J 26*: 607-612.

Ito T, Katayama Y, Asada K, Mori N 2001. Structural comparison of three types of staphylococcal cassette chromosome *mec* integrated in the chromosome in methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother 45*: 1323-1336.

Ito T, Katayama Y, Hiramatsu K 1999. Cloning and nucleotide sequence determination of the entire *mec* DNA of pre-methicillin-resistant *Staphylococcus aureus* N315. *Antimicrob Agents Chemother 43*: 1449-1458.

IWG-SCC - International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements 2009. Classification of staphylococcal cassette chromosome *mec* (SCC*mec*): guidelines for reporting novel SCC*mec* elements. *Antimicrob Agents Chemother 53*: 4961-4967.

Kawaguchi E, Minamide W, Mori H, Igmi H 1996. The taxonomic distribution, characteristic and susceptibility against antimicrobial agents of methicillin-resistant staphylococci isolated from blood. *Kansenshogaku Zasshi 70*: 1147-1153.

Kloos WE, Bannerman TL 1994. Update on clinical significance of coagulase-negative staphylococci. *Clin Microbiol Rev 7*: 117-140.

Krediet TG, Mascini EM, Rooij EV, Vlooswijk J, Paauw A, Gerards LJ, Fleer A 2004. Molecular epidemiology of coagulase-negative staphylococci causing sepsis in a neonatal intensive care unit over an 11-year period. *J Clin Microbiol 42*: 992-995.

Kuehnert MJ, Kruszon-Moran D, Hill HA, McQuillan G, McAllister K, Fosheim G, McDougal LK, Chaitram J, Jensen B, Fridkin SK, Killgore G, Tenover FC 2006. Prevalence of *Staphylococcus aureus* nasal colonization in the United States, 2001-2002. *J Infect Dis 193*: 172-179.

Maayan-Metzger A, Linder N, Marom D, Vishne T, Ashkenazi S, Sirota L 2000. Clinical and laboratory impact of coagulase-negative staphylococci bacteremia in preterm infants. *Acta Paediatr 89*: 690-693.

Machado ABMP, Reiter KC, Paiva RM, Barth AL 2007. Distribution of staphylococcal cassette chromosome *mec* (SCC*mec*) types I, II, III and IV in coagulase-negative staphylococci from patients attending a tertiary hospital in southern Brazil. *J Med Microbiol 56*: 1328-1333.

Murakami K, Minamide K, Wada K, Nakamura E, Teraoka H, Watanabe S 1991. Identification of methicillin-resistant strains of staphylococci by polymerase chain reaction. *J Clin Microbiol 29*: 2240-2244.

Oliveira DC, Lencastre H 2002. Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother 46*: 2155-2161.

Otto M 2009. *Staphylococcus epidermidis*-the 'accidental' pathogen. *Nat Rev Microbiol 7*: 556-567.

Tee WSN, Soh SY, Lin R, Loo LH 2003. *Staphylococcus lugdunensis* carrying the *mecA* gene causes catheter-associated bloodstream infection in premature neonate. *J Clin Microbiol 41*: 519-520.