Clinical significance of coagulase-negative staphylococci isolated from neonates

Maria de Lourdes R.S. Cunha¹, Carlos A.M. Lopes², Ligia M.S.S. Rugolo³, Liciana V.A.S. Chalita⁴

Abstract

Objective: to evaluate the clinical significance of coagulase-negative staphylococci (CNS) isolated from newborns' infections at Neonatal Unit of Hospital das Clínicas da Faculdade de Medicina de Botucatu.

Methods: the CNS strains isolated were identified and classified as clinically significant and contaminant, based on a series of clinical and laboratory data obtained from patients who stayed in the Neonatal Unit. The following data were analyzed: risk factors for infections, clinical evolution, abnormal blood cell counts and/or C-reactive protein e antibiotic therapy.

Results: among the 117 CNS strains isolated, 60 (51.3%) were classified as significant and 57 (48.7%) as contaminant. Among the 54 infants infected by CNS, 43 (79.6%) presented very low birthweight (< 1,500g). Most of the infants infected by CNS were submitted to two or more invasive procedures (77.8%), including use of catheter (88.9%), parenteral nutrition (64.8%) and mechanical ventilation (61.1%). Staphylococcus epidermidis was the most frequently isolated species (77.8%) and more often associated with infection (86.7%) than with contamination (68.4%). Other species of CNS, including two strains of S. haemolyticus, three strains of S. lugdunensis, one strain of S. simulans, one strain of S. warneri and one strain of S. xylosus were also isolated from infants with clinical evidence of pneumonia, necrotizing enterocolitis and sepsis.

Conclusions: most newborns infected by CNS presented important risk factors for infection onset, including birthweight < 1,500g, foreign body presence and previous use of antibiotics. The identification of CNS species constitutes a useful marker of infection, since S. epidermidis was the species more frequently associated with infection.


Introduction

Staphylococcus aureus, which is coagulase-positive and produces a series of other enzymes and toxins, is the most widely known species of the genus Staphylococcus. This species usually causes a number of infections and intoxications in men and animals, whereas coagulase-negative staphylococci (CoNS) have been considered to be saprophytic or rarely pathogenic.¹

Nevertheless, in the last decade, remarkable progress in the systematic classification of staphylococci and in the development of methods for the identification of genus, species and subspecies has allowed clinicians to get acquainted with the variety of CoNS found in clinical samples and to consider them as etiologic agents of an array of infectious processes.² Currently, these organisms are regarded as essentially opportunistic, since they take advantage of various organic situations in order to produce severe infections.³
These microorganisms pose a highly potential risk of causing nosocomial bacteremia in low-weight newborns, who are immunologically immature and often require invasive procedures for the administration of nutrients and drugs. The increased incidence of nosocomial bacteremia by CoNS in newborns in the last 20 years has been associated with the increase of survival rates among preterm newborns weighing less than 1,500g at birth and with their long stay at hospital.

However, the interpretation of positive blood cultures of CoNS is quite difficult, since these microorganisms colonize the skin and the mucous membranes as commensals and also because they can contaminate blood cultures during blood sampling. In this respect, researchers have used a varied number of clinical and laboratory criteria to distinguish between contamination and bacteremia. Therefore, the diagnosis of bacteremia has been based on the patients’ clinical data and on the isolation of identical microorganisms in two or more blood cultures. The cultures that present growth of multiple strains or species of CoNS in association with other species of microorganisms are classified as contaminants. However, as blood volume is small in low-weight preterm newborns, only one blood culture is carried out, so as to avoid the necessity for and the risk of blood transfusions due to constant venipunctures. Thus, neonatologists have used clinical and laboratory criteria, such as lethargy, food intolerance, abdominal distension, deterioration of respiratory function, unstable body temperature, perinatal risk factors and hematologic data, among others, as diagnostic basis.

CoNS species can be easily identified by their biochemical characteristics, however, most laboratories of clinical microbiology do not use this method as daily routine. There is some controversy over the specific literature on the clinical relevance of CoNS identification; according to some authors, this procedure is not clinically significant, although other researchers believe this identification is important to distinguish between contamination and infection. According to Archer, CoNS identification is of paramount importance to the association of certain species with specific infections, given the fact that some data suggest that, apart from *S. epidermidis* and *S. saprophyticus*, which have been considered to be pathogenic, some other species, such as *S. haemolyticus*, *S. lugdunensis* and *S. schleiferi* are more associated with infections than other species.

