Molecular epidemiology of coagulase-negative *Staphylococcus* carriage in neonates admitted to an intensive care unit in Brazil

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Abstract

Background

Nasal colonization with coagulase-negative *Staphylococcus* (CoNS) has been described as a risk factor for subsequent systemic infection. In this study, we aimed to evaluate the profile of CoNS isolates colonizing the nares of children admitted to a neonatal intensive care unit (NICU) of a major city located in central Brazil.

Methods

We assessed CoNS carriage at admittance and discharge among newborns admitted to an NICU from July 2007 through May 2008 in one of the major municipalities of Brazil. Isolates were screened on mannitol salt agar and tryptic soy broth and tested
for susceptibility to antimicrobials using the disc diffusion method. Polymerase chain
reaction (PCR) was used to determine the species, to assay for the presence of the
mecA gene, and to perform SCCmec typing. *S. epidermidis* and *S. haemolyticus*
isolated from the same child at both admission and discharge were characterized by
PFGE.

**Results**

Among 429 neonates admitted to the NICU, 392 (91.4%) had nasal swabs collected at
both admission and discharge. The incidence of CoNS during the hospitalization
period was 55.9\% (95\% confidence interval [CI]: 50.9-60.7). The most frequently
isolated species were *S. haemolyticus* (37.6\%) and *S. epidermidis* (34.6\%). Multidrug
resistance (MDR) was detected in 29.9\% of the CoNS isolates at discharge. The mecA
gene was more prevalent among strains isolated at discharge (83.6\%) than those
isolated at admission (60\%), and overall, SCCmec type I was isolated most
frequently. The length of hospitalization was associated with colonization by MDR
isolates. Great genetic diversity was observed among *S. epidermidis* and *S. haemolyticus*.

**Conclusions**

In conclusion, the NICU represents a risk factor for colonization by MDR CoNS.
Neonates admitted to the NICU can become a reservoir of CoNS strains with the
potential to spread MDR strains into the community.

**Keywords:** coagulase-negative *Staphylococcus*, mecA, SCCmec, neonatal intensive
care units, neonates

**Background**
Coagulase-negative Staphylococcus (CoNS) are the most common bacteria associated with neonatal healthcare-associated infections (HAIs) [1]. Colonization with CoNS is a risk factor associated with infection among neonates [2]. The neonatal hospital population has several characteristics that favor the development of infections; primarily, these include the immature immune system of the neonates, the use of invasive procedures, and aggressive antibiotic therapy protocols [3, 4]. Consequently, CoNS strains play an important role in the environment of neonatal intensive care units (NICUs) [5].

A methicillin resistance rate of up to 80% has been observed among CoNS isolated from bloodstream infections in NICU patients [1]. Resistance to other antibiotics has also emerged in the last few decades [6, 7]. The main reservoirs for multidrug resistant (MDR), Staphylococcus epidermidis have been isolated from the skin of patients and healthcare staff, medical equipment, healthcare professionals’ clothing, and hospital surfaces [8]. S. epidermidis is the predominant CoNS species in infections associated with immune deficiency, catheters, and other invasive procedures [9, 10]; in most cases, infection occurs due to migration of the microorganism on the skin to the vascular catheter insertion point, which favors hematogenic dissemination and infection [4].

Infections caused by CoNS are related to a significant increase in acute neonatal mortality; in some cases, they may be associated with the development of respiratory complications [11, 12]. The identification of nasal colonization by CoNS could be useful for the prevention of future infections, as shown by others [13].

Most of the studies on CoNS nasal colonization in neonates were conducted in the 1990s [2] and showed a colonization prevalence ranging from 13% to 56% [14, 15].

A recent study investigated early colonization by CoNS in 46 preterm neonates at the
NICU and found that 55% of the neonates were colonized on days 2-3 of hospitalization [12].

In Latin America, information regarding both the characteristics of CoNS microflora in neonates as well as the relationship between colonizing and invasive CoNS isolates is scarce [16]. In this study, we assessed different species of CoNS and examined the antibiotic susceptibility profile of CoNS isolates colonizing the nares of children admitted to an NICU of a major city located in Central Brazil. Potential risk factors for CoNS colonization and the genetic relatedness among the CoNS strains isolated at admission and discharge were also evaluated.

Methods

Patient enrollment and sample collection

This prospective study was carried out from July 2007 through May 2008 in the NICU of Hospital da Criança (26 neonatal beds), located in Goiania (~1,300,000 inhabitants), one of the major cities in Brazil. All children admitted to the NICU during the study period were eligible for nasal swab collection. The study was approved by the Regional Ethical Committee of Hospital Materno Infantil (CEP-HMI #006/07), and written consent was obtained from the neonates’ parents. A nasal swab (Copan®, CA, USA) was collected from the neonates at admittance and discharge, and the swab was transported to the microbiology laboratory in Stuart’s medium.

Risk factors

Potential risk factors for CoNS colonization, which were obtained from medical records by physicians and nurses, included type of birth, sepsis occurrence, prematurity, age (days) at hospitalization, antimicrobial use during hospitalization, low birth weight, length of hospitalization (days), use of continuous positive airway pressure (CPAP), gender, and comorbidities (chronic, genetic, and infectious diseases;
fetal malformation). The criteria for sepsis definition followed the Centers for Disease Control and Prevention (CDC) guidelines [17] with local adaptations.

**Microbiological procedures**

The nasal swabs were inoculated onto mannitol salt agar and one suggestive colony from each patient was submitted to screening tests [18]. Each colony was identified by standard methods [18].

**Susceptibility tests**

CoNS isolates were submitted to a disk diffusion susceptibility test with the following antibiotics: oxacillin (1 µg), cefoxitin (30 µg), erythromycin (15 µg), clindamycin (2 µg), quinupristin-dalfopristin (15 µg), rifampicin (15 µg), ciprofloxacin (5 µg), tetracycline (30 µg), sulfamethoxazole-trimethoprim (23.75/1.25 µg), linezolid (30 µg), and penicillin (10 µg) (Oxoid®, Basingstoke, England). Inhibition halos were interpreted according to the Clinical Laboratory and Standards Institute (CLSI) guidelines [19]. The D test for macrolide-lincosamide-streptogramin B (MLSb) resistance was performed according to Fiebelkorn et al. [20]. Resistance to at least four classes of antibiotics was defined as MDR [21]. Vancomycin and teicoplanin susceptibility were not tested.

**DNA Extraction**

DNA was extracted from the isolates identified as CoNS according to the method of van Embden et al. [22].

**Identification of CoNS species**

Internal transcribed spacer (ITS) PCR was performed according to Couto et al. [22] to identify the staphylococcal species. Reference strains for *S. epidermidis* (DEN125), *S. haemolyticus* (ICE145), *S. warneri* (DEN157), *S. lugdunensis* (ICE187), *S. saprophyticus* (DEN177), *S. caprae* (COB-25), *S. cohnii* (ITL152), *S. simulans*...
AGT121), and *S. carnosus* (TAW88) were kindly provided by Dr. Hermínia de Lencastre (Laboratory of Molecular Genetics, Institute for Chemical and Biological Technology, Nova Lisboa University, Oeiras, Portugal) and used as controls. The PCR product profile of each strain was compared to those of the reference strains, and a species was assigned when a perfect match was determined [23].

**SCCmec typing**

All CoNS strains were analyzed for the presence of the *mecA* gene by PCR, as previously described by Geha et al. [24]. Staphylococcal cassette chromosome *mec* (SCCmec) typing was performed by multiplex PCR [25]. The regions identified were as follows: type I (J1 region); type II (J1 and J3 regions, *ccr* complex, and *mec* complex); type III (J1 and J3 regions and *mec* complex); type IV (J3 region and *ccr* complex); type V (J1 region and *ccr* complex); and type VI (J3 region). PCR was performed in a thermocycler (Biocycler®, Zhejiang, China), and the PCR products were resolved by electrophoresis on a 1.5% agarose gel stained with ethidium bromide. A Gel Doc XR System (Bio-Rad® Laboratories, Hercules, CA, USA) was used for visualization and analysis. Strains harboring the *mecA* gene that did not produce amplification of any SCCmec gene were assigned as nontypeable (NT).

**Multilocus Sequence Typing (MLST) of *Staphylococcus epidermidis***

Determination of MLST was performed by sequencing the PCR amplicons of the following housekeeping genes: shikimate dehydrogenase (*aroE*), carbamate kinase (*arcC*), ABC transporter (*gtr*), DNA mismatch repair protein (*mutS*), pyrimidine operon regulatory protein (*pyrR*), triphosphate isomerase (*tpiA*), and acetyl coenzyme A acetyltransferase (*yqiL*). Primers used for amplification of the seven housekeeping genes were those recommended the unified MLST scheme (http://sepidermidis.mlst.net) that employs the internal fragments of *arcC* and *aroE*
from the Wisplinghoff et al. MLST scheme [26], gtr, mutS and pyrR loci from the Thomas et al. MLST scheme [27] and sequences from the tpiA and yqiL genes used by the Wang et al. MLST scheme [28]. Sequencing of purified amplicons was performed on an ABI 3130 XL genetic analyzer (Applied Biosystems, Foster City, CA, USA). Sequences obtained were assigned allele numbers that were used to generate sequence types (STs) for each isolate.

Pulsed field gel electrophoresis (PFGE) analysis

The genetic relatedness of the most prevalent species (S. epidermidis and S. haemolyticus) was determined by PFGE according to Chung et al. [26]. Band patterns were interpreted based on the criteria described by Tenover et al. [26], and analyses were performed using BioNumerics® software version 5.0 (Applied Maths, Kortrijk, Belgium). Isolates that presented 100% similarity were considered clones, and those with genetic similarity greater than or equal to 80% were considered to belong to the same cluster [28].

Data analysis

All data were analyzed with SPSS® software (version 18.0; Chicago, USA). Neonates carrying CoNS at admission were removed from the analysis for incidence rate calculations. Univariate analyses were performed with the Chi-square and Mann-Whitney tests to detect the relative risk and respective 95% confidence intervals. A multivariate logistic regression model was used to measure the effect of the exposure variables on MDR CoNS carriage using backward stepwise elimination. The Hosmer–Lemeshow statistical test was used to determine the goodness-of-fit of the logistic regression model. P-values less than 0.05 were considered significant for all analyses.

Results
A total of 429 neonates were admitted to the NICU during the study period. Nasal swabs were collected from 392 (91.4%) neonates at both admission and discharge. The frequency of nasal carriage by CoNS was 69.9% (95% confidence interval [CI]: 65.2-74.3). The incidence of colonization by CoNS in the neonatal cohort during the hospitalization period was 55.9% (95% CI: 50.9-60.7).

The antibiotics associated with MDR were oxacillin/cefotaxim, erythromycin, clindamycin, ciprofloxacin, tetracycline, and sulfamethoxazole-trimethoprim. There was a significant increase in the frequency of MDR CoNS isolates at discharge compared with the frequency at admission. The increase in resistance to all antimicrobials tested was higher among the CoNS isolated at discharge compared with the CoNS isolated at admission. Tetracycline resistance decreased amongst the CoNS from admission to discharge. Nasal carriage of MDR CoNS was significantly associated with length of hospitalization (Fig. 1).

To evaluate the progression of drug resistance among CoNS, the susceptibility profiles of the isolates at admission and discharge were analyzed. The prevalence of β-lactam antibiotic (cefotaxim and oxacillin) resistance among 45 CoNS isolates at admission was 57.8% and increased significantly to 89.3% among 244 isolates at discharge; two neonates were colonized by CoNS with a MLSb-resistant phenotype (one at admission and one at discharge). The mecA gene was detected at frequencies of 60.0% and 83.6% at admission and at discharge, respectively. Antimicrobial susceptibility for isolates from twenty-four children was not performed due to failure to recover viable culture. The frequencies of the mecA gene and bacterial resistance among CoNS isolates were significantly higher at discharge compared with admission (Table 1).
SCCmec typing revealed similar frequencies of types I and IV at both admission and discharge. Types I, II, and III accounted for the majority of SCCmec identified in this study. Several CoNS isolates presented more than one SCCmec type (43.2% of the isolates from admission and 46.8% from discharge) (Table 2).

*S. epidermidis* (38.3%) and *S. haemolyticus* (38.0%) were the most frequent species colonizing the anterior nares of neonates followed by *S. capitis* (4.7%), *S. warneri* (4.4%), *S. kloosii* (1.4%), *S. caprae* (1.4%), *S. schleiferi* (1.1%), *S. hominis* (0.7%), *S. lugdunensis* (0.7%), and *S. saprophyticus* (0.4%). *S. epidermidis* was primarily observed at admission, whereas *S. haemolyticus* predominated at discharge. Twenty-four isolates (8.2%) showed ITS-PCR patterns that differed from those of the reference strains and could therefore not be assigned to a species.

The persistence of the same strain within a particular individual during the hospitalization period was evaluated by PFGE for *S. epidermidis* and *S. haemolyticus*, which were the most prevalent species isolated in this study. *S. epidermidis* was isolated from eight patients and *S. haemolyticus* was isolated from four patients at both admission and discharge, and the PFGE dendrogram revealed significant genetic diversity between the strains (Figs. 2 and 3). Two patients (#507 and #119) were colonized by the same *S. epidermidis* clone during their entire hospitalization period. One patient (#608) presented strains with a mixed SCCmec type (III/IV) at both admission and discharge despite the low similarity (<50%) between them. Only one patient (#584) was persistently colonized by the same *S. haemolyticus* clone, which can be explained by the short duration of hospitalization (one day). Two patients (#779-discharge, #585-admission) presented strains with different SCCmec types (III and I, respectively) despite the high similarity (>90%) between them. Among the
studied risk factors associated with MDR CoNS colonization, the length of hospitalization was the only significant variable ($p < 0.001$) (Table 3).

The molecular characterization of eight *S. epidermidis* strains representatives of different PFGE patterns by MLST identified 1 ST and 7 approximate STs (Additional files 1 and 2). One strain was assigned as ST 59, and the remaining were single-locus variants (SLV) and/or double-locus variants (DLV) of ST 59 (ST 81, 88, 89, 255). The observed STs are included in the major clonal complex (CC2) of the *S. epidermidis* lineages.

**Discussion**

Few investigations have been performed to examine CoNS neonatal colonization in NICU settings. We observed a lower incidence of CoNS colonization in neonates during hospitalization (55.9%) compared with Campeotto et al. [32], who observed a 66.1% incidence of colonization in nasal specimens from children admitted to the NICU. However, we collected samples only at admittance and discharge, whereas the cited study sampled the children weekly.

We found that the prevalence of MDR was increased among CoNS strains isolated at discharge, which could most likely be explained by the intensive selective pressure caused by antibiotic therapy administered to patients admitted to the NICU, as reported by others [33]. The increase of MDR prevalence could not be explained by an increase in frequency of colonization by *S. haemolyticus* alone, as susceptibility profile of *S. haemolyticus* isolated at admission showed that they were as susceptible as other species (data not shown). We also detected a high rate of antimicrobial resistance to β-lactam antibiotics (oxacillin and cefoxitin), which is conferred by the *mecA* gene. Similar to our results, studies of colonization and sepsis among hospitalized neonates have reported that most CoNS species carry the *mecA* gene and
that microorganisms isolated from systemic diseases have even higher resistance rates
[1, 34]. Taken together, these observations support the hypothesis that CoNS may be
a genetic repository of antibiotic resistance genes, particularly mecA, and may serve
as a carrier and/or reservoir of antibiotic resistance, which increases the risk of
staphylococcal infection in the hospital environment [35]. In Brazil, the use of
tetracycline in pediatrics is very limited, which may explain the lower prevalence of
tetracycline resistance among CoNS isolated at discharge observed in our study.
Studies have shown that the frequency of SCCmec among CoNS is increasing due to
the wide dissemination of the microorganism in hospital-acquired infections (HAIs),
which is associated with the potential genetic transfer of resistance elements among
Staphylococcus spp. [36]. We observed a high frequency (68.1%) of SCCmec types I,
II, and III among CoNS isolates at discharge. These types are commonly related with
healthcare-associated methicillin-resistant Staphylococcus aureus (MRSA) [37],
although it is not clear what SCCmec types are associated with particular CoNS
species. Although CoNS isolates often present SCCmec types similar to those of S.
aureus, they may also present unique, complex groups of SCCmecs [13], thereby
acting as reservoirs for particular SCCmec genes and elements. The diverse SCCmec
type profile among CoNS strains, as described previously [38], is due to the higher
capacity for genetic transfer between the species. In addition, CoNS strains present a
combination of SCCmec types, with SCCmec type IV being most frequently
associated with other types of chromosomal cassettes. Due to the various
combinations of different SCCmec types in the CoNS observed here and reported by
others [39, 40], there is a clear need to develop a unique typing system for this group
of bacteria.
Although *S. epidermidis* has been reported as the most frequent CoNS species colonizing neonates, the most prevalent CoNS species colonizing the neonates at discharge in our study was *S. haemolyticus*. This observation could be related to the ability of *S. haemolyticus* to adapt to selective pressures, such as antimicrobials and biocides present in the hospital environment [8, 41].

The high degree of genetic diversity among CoNS, as revealed by PFGE, and the presence of different SCC\textit{mec} types at both admittance and discharge suggest that nasal colonization by these species is a very dynamic process in the NICU environment. Although a neonate is typically admitted to the hospital with a particular strain of *S. epidermidis*, there is a high probability that the nasal colonization will be replaced by a *Staphylococcus* species that is highly drug resistant and likely acquired from the hospital environment or professional staff [13]. The length of hospitalization could be associated with MDR CoNS, as a longer stay would result in greater exposure to these factors.

Although there are scarce data regarding CoNS colonization, blood culture-positive newborns have been associated with CoNS colonization [12]. Further studies are needed to address the effect of colonization with MDR CoNS on infection among neonates that are admitted to intensive care and undergo invasive procedures.

The present study has some limitations. Ideally, several nasal swabs should be collected during the neonatal hospitalization period to assess the dynamic characteristics of CoNS carriage status. Additionally, the currently available techniques for SCC\textit{mec} characterization of CoNS are based on the *S. aureus* prototype.

The identification of a single genetic lineage (CC2) comprising the majority of STs was showed worldwide [10]. The CC2 is considered the most prevalent complex in
the nosocomial population of *S. epidermidis* and has been characterized by a high level of genetic diversity, an increased recombination/mutation rate, and a high number of SCC\textit{mec} elements [10, 42] as demonstrated in this study.

**Conclusion**

In conclusion, we have shown that children admitted to the NICU have a high rate of nasal colonization by CoNS with an increased frequency of MDR strains during the hospitalization period, primarily at discharge. In addition, we observed a high rate of SCC\textit{mec} dissemination in the healthcare environment. An extended stay in the NICU could result in a greater risk for neonate colonization by MDR CoNS strains and consequently an increased risk of systemic infection by these microorganisms.

**Competing interests**

There are no competing interests to be disclosed.

**Authors’ contributions**

ALA and AK participated on the conception and design of the study, analysis and interpretation of data and drafting the manuscript. YMT and JLC participated in acquisition of data, molecular tests, interpreting results and revised the manuscript. MCDBPA participated in acquisition of data, molecular tests, and critically contributed to the draft of the manuscript. VPPJ and MASV participated in patients’ recruitment, supervision of all epidemiological data collection, interpretation of data and revising the manuscript. RM contributed on the study design, analysis and interpretation of epidemiological data, managing the database, and critically revised the manuscript.

All authors read and approved the final version of the manuscript.

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References


obtained in a multilaboratory effort using identical protocols and MRSA strains.


Table 1. Cefoxitin resistance and presence of the *mecA* gene among CoNS nasal isolates obtained from neonates in the NICU at admission and discharge from July 2007 through May 2008.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Coagulase-negative <em>Staphylococcus</em> isolates</th>
<th>Adm</th>
<th>Dis</th>
<th>Adm</th>
<th>Dis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td></td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>Cefoxitin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistant</td>
<td>26</td>
<td>57.8</td>
<td>218</td>
<td>89.3</td>
<td></td>
</tr>
<tr>
<td>Susceptible</td>
<td>19</td>
<td>42.2</td>
<td>26</td>
<td>10.7</td>
<td></td>
</tr>
<tr>
<td><em>mecA</em> genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>27</td>
<td>60.0</td>
<td>204</td>
<td>83.6</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>18</td>
<td>40.0</td>
<td>40</td>
<td>16.4</td>
<td></td>
</tr>
<tr>
<td>Multidrug resistance*</td>
<td></td>
<td>1</td>
<td>2.2</td>
<td>73</td>
<td>29.9</td>
</tr>
<tr>
<td>No</td>
<td>44</td>
<td>97.8</td>
<td>171</td>
<td>70.1</td>
<td></td>
</tr>
</tbody>
</table>

*a* Multidrug resistance was considered as resistance to at least four classes of antibiotics
Table 2. SCC\textit{mec} frequencies at admission and discharge, among the most prevalent \textit{mecA} strains isolated from neonates from July 2007 through May 2008.

<table>
<thead>
<tr>
<th>SCC\textit{mec} type</th>
<th>\textit{S. epidermidis} (%)</th>
<th>\textit{S. haemolyticus} (%)</th>
<th>\textit{S. capitis} (%)</th>
<th>\textit{S. warneri} (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1 (3.7)</td>
<td>5 (8.5)</td>
<td>7 (26.9)</td>
<td>52 (73.2)</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1 (1.7)</td>
<td>0</td>
<td>0</td>
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<tr>
<td>II</td>
<td>2 (7.4)</td>
<td>2 (3.4)</td>
<td>2 (7.7)</td>
<td>2 (11.8)</td>
</tr>
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<td></td>
<td>4 (14.8)</td>
<td>9 (15.2)</td>
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<td>0</td>
</tr>
<tr>
<td></td>
<td>1 (3.7)</td>
<td>1 (1.7)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>16 (59.3)</td>
<td>25 (42.4)</td>
<td>6 (23.1)</td>
<td>5 (7.1)</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>13 (22.0)</td>
<td>7 (26.9)</td>
<td>8 (11.2)</td>
</tr>
<tr>
<td></td>
<td>3 (11.1)</td>
<td>3 (5.1)</td>
<td>4 (15.4)</td>
<td>5 (7.1)</td>
</tr>
<tr>
<td></td>
<td>3 (11.1)</td>
<td>3 (5.1)</td>
<td>4 (15.4)</td>
<td>5 (7.1)</td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>59</td>
<td>26</td>
<td>71</td>
</tr>
</tbody>
</table>

* Adm: Admission
** Disch: Discharge
Table 3 Risk factors for multidrug resistance in CoNS isolates among children admitted to the NICU in Brazil from July 2007 through May 2008

<table>
<thead>
<tr>
<th>Variables</th>
<th>Incident cases of MDR CoNS&lt;sup&gt;a&lt;/sup&gt;</th>
<th>RR  95% CI</th>
<th>P value</th>
<th>OR&lt;sup&gt;b&lt;/sup&gt; 95% CI</th>
<th>P value</th>
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</thead>
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<td></td>
<td>yes</td>
<td>no</td>
<td></td>
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<td></td>
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<tr>
<td>Sex</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Male</td>
<td>46</td>
<td>184</td>
<td>1.29</td>
<td>0.80-2.34</td>
<td>0.256</td>
</tr>
<tr>
<td>Female</td>
<td>25</td>
<td>136</td>
<td></td>
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<td>Antimicrobial use</td>
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<td>Yes</td>
<td>62</td>
<td>241</td>
<td>2.42</td>
<td>1.10-5.37</td>
<td>0.011</td>
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<tr>
<td>No</td>
<td>6</td>
<td>65</td>
<td></td>
<td></td>
<td>0.51</td>
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<tr>
<td>Low birthweight</td>
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<tr>
<td>Yes</td>
<td>43</td>
<td>162</td>
<td>1.26</td>
<td>0.88-2.09</td>
<td>0.162</td>
</tr>
<tr>
<td>No</td>
<td>28</td>
<td>153</td>
<td></td>
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<td>Age at hospitalization</td>
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<tr>
<td>&lt;24 hours</td>
<td>55</td>
<td>241</td>
<td>1.12</td>
<td>0.67-1.85</td>
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</tr>
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<td>80</td>
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<td>Type of birth</td>
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<tr>
<td>Caesarean</td>
<td>39</td>
<td>219</td>
<td>0.61</td>
<td>0.40-0.93</td>
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</tr>
<tr>
<td>Vaginal</td>
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<tr>
<td>CPAP&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>Yes</td>
<td>30</td>
<td>97</td>
<td>1.53</td>
<td>1.00-2.35</td>
<td>0.056</td>
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<td>208</td>
<td></td>
<td></td>
<td>1.08</td>
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<tr>
<td>Hospitalization&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>Median hospitalization</td>
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<td>2</td>
<td>&lt;0.05</td>
<td>0.97</td>
<td>0.96-0.98</td>
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<td>Interquartile range</td>
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</table>

<sup>a</sup>Multidrug-resistant (resistance to four or more classes of antimicrobials), coagulase-negative *Staphylococcus*<br><sup>b</sup>Odds Ratio adjusted for age at hospitalization and sex<br><sup>c</sup>Continuous positive airway pressure<br><sup>d</sup>Length of hospitalization (days)
Figure 1. Length of hospitalization among neonates admitted to Neonatal Intensive Care Units according to CoNS multidrug resistance. The line across the box indicates the median values. The circles represent the outliers for length of stay.

Figure 2. PFGE dendrogram of *S. epidermidis*. Genetic similarity of eight isolates from eight patients at hospital admission (A) and discharge (D).

NT: Nontypeable;
-: not applicable, *mecA* negative;
*: strain with MDR phenotype

Figure 3. PFGE dendrogram of *S. haemolyticus*. Genetic similarity isolates from four patients at hospital admission (A) and discharge (D).

NT: Nontypeable;
*: strain with MDR phenotype

Additional file 1: Supplementary Table 1. MLST analysis from eight *S. epidermidis* strains isolated from neonates in this study. Sequencing analysis of each locus resulted in an allele determination. Based on the allelic profile for each sample, exact or approximate sequence types (STs) were assigned.

Additional file 2: Supplementary Figure 1. Dendrogram based on MLST from eight *S. epidermidis* samples. Samples identification numbers preceded by UFG are samples isolated in this study. Samples preceded by ST are from MLST database (http://sepidermidis.mlst.net).
Figure 1

Length of hospitalization (days)

Carriage of MDR CoNS

p < 0.000
Figure 2: Phylogenetic tree showing the Dice coefficient for *S. epidermidis*. The table lists the ID, A/D, SCCmec, and length of hospitalization (days) for each strain.

<table>
<thead>
<tr>
<th>ID</th>
<th>A/D</th>
<th>SCCmec</th>
<th>Length of hospitalization (days)</th>
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<tbody>
<tr>
<td>420</td>
<td>D</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>665</td>
<td>D</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>724</td>
<td>A</td>
<td>I, III</td>
<td>6</td>
</tr>
<tr>
<td>608</td>
<td>D</td>
<td>III, IV</td>
<td>5</td>
</tr>
<tr>
<td>507</td>
<td>A</td>
<td>NT</td>
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<td>D</td>
<td>II, IV</td>
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<td>IV</td>
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<tr>
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<td>D</td>
<td>IV, V</td>
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<td>A</td>
<td>NT</td>
<td>4</td>
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<tr>
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<td>III, IV</td>
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<td>D</td>
<td>NT</td>
<td>4</td>
</tr>
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<td>723</td>
<td>A</td>
<td>I</td>
<td>24</td>
</tr>
<tr>
<td>723</td>
<td>D</td>
<td>-</td>
<td>24</td>
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</tr>
<tr>
<td>724</td>
<td>D</td>
<td>I</td>
<td>6</td>
</tr>
</tbody>
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### Figure 3

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<th>Length of hospitalization (days)</th>
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<td>A</td>
<td>I</td>
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Additional files provided with this submission:

Additional file 1: Yves Ternes additional file 1 table_rev_al 09_09_2013.doc, 35K
http://www.biomedcentral.com/imedia/2072364391107530/supp1.doc
Additional file 2: Ternes additional file 2 figure .doc, 29K
http://www.biomedcentral.com/imedia/1113888422107530/supp2.doc