JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

High rate of neonates colonized by methicillin-resistant *Staphylococcus* species in an Intensive Care Unit

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Abstract

Introduction: Staphylococcal colonization is a risk factor for healthcare-associated infections, which are frequent in Neonatal Intensive Care Units (NICU). This study analyzed microbiology, epidemiology and clinical aspects of *Staphylococcus* spp. colonizing neonates.

Methodology: Nasal or periumbilical swabs were evaluated from 175 newborns admitted to a NICU of a Rio de Janeiro hospital from March to September 2009. Clinical data were obtained from the medical records. SCC*mec* typing and the *mecA* and Panton-Valentine Leukocidin (PVL) genes were detected by PCR. Clonal diversity was evaluated by pulsed-field gel electrophoresis.

Results: *Staphylococcus* spp. isolates were detected in 98 (56%) neonates, 66.3% of them had birth weight ≤ 2500 g, 62.2% were preterm (< 37 weeks) and the mean length of hospitalization was 14.9 days. Among the 133 isolates identified, 48.1% were *S. epidermidis,* 23.3% *S. haemolyticus* and 13.5% *S. aureus*. Methicillin-resistant *Staphylococcus* isolate was detected in 77.6% of neonates. The methicillin-resistant *S. aureus* isolates carried the SCC*mec* type IV, while 94.6% of *S. epidermidis* and 85.7% of *S. haemolyticus* presented non-typeable cassettes. Among the *S. aureus*, 55.6% had PVL genes and the USA800 genotype was prevalent. Two genotypes of *S. epidermidis* and one of *S. haemolyticus* clustered 42.2% and 25.8% of the isolates, respectively. *S haemolyticus* colonization was associated with the use of parenteral nutrition and mechanical ventilation.

Conclusion: High rate of neonates colonized by methicillin-resistant *Staphylococcus* species and the permanence of clones circulating in the NICU highlight the importance for continuous and preventive surveillance in this high-risk population.

Key words: *Staphylococcus* species; colonization; neonates; multiresistance; clonal diversity; clinical aspects.

J Infect Dev Ctries 2019; 13(9):810-816. doi:10.3855/jidc.11241

(Received 15 January 2019 – Accepted 18 May 2019)

Introduction

Neonates admitted to neonatal intensive care units (NICU) are susceptible to health care associated infections (HCAI) due to low birth weight, poor skin integrity, immature immune system and frequent use of invasive devices [1]. *Staphylococcus aureus* is the etiologic agent of 10% to 25% of HCAI in NICUs [1-4], while methicillin-resistant *S. aureus* (MRSA) isolates are responsible of 2 to 5% of these infections [5,6] and are associated with significant morbidity and financial burden [7]. Another disturbing factor is the presence of the Panton-Valentine leukocidin (PVL), a pore-forming toxin associated to a wide range of infections [8] that could exacerbate the prognosis of infected neonates.

Coagulase Negative *Staphylococcus* (CoNS) isolates are normally skin and mucosal surface colonizers. However, their access to deep tissues, usually through indwelling devices, can promote an opportunistic infection [1]. They are responsible for 10% to 40% of the bloodstream infections (BSI) [1-3,6], and a high mortality rate of 46% was already associated to infections by CoNS in an intensive care unit [3]. Among neonates, *S. aureus* and CoNS are responsible for 18% and 54% of late onset sepsis, respectively [9], and *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* are the most frequently isolated CoNS species [10].

Staphylococcus colonization is known to be an important and independent risk factor for infection, mainly by multiresistant isolates [11], and, therefore,

efforts to reduce these pathogens remains a high priority in NICUs. In this study, we characterized *Staphylococcus* spp. isolates colonizing patients in a Neonatal Intensive Care Unit in Rio de Janeiro in order to verify which species of this genus are circulating in the unit, their antimicrobial susceptibility profile, clonal diversity and associated clinical aspects.

Methodology

Patients and specimen collection

The study was conducted at the NICU of a public hospital in Rio de Janeiro from March to September 2009, under Ethics and Research Committee number 372A/2007. This NICU has five ICU beds and 15 intermediate unit beds, located in the same physical space. All neonates consecutively admitted with up to 28 days old were included. Patients who were discharged before the swab collection, born in other units or from home birth were excluded. Clinical data were collected from the patients charts and included: date of birth, weight at birth, gestational age, length of hospitalization and underlying conditions. The Infection Control Commission staff collected one nasal or periumbilical swab per neonate. Periumbilical swabs were obtained from neonates with no access to the nasal site. The specimens were immediately inoculated onto mannitol salt agar and incubated at 37°C for 24 hours.

Identification of isolates

All isolates were initially analyzed and identified by a simplified phenotypic test [12]. *S. aureus, S. epidermidis* and *S. haemolyticus* species were additionally confirmed by a multiplex PCR test [13]. Mass spectrometry technology using the Matrix Assisted Laser Desorption Technology/Ionization Time of Flight (MALDI-TOF) technique (Bruker Daltonics, Billerica, MA, USA) was used to confirm CoNS species. Runs were carried out using the FlexControl software in the default mode and identification scores were generated with the Biotyper version 3.1, considering 2.0 as the cutoff score.

Detection of PVL and mecA genes and SCCmec typing

After bacterial DNA extraction [14], the detection of the *S. aureus pvl* genes was performed as previously described [15], as well as the *mecA* gene detection [13] and SCC*mec* typing [16]. *S. aureus* strains were used as positive controls: Mu50 (SCC*mec* II) [17], 63a (SCC*mec* III) [18], and 526a (SCC*mec* IV and *pvl*-positive) [19]. *S. lugdunensis* strain (468s) was used as positive control of SCC*mec* V [20].

Clonal relatedness

Clonal relatedness of the isolates was evaluated by pulsed field gel electrophoresis (PFGE) [18]. Bacterial DNA was extracted and digested with 20 U of *Sma*I enzyme (New England Biolabs, Rowley, MA, USA). The restriction fragments were separated using a BioRad CHEF DR III apparatus. The PFGE profiles were analyzed with Bio-Numerics 6.0 software (Applied Maths, Biomérieux, Sint-Martens-Latem, Belgium). Similarity percentage was identified on a dendrogram derived from the unweighted pair group method using arithmetic averages and based on Dice coefficients. Isolates showing a similarity coefficient < 80% or differences of five or more bands were considered genetically unrelated.

Statistical methods

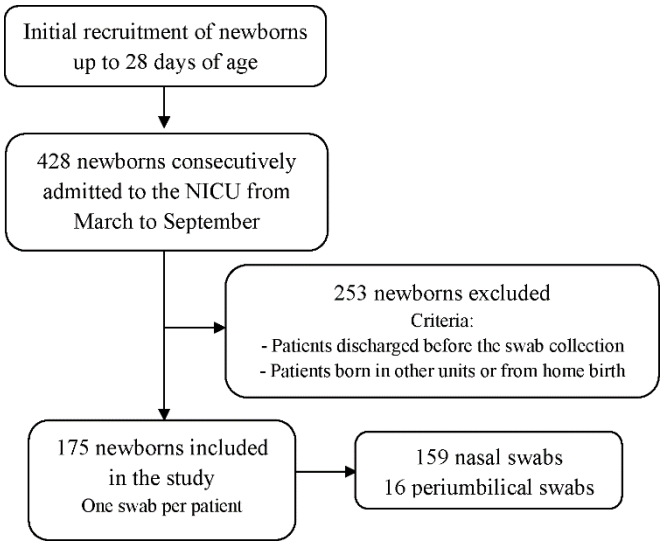
Statistical analysis was performed in order to detect possible associations between the clinical data and *Staphylococcus* species, prevalent clones, PVL genes carriage, methicillin-resistance and SCC*mec* types. The Fischer's exact test was used and differences with $p < 0.05$ values were considered statistically significant.

Results

Clinical data and Staphylococcus spp. identification

During the study, 428 newborns were admitted to the NICU, and 253 (59.1%) were excluded (Figure 1). One hundred seventy-five swabs were obtained (159 nasal and 16 periumbilical), and *Staphylococcus* species were isolated from 98 (56%) of them. Sixty-six (67.3%) neonates presented a single staphylococcal species colonizing, while two or three species were

Figure 1. Flowchart of the patients selected to the study.



found in 32 (32.7%) neonates (Table 1). Among 98 colonized neonates, 66.3% had birth weight ≤ 2500g, 62.2% were preterm (< 37 weeks) and 68.4% presented respiratory distress or neonatal sepsis. The mean length of hospitalization was 14.9 days. About 84% of the positive swabs for *Staphylococcus* spp. were collected in the first ten days of hospitalization and the median time was 4, 5 and 7 days for *S. aureus*, *S. haemolyticus* and *S. epidermidis*, respectively. Twelve (66.7%) *S. aureus* isolates were detected in the first five days of hospitalization. There was no relation between birth weight, gestational age, underlying conditions and the number or species of *Staphylococcus* identified. Statistical association was found between colonization by *S. haemolyticus* and use of parenteral nutrition (p = 0.04).

A total of 133 isolates were identified: *S. epidermidis* (64 isolates; 48.1%), *S. haemolyticus* (31; 23.3%) and *S. aureus* (18; 13.5%) were the most frequent species (Table 2). Another five CoNS species were detected from 20 samples, 70% of them were classified as *S. capitis* subsp. *capitis* or *S. hominis* subsp. *hominis*. *S. epidermidis* was the most frequent species found colonizing as a single microorganism (35.7%).

Detection of mecA and PVL genes and SCCmec typing

Seventy-six (77.6%) neonates had at least one methicillin-resistant *Staphylococcus* species. The *mecA* gene was detected in 71.4% of the isolates (Table 2). *S. haemolyticus* (90.3%) and *S. epidermidis* (87.5%) presented the highest rates of methicillin-resistance, while two *S. aureus* isolates carried the *mecA* gene. The MRSA isolates had SCC*mec* type IV, while 94.6% and 85.7% of the *S. epidermidis* and *S. haemolyticus* isolates, respectively, were classified as non-typeable (NT). All eight *S. capitis* subsp. *capitis* isolates had the *mecA* gene and the SCC*mec* IV, while most of the other CoNS were sensitive to methicillin. Among the 18 *S. aureus* isolates, 55.6% harbored *pvl* genes, including the two MRSA type IV isolates. No statistical association was found between the presence of PVL genes or methicillin resistance with clinical data of the patients.

Clonal profile of the prevalent Staphylococcus spp. species

The three most frequent species were evaluated by PFGE, and for 18 *S. aureus* isolates, eight clones (A'-H') were detected, being 44.4% of them identified as Pediatric clone or USA800 (Figure 2). This clone was found in almost every month of the study and 62.5%

Table 1. Distribution of *Staphylococcus* species identified colonizing 98 neonates admitted in the NICU of a public hospital in Rio de Janeiro according to the clinical data.

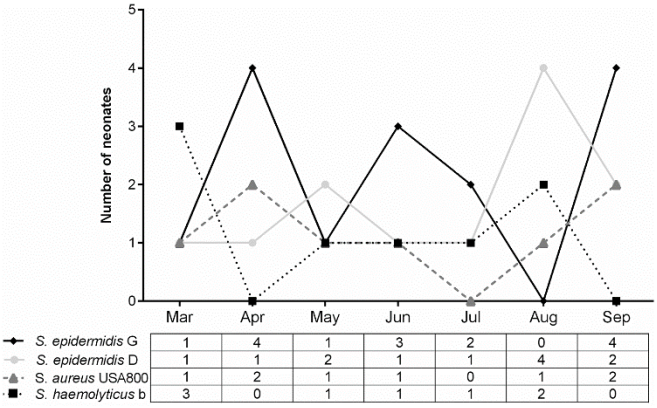
Clinical data	Number of neonates colonized by *Staphylococcus* spp (N of *Staphylococcus aureus*[*])			
	One species (66/67.3%)	Two species (29/29.6%)	Three species (3/3.1%)	Total/% (98/100%)
Birth weight (grams)				
< 1000	6 (1)	1	0	7/7.1
1000 - 1499	17	6	2 (1)	25/25.5
1500 - 2500	21 (5)	12 (4)	0	33/33.7
> 2500	20 (3)	9 (3)	1 (1)	30/30.6
ND	2	1	0	3/3.1
Gestational age (weeks)				
< 30	4	0	0	4/4.1
30 – 36	41 (5)	15 (4)	1 (1)	57/58.1
≥ 37	16 (4)	12 (2)	1	29/29.6
ND	5	2 (1)	1 (1)	8/8.2
Underlying conditions				
Respiratory distress	25 (5)	18 (3)	0	43/43.9
Sepsis	19 (2)	3 (1)	2 (1)	24/24.5
Other[**]	10	4 (2)	0	14/14.3
ND	12 (2)	4 (1)	1 (1)	17/17.3
Length of hospitalization (days)				
1 – 10	28 (7)	15 (5)	1 (1)	44/45
11 – 20	21 (1)	10 (1)	0	31/31.6
> 20	13 (1)	2 (1)	2 (1)	17/17.3
ND	4	2	0	6/6.1

[*]Data of the *Staphylococcus aureus* species was highlighted in parentheses; [**]Other conditions: congenital heart disease, metabolic distress; N: number; ND: no data available.

(5/8 isolates) of them were positive for the *pvl*-genes. Genotype B', identified as the Oceania clone was the second most frequent, and among four isolates detected, two were *pvl*-positive isolates.

The 64 *S. epidermidis* isolates were distributed into 18 clones (A - R), and the most frequent G and D genotypes comprised 42,2% of the isolates, and colonized 15 and 12 neonates, respectively (Figure 2). These two genotypes persisted throughout the months of the study, and all these isolates were methicillin-resistant. Among 31 *S. haemolyticus* isolates 14 clones (a - n) were detected, with the genotype b as the most frequent and persistent, grouping 8 (25.8%) isolates. The *S. epidermidis* clone D was more frequent in newborns with some underlying disease ($p = 0.04$) or when they were born by cesarean section ($p = 0.0004$). Colonization by *S. haemolyticus* clone b isolates was associated with the use of mechanical ventilation ($p = 0.02$).

Figure 2. Distribution of the prevalent *Staphylococcus* species clones in the NICU of a public hospital in Rio de Janeiro. The values represent the number of isolates recovered per month.



Discussion

The association between staphylococcal colonization and the development of infection is evident, especially among neonates [4,21,22], justifying the characterization of *Staphylococcus* species colonizing neonates in a NICU of Rio de Janeiro. In this study, the majority of neonates were preterm and had birth weights ≤ 2500g. These facts may be the reflection that the study encompasses children of a low-income population in a hospital in a developing country. Fifty-six percent of the neonates evaluated were colonized by *Staphylococcus* species. An overall prevalence of 50.9% and 10.3% were detected for CoNS species and *S. aureus*, respectively, consistent with other studies developed in Brazilian NICUs [23,24]. We did not identify any relation between clinical data and number or species of *Staphylococcus* isolated. However, in our study, *S. haemolyticus* colonization was associated with the use of parenteral nutrition, and specifically the clone b was found be related with use of mechanical ventilation. Brito and colleagues reported the use of central venous catheters, mechanical ventilation and total parenteral nutrition, as significant high-risk factors for acquisition of HCAI in a Brazilian NICU [2]. It should be noted that neonates are very vulnerable and frequently handled by health professionals. In addition, the detection of CoNS species colonizing neonates is common [23] and, the same pattern of *Staphylococcus* species is found as cause of bloodstream infections [10], highlighting the importance of the colonization as risk factor in the development of infections by *Staphylococcus* species.

Among 98 neonates colonized 77.6% had at least one methicillin-resistant *Staphylococcus* species. Despite the low prevalence of MRSA isolates in our study (2%), similar to that observed by other authors [11,24], the majority of the *S. aureus* isolates (55.6%) harbored *pvl* genes. Such a high frequency was previously reported by us, however in a study with pediatric outpatients [25]. Panton-Valentine leukocidin

Table 2. *Staphylococcus* species identified and presence of *mecA* gene and SCC*mec* type among 133 colonization isolates from neonates admitted in the NICU.

***Staphylococcus* species**	**N (%) of isolates (N = 133)**	**N (%) of *mecA* positive isolates (N = 95)**	**SCC*mec* type (% of isolates)**
Staphylococcus epidermidis	64 (48.1)	56 (87.5)	V (5.4); NT (94.6)
Staphylococcus haemolyticus	31 (23.3)	28 (90.3)	V (14.3); NT (85.7)
Staphylococcus aureus	18 (13.5)	2 (11.1)	IV (100)
Staphylococcus capitis subsp. *capitis*	8 (6)	8 (100)	V (100)
Staphylococcus hominis subsp. *hominis*	6 (4.5)	1 (16.7)	NT (100)
Staphylococcus lugdunensis	2 (1.5)	0	na
Staphylococcus warneri	2 (1.5)	0	na
Staphylococcus saprophyticus subsp. *saprophyticus*	1 (0.8)	0	na
Staphylococcus caprae	1 (0.8)	0	na

N: number; NT: non-typeable; na: not applicable; subsp.: sub-species.

is one of the major virulence factors in *S. aureus* and its leukotoxic action is responsible for high mortality rates associated with necrotizing pneumonia [8]. To the best of our knowledge, this is the first report of this alarming PVL carriage rate in a Brazilian NICU.

Among the CoNS, 87.5% and 90.3% of the *S. epidermidis* and *S. haemolyticus* isolates harbored the *mecA* gene, respectively. Ternes and coworkers found 60% of *mecA* gene positive CoNS isolates colonizing neonates in a Brazilian NICU, increasing to 83.6% among isolates taken during discharged [23]. High rates of resistance among CoNS is probably the result of the selective pressure exerted by the hospital environment. Moreover, CoNS have been the major reservoirs of resistant genes and their ability to transfer them to *S. aureus* may influence therapy for patients as vulnerable as neonates. Unlike Ternes *et al*., who reported SCC*mec* types I, II, and III as prevalent among nasal CoNS isolates [24], we detect the type V among the CoNS isolates, demonstrating a striking difference for the SCC*mec* carriage between regions of the same country. Furthermore, 91.7% of our methicillin-resistant isolates were non-typeable, reinforcing that CoNS isolates present high variability in their SCC*mec* structure conformation. Some studies have reported the emergence of *S. capitis* as cause of HCAIs, especially the SCC*mec* V NRCS-A clone with reduced susceptibility to antimicrobials [26], highlighting the emergence of this staphylococcal species, also detected among 8.2% of the neonates colonized in our study.

For all *Staphylococcus* isolates the median time for patient colonization ranged from 4 to 7 days, reflecting a rapid colonization by these microorganisms in the study NICU. According to Ghirardi and coworkers, once multiresistant organisms are introduced into a healthcare setting, some aspects that allow their transmission and persistence are the availability of vulnerable patients, antimicrobials selective pressure and the potential of intra hospital transmission [27]. We identified staphylococcal clones that persisted in the NICU of this study. Worldwide reports for *S. aureus* isolates show the presence of persistent clones, such as the Brazilian endemic clone (BEC)/ST239 in Brazilian hospitals [18,24], and USA300/ST8 in USA [21]. Unlike another Brazilian NICU report that found BEC as the prevalent clone among nasal isolates [24], the Pediatric clone was the most frequent lineage in our study. It is evident that *S. aureus* epidemiology is changing worldwide, as we have also seen in Rio de Janeiro hospitals [28], with the substitution of BEC by other MRSA lineages. Among the CoNS, we identified three prevalent clones (two of *S. epidermidis* and one of *S. haemolyticus*) disseminated and persistent in this NICU during the study period. The presence of persistent clones of *Staphylococcus* species colonizing neonates and their association with subsequent infection has already been reported [29]. It is important to highlight that the *S. epidermidis* clone D and *S. haemolyticus* clone b were more frequent in neonates presenting some underlying disease and need for mechanical ventilation, respectively. Klingenberg *et al*. identified *Staphylococcus* endemic clones more frequently in very pre-term infants, probably as a result of frequent therapeutic handling, enhancing the risk of cross transmission within the NICU [29]. Therefore, we believe that frequent control practices should be implemented especially in NICUs where persistent *Staphylococcus* clones have been identified and are associated with relevant clinical aspects.

Our study had limitations since only one swab per patient was collected and it was performed on days established by the surveillance survey staff. Thus, we could not standardize or systematize the sampling, which may have influenced our results. Moreover, the comparison with groups of patients without *Staphylococcus* colonization could allow us to better understand about the dynamics of the colonization by staphylococci.

Conclusion

In conclusion, there was a high rate of colonization by methicillin-resistant *Staphylococcus* species in neonates and the presence of prevalent clones circulating in the NICU during the study period associated with relevant clinical aspects. Therefore, this study highlights the importance for continuous surveillance and use of preventive measures against the acquisition of *Staphylococcus* species in high-risk population.

Acknowledgements

This work was supported by Brazilian grants from Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro, Conselho Nacional de Desenvolvimento Científico e Tecnológico, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) – Finance Code 001

References

1. Polin RA, Denson S, Brady MT; Committee on Fetus and Newborn; Committee on Infectious Diseases (2012) Epidemiology and diagnosis of health care-associated infections in the NICU. Pediatrics 129: e1104-9.
2. Brito DV, Brito CS, Resende DS, Moreira do Ó J, Abdallah VO, Gontijo Filho PP (2010) Nosocomial infections in a Brazilian neonatal intensive care unit: a 4-year surveillance study. Rev Soc Bras Med Trop 43: 633-637.
3. Marra AR, Camargo LF, Pignatari AC, Sukiennik T, Behar PR, Medeiros EA, Ribeiro J, Girão E, Correa L, Guerra C, Brites C, Pereira CA, Carneiro I, Reis M, de Souza MA, Tranchesi R, Barata CU, Edmond MB; Brazilian SCOPE Study Group (2011) Nosocomial bloodstream infections in Brazilian hospitals: analysis of 2,563 cases from a prospective nationwide surveillance study. Microbiol 49: 1866-1871.
4. Reich PJ, Boyle MG, Hogan PG, Johnson AJ, Wallace MA, Elward AM, Warner BB, Burnham CA, Fritz SA (2016) Emergence of community-associated methicillin-resistant *Staphylococcus aureus* strains in the neonatal intensive care unit: an infection prevention and patient safety challenge. Clin Microbiol Infect 22: 645.e1-8.
5. Chen YC, Lin CF, Rehn YF, Chen JC, Chen PY, Chen CH, Wang TM, Huang FL (2017) Reduced nosocomial infection rate in a neonatal intensive care unit during a 4-year surveillance period. J Chin Med Assoc 80: 427-431.
6. Mohsen L, Ramy N, Saied D, Akmal D, Salama N, Abdel Haleim MM, Aly H (2017) Emerging antimicrobial resistance in early and late-onset neonatal sepsis. Antimicrob Resist Infect Control 6: 63.
7. Song X, Perencevich E, Campos J, Short BL, Singh N (2010) Clinical and economic impact of methicillin-resistant *Staphylococcus aureus* colonization or infection on neonates in intensive care units. Infect Control Hosp Epidemiol 31: 177-182.
8. Boan P, Tan HL, Pearson J, Coombs G, Heath CH, Robinson JO (2015) Epidemiological, clinical, outcome and antibiotic susceptibility differences between PVL positive and PVL negative *Staphylococcus aureus* infections in Western Australia: a case control study. BMC Infect Dis 9; 15:10.
9. Vergnano S, Menson E, Kennea N, Embleton N, Russell AB, Watts T, Robinson MJ, Collinson A, Heath PT (2011) Neonatal infections in England: the NeonIN surveillance network. Arch Dis Child Fetal Neonatal Ed 96: F9-F14.
10. Salgueiro VC, Azevedo MB, Iorio NL, Amorim E de L, dos Santos KR (2014) Staphylococcal cassette chromosome *mec* elements in methicillin-resistant coagulase-negative staphylococci from a Brazilian neonatal care unit. Pediatr Infect Dis J 33: 1089-1090.
11. Zervou FN, Zacharioudakis IM, Ziakas PD, Mylonakis E (2014) MRSA colonization and risk of infection in the neonatal and pediatric ICU: a meta-analysis. Pediatrics 133 :e1015-1023.
12. Iorio NL, Ferreira RB, Schuenck RP, Malvar KL, Brilhante AP, Nunes AP, Bastos CC, Dos Santos KR (2007) Simplified and reliable scheme for species-level identification of *Staphylococcus* clinical isolates. J Clin Microbiol 45: 2564–2569.
13. Pereira EM, Schuenck RP, Malvar KL, Iorio NL, Olendzki AN, Oelemann WM, dos Santos KR (2010) *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Staphylococcus haemolyticus*: methicillin-resistant isolates are detected directly in blood cultures by multiplex PCR. Microbiol Res 165: 243-249
14. Pitcher DG, Sauders NA, Owen RJ (1989) Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. Lett Appl Microbiol 8: 1551–1556.
15. Lina G, Piémont Y, Godail-Gamot F, Bes M, Peter MO, Gauduchon V, Vandenesch F, Etienne J (1999) Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clin Infect Dis 29: 1128-1132.
16. Kondo Y, Ito T, Ma XX, Watanabe S, Kreiswirth BN, Etienne J, Hiramatsu K (2007) Combination of multiplex PCRs for staphylococcal cassette chromosome *mec* type assignment: rapid identification system for *mec*, *ccr*, and major differences in junkyard regions. Antimicrob Agents Chemother 51: 264-274.
17. Hiramatsu K, Hanaki H, Ino T, Yabuta K, Oguri T, Tenover FC (1997) Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. J Antimicrob Chemother 40: 135-136.
18. Vivoni AM, Diep BA, de Gouveia Magalhães AC, Santos KR, Riley LW, Sensabaugh GF, Moreira BM (2006) Clonal composition of *Staphylococcus aureus* isolates at a Brazilian university hospital: identification of international circulating lineages. J Clin Microbiol 44: 1686-1691.
19. Schuenck RP, Nouér SA, Winter C de O, Cavalcante FS, Scotti TD, Ferreira AL, Giambiagi de Marval M, dos Santos KR (2009) Polyclonal presence of non-multiresistant methicillin-resistant *Staphylococcus aureus* isolates carrying SCC*mec* IV in health care-associated infections in a hospital in Rio de Janeiro, Brazil. Diagn Microbiol Infect Dis 64: 434-441.
20. Pereira EM, Schuenck RP, Nouér SA, Santos KR (2011) Methicillin-resistant *Staphylococcus lugdunensis* carrying SCC*mec* type V misidentified as MRSA. Braz J Infect Dis 15: 293-295.
21. Popoola VO, Budd A, Wittig SM, Ross T, Aucott SW, Perl TM, Carroll KC, Milstone AM (2014) Methicillin-resistant *Staphylococcus aureus* transmission and infections in a neonatal intensive care unit despite active surveillance cultures and decolonization: challenges for infection prevention. Infect Control Hosp Epidemiol 35: 412-418.
22. Simon A, Dresbach T, Müller A (2018) Methicillin-resistant *Staphylococcus aureus* Decolonization in Neonates and Children. Pediatr Infect Dis J 37: 612-614.
23. Ternes YM, Lamaro-Cardoso J, André MC, Pessoa VP Jr, Vieira MA, Minamisava R, Andrade AL, Kipnis A (2013) Molecular epidemiology of coagulase-negative *Staphylococcus* carriage in neonates admitted to an intensive care unit in Brazil. BMC Infect Dis 13: 572.
24. Vieira MA, Minamisava R, Pessoa-Júnior V, Lamaro-Cardoso J, Ternes YM, Andre MC, Sgambatti S, Kipnis A, Andrade AL (2014) Methicillin-resistant *Staphylococcus aureus* nasal carriage in neonates and children attending a pediatric outpatient clinics in Brazil. Braz J Infect Dis 18: 42-47.
25. Cavalcante FS, Abad ED, Lyra YC, Saintive SB, Ribeiro M, Ferreira DC, Santos KR (2015) High prevalence of methicillin resistance and PVL genes among *Staphylococcus aureus* isolates from the nares and skin lesions of pediatric patients with atopic dermatitis. Braz J Med Biol Res 48: 588-594.
26. Butin M, Rasigade JP, Martins-Simões P, Meugnier H, Lemriss H, Goering RV, Kearns A, Deighton MA, Denis O, Ibrahimi A, Claris O, Vandenesch F, Picaud JC, Laurent F (2016) Wide geographical dissemination of the multiresistant

Staphylococcus capitis NRCS-A clone in neonatal intensive-care units. Clin Microbiol Infect 22: 46-52.
27. Ghirardi B, Pietrasanta C, Ciuffini F, Manca MF, Ucella S, Lavizzari A, Pugni L, Mosca F (2013) Management of outbreaks of nosocomial pathogens in neonatal intensive care unit. Pediatr Med Chir 35: 263-268.
28. Chamon RC, Ribeiro SD, da Costa TM, Nouér AS, Dos Santos KR (2017) Complete substitution of the Brazilian endemic clone by other methicillin-resistant *Staphylococcus aureus* lineages in two public hospitals in Rio de Janeiro, Brazil. Braz Infect Dis 21: 185-189.
29. Klingenberg C, Rønnestad A, Anderson AS, Abrahamsen TG, Zorman J, Villaruz A, Flaegstad T, Otto M, Sollid J (2007) Persistent strains of coagulase-negative staphylococci in a neonatal intensive care unit: virulence factors and invasiveness. Clin Microbiol Infect 13: 1100–1111.

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Conflict of interests: No conflict of interests is declared.