Even though the ability of CoNS to cause infection has been well documented, these microorganisms have been often neglected in terms of clinical importance. Therefore, we decided to assess this issue in newborns at the neonatal unit of Hospital das Clínicas da Faculdade de Medicina de Botucatu, UNESP (School of Medicine of Botucatu), with the principal aim of evaluating the clinical relevance of CoNS species and strains isolated from infectious processes.

**Materials and methods**

**Strains**

This retrospective study included 117 strains of CoNS isolated from clinical material obtained from 107 newborns admitted to the neonatal unit of Hospital das Clínicas da Faculdade de Medicina de Botucatu, between 1990 and 1996. The procedures were approved by the Ethics and Research Committee of the School of Medicine.

**Inclusion criteria**

Strains of CoNS isolated from internal fluids (blood, urine and secretions) and foreign bodies (cannulae, drains and catheters) were included in the study.

In cases of biological material in which normal microbiota was observed and in which CoNS were in associative culture with other bacteria, a clear numerical prevalence was always requested.

**Identification of coagulase-negative staphylococci**

The isolates, obtained from clinical samples, were inoculated onto blood agar and were Gram stained, in order to determine their purity and to observe their morphology and specific color. After these characteristics were analyzed, the strains were submitted to catalase and coagulase tests. Genus *Staphylococcus* was distinguished from *Micrococcus* by means of the oxidation and glucose fermentation test and by bacitracin resistance (0.04 U), indicated by the absence of inhibition zone diameters or formation of a zone diameter of up to 9 mm, and by furazolidone (100 mg) sensitivity characterized by inhibition zone diameters between 15 and 35 mm.

The identification of coagulase-negative staphylococci followed the criteria proposed by Kloos & Schleifer, and Kloos & Bannerman, according to the simplified scheme of biochemical tests, which includes the assessment of the following sugars: xylose, arabinose, sucrose, trehalose, mannitol, maltose, lactose, xylitol, ribose and fructose. In addition, the criteria also include characterization of hemolysins, nitrate reduction, urease, ornithine decarboxylase and novobiocin resistance.

**Clinical relevance**

The clinical data that have supported the definition of clinical relevance were obtained from the analysis of medical histories. The major data regarding perinatal risk factors for infection were prolonged membrane rupture (> 24 hours); gestational age (GA); birthweight; invasive procedures, such as arterial catheterization or umbilical venous catheterization, venous central or peripheral catheterization, endotracheal intubation (mechanical ventilation), surgical procedures, peritoneal dialysis, parenteral nutrition, thoracic drain and ventricular-peritoneal shunt. The removal or not of a foreign body during the infection episode caused by CoNS was also taken into consideration.
The clinical outcome of newborns was assessed one week before and one week after the isolation of CoNS. The diagnoses and clinical status suggestive of CoNS infection, which are characterized by insidious and nonspecific signs and symptoms, were taken into account. These signs and symptoms usually include involvement of the general health status, unstable temperature and apneas.

In addition to clinical outcome, abnormal hemogram results and/or positive C-reactive protein were considered relevant when isolating the agent. The normal hematological parameters were those proposed by Manroe et al.18

Deaths were attributed to CoNS infection within the first 72 hours of isolation and to the possible association with CoNS if they occurred between four and seven days after CoNS isolation.

Another aspect that was investigated and that helped to determine clinical relevance was the previous use of antibiotics, CoNS-specific antibiotics after the result of bacteriological diagnosis, as well as the use of specific antibiotics, such as vancomycin, oxacillin or teicoplanin. The indication of antibiotics was always based upon the results of the antibiogram performed during the study.

The CoNS strains included in the study were classified as significant and contaminant, according to the modified CDC19 criterion:

- **significant strains**: strains isolated from newborns with three or more of the following: risk factors for infection, clinical status, abnormal hemogram results and proper antibiotic therapy. The strains isolated from patients with two of the criteria and who had not received proper antibiotic therapy and died were also considered significant;

- **contaminant strains**: strains isolated from newborns who only showed risk factors for infection and/or presented one of the other criteria (clinical status or abnormal hemogram results, or proper antibiotic therapy). The strains isolated from newborns who showed three of the criteria, but who had satisfactory improvement of the infection without the use of proper antibiotics were also considered contaminant. The isolation of another etiologic agent from internal fluids and foreign bodies at the same time of CoNS isolation was also used as a criterion for the classification of contamination.

**Statistical analysis**

The data regarding the clinical relevance of CoNS strains were analyzed by the chi-square test.

The nonparametric Mann-Whitney test was used for the analysis of birthweight and age of newborns.

The data that had a \( P < 0.25 \) on the univariate analysis were later submitted to multivariate analysis by means of a logistic regression model,20 with the aim of simultaneously assessing the influence of several variables over the incidence of CoNS infection.

The significance level for all tests was established at \( P < 0.05 \).21

**Results**

**Strains**

A total of 117 CoNS strains were isolated from different clinical materials obtained from 107 newborns. The referred sample consisted of 60 isolates from blood cultures, 41 from foreign bodies (30 from catheter tip, 10 from cannula tip, one from thoracic drain), 13 from secretions (two from the drain, five from the stomach and six from the trachea) and three from urine.

**Identification of coagulase-negative staphylococci**

Figure 1 shows the results of the distribution of isolated CoNS strains according to species. *S. epidermidis* was the most commonly isolated species (77.8%).

Figures 2 and 3 show the distribution of CoNS species classified respectively as significant and contaminant. Of the 117 strains included in the study, 60 (51.3%) were classified as clinically significant and 57 (48.7%) as contaminant.

The results revealed a higher frequency of *S. epidermidis* associated with infection (86.7%) than with contamination (68.4%) \( (P<0.05) \). The statistical analysis of the distribution of other species did not show any significant difference.

Among 54 newborns with CoNS infection, *S. epidermidis* was isolated from 46 infants (85.2%), *S. haemolyticus* was responsible for infection in two infants (3.7%), *S. lugdunensis* in three (5.6%), and *S. simulans* (1.8%), *S. warneri* (1.8%) and *S. xylosus* (1.8%) in one.

**Clinical relevance**

Of 107 newborns, 54 were considered to be infected by CoNS and 53 were considered uninfected. Among the 60 strains isolated from blood cultures and analyzed in terms of clinical relevance, 35 (58.3%) were interpreted as significant and 25 (41.7%) as contaminant (Figures 2 and 3). Of the 41 strains isolated from foreign bodies, 21 (51.2%) were interpreted as significant, of which 14 were obtained from catheter tip (66.7%), six from cannulae (28.6%) and one from thoracic drain (4.7%) (Figure 2). Of the 13 strains isolated from secretions, four (30.8%) strains, one from the secretion of a thoracic drain and three from tracheal secretion, were considered significant. The strains interpreted as clinically significant were isolated in a larger number from the blood than from secretions \( (P<0.05) \), but with no statistically significant difference when compared to those obtained from foreign bodies. On the other hand, the children classified as uninfected presented a greater
number of strains isolated from secretions than from the
ingood, birthweight, and personal data is shown in Table 1.
Among 54 children with CoNS infection, 43 (79.6%) were
premature, of which 18 (33.3%) were extremely premature
(gestational age < 31 weeks), compared with seven (13.2%)
in the group of infection-free children. However, this
difference in the number of patients with gestational age
less than 31 weeks between the two groups was not
statistically significant (Table 1). Table 1 shows that 27
(50.0%) newborns with CoNS infection had birthweight
<1,500g, with significant difference from the group of
infection-free newborns (20.8%). The median birthweight
also showed statistically significant difference between the
group of CoNS-infected children (1,495g) and the group of
infection-free children (2,270g).

The median age at the time of CoNS isolation was
significantly different between the group of CoNS-infected
children (10 days of life) and infection-free children (4 days
of life). No statistically significant difference was observed
in terms of gender and origin of the children in both groups.

The univariate analysis of clinical data of the patients in
relation to perinatal risk factors for infection revealed
significant difference between the groups of CoNS-infected
and infection-free children with regard to ICU admission,
catheterization, mechanical ventilation, parenteral nutrition,
non-removal of foreign bodies and presence of two or more
foreign bodies (Table 2).

As for hematological data, we observed a greater number
of newborns with neutrophilia in the CoNS-infected group,
when compared to the infection-free group (Table 2).

Table 4 shows the results of the multivariate analysis by
means of a logistic regression model, in which the significant
risk factors for CoNS infection were very low birthweight,
non-removal of foreign body, and use of previous antibiotic
therapy. The estimation of recurrence risk by means of
Odds Ratio showed that newborns weighing less than 1,500g
at birth had a risk of CoNS infection 5.98 times higher than
newborns with greater birthweight, whereas the non-removal
of foreign bodies increased the risk of infection by 4.40
times. Previous treatment with antibiotics increased the
probability of recurrence of infection by 5.38 times.

During hospitalization, a 37% death rate was observed
in the CoNS-infected group, compared to 17.0% in the
group of infection-free newborns. However, seven newborns
who died in the first group had infection caused by gram-
positive bacteria and four were infected by a fungus after the
episode of CoNS infection. In addition, the deaths of two
newborns were not associated with infection and other two
newborns died between four and seven days after CoNS
isolation. Therefore, CoNS contributed to the death of
seven newborns (13.0%). Of these seven newborns, five
had foreign bodies infected with CoNS, which were removed at the time of death, and four children were extremely premature with birthweight less than 1,000g.

Figure 4 presents the clinical diagnosis of the patients one week before and one week after CoNS isolation. Although 54 newborns considered infected by CoNS presented clinical signs of sepsis, laboratory confirmation was obtained in 26 (48.1%) newborns. Forty-one (75.9%) radiological episodes of pulmonary infection, 14 (25.0%) clinical and radiological episodes of necrotizing enterocolitis (NEC), and five (9.2%) episodes of meningitis were confirmed. S. epidermidis was the predominant etiologic agent. On top of that, S. haemolyticus was associated with an episode of pneumonia and two episodes of NEC, whereas S. lugdunensis was the etiologic agent of three episodes of pneumonia, S. simulans was found in one case of pneumonia.
and in one case of sepsis, *S. warneri* was the causative agent of one case of pneumonia, and *S. xylosus* was associated with one episode of pneumonia and one episode of sepsis.

### Discussion

Our analysis of 117 strains revealed that *S. epidermidis* was the most frequently isolated species (77.8%), which is in agreement with the findings of other authors(5,8,22).

According to the data presented by Oren & Merzbach,10 the identification of different species of CoNS is not clinically significant. However, our results show that the isolated strains of *S. epidermidis* were significantly more often associated with infection (86.7%) than with contamination (68.4%). Silbert et al.,23 conducted a study to assess the prevalence of infection versus contamination in patients younger than 60 days, with positive blood cultures for CoNS, and found out that 91.1% of the isolates belonged to

---

**Table 1 - Newborns’ personal data**

<table>
<thead>
<tr>
<th>Personal data</th>
<th>Infected</th>
<th>Noninfected</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>GA &lt; 31</td>
<td>18</td>
<td>33.3</td>
<td>7</td>
<td>13.2</td>
</tr>
<tr>
<td>GA 31-36</td>
<td>21</td>
<td>38.9</td>
<td>26</td>
<td>49.0</td>
</tr>
<tr>
<td>GA 37</td>
<td>4</td>
<td>7.4</td>
<td>3</td>
<td>5.7</td>
</tr>
<tr>
<td>GA ≥ 38</td>
<td>8</td>
<td>14.8</td>
<td>16</td>
<td>30.2</td>
</tr>
<tr>
<td>BW &lt; 1,500 g</td>
<td>27</td>
<td>50.0</td>
<td>11</td>
<td>20.8</td>
</tr>
<tr>
<td>BW median (g)</td>
<td>1,495</td>
<td>2,270</td>
<td>1,985</td>
<td></td>
</tr>
<tr>
<td>Age median (days)</td>
<td>10</td>
<td>4</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>24</td>
<td>44.4</td>
<td>27</td>
<td>50.9</td>
</tr>
<tr>
<td>Born at HC/FMB</td>
<td>34</td>
<td>62.9</td>
<td>31</td>
<td>58.5</td>
</tr>
<tr>
<td>Total</td>
<td>54</td>
<td>50.5</td>
<td>53</td>
<td>49.5</td>
</tr>
</tbody>
</table>

GA: gestational age (weeks). Note: 4 newborns with unknown gestational age. ns: no significance (P >0.05), NB: newborn, BW: birthweight, HC/FMB: Hospital das Clínicas da Faculdade de Medicina of Botucatu.
Table 2 - Risk factors for CoNS infection

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Infected n</th>
<th>Infected %</th>
<th>Noninfected n</th>
<th>Noninfected %</th>
<th>Total n</th>
<th>Total %</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICU admission</td>
<td>53</td>
<td>98.1</td>
<td>33</td>
<td>62.3</td>
<td>86</td>
<td>80.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Catheterization</td>
<td>48</td>
<td>88.9</td>
<td>33</td>
<td>62.3</td>
<td>81</td>
<td>75.7</td>
<td>0.0004</td>
</tr>
<tr>
<td>Catheter complication</td>
<td>4</td>
<td>7.4</td>
<td>1</td>
<td>1.9</td>
<td>5</td>
<td>4.7</td>
<td>ns</td>
</tr>
<tr>
<td>Drain</td>
<td>10</td>
<td>18.5</td>
<td>5</td>
<td>9.4</td>
<td>15</td>
<td>14.0</td>
<td>ns</td>
</tr>
<tr>
<td>Mechanical ventilation</td>
<td>33</td>
<td>61.1</td>
<td>18</td>
<td>34.0</td>
<td>51</td>
<td>47.7</td>
<td>0.0138</td>
</tr>
<tr>
<td>Parenteral nutrition</td>
<td>35</td>
<td>64.8</td>
<td>22</td>
<td>41.5</td>
<td>57</td>
<td>53.3</td>
<td>0.0040</td>
</tr>
<tr>
<td>Ventricular-peritoneal shunt</td>
<td>3</td>
<td>5.5</td>
<td>2</td>
<td>3.8</td>
<td>5</td>
<td>4.7</td>
<td>ns</td>
</tr>
<tr>
<td>Non-removal of foreign bodies</td>
<td>31</td>
<td>54.7</td>
<td>17</td>
<td>32.1</td>
<td>48</td>
<td>44.8</td>
<td>0.0010</td>
</tr>
<tr>
<td>Surgery</td>
<td>9</td>
<td>16.7</td>
<td>6</td>
<td>11.3</td>
<td>15</td>
<td>14.0</td>
<td>ns</td>
</tr>
<tr>
<td>Dialysis</td>
<td>4</td>
<td>7.4</td>
<td>3</td>
<td>5.7</td>
<td>7</td>
<td>6.5</td>
<td>ns</td>
</tr>
<tr>
<td>Membrane rupture &gt; 24 hours</td>
<td>18</td>
<td>33.3</td>
<td>17</td>
<td>32.1</td>
<td>35</td>
<td>32.7</td>
<td>ns</td>
</tr>
<tr>
<td>Two or more foreign bodies</td>
<td>42</td>
<td>77.8</td>
<td>20</td>
<td>37.7</td>
<td>62</td>
<td>57.9</td>
<td>0.0001</td>
</tr>
<tr>
<td>Total NB</td>
<td>54</td>
<td>50.5</td>
<td>53</td>
<td>49.5</td>
<td>107</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

NB: newborn, ns: no significance (P>0.05)

The species *S. epidermidis*, three (6.7%) belonged to the species *S. hominis* and one (2.2%) to the species *S. warneri*, but only *S. epidermidis* was found in the group of infected patients. These results confirm the findings of Lowy & Hammer,11 who believe in the importance of identifying CoNS species so that the distinction between contamination and infection is possible.

Even though *S. epidermidis* is the most widely characterized and etiologically involved species, other species of pathogenic CoNS have been isolated from a broad series of clinical sources.2 In our study, other species were associated with infection, including *S. haemolyticus*, *S. lugdunensis*, *S. simulans*, *S. warneri* and *S. xylosus*. Hall et al.8 have also isolated strains of *S. haemolyticus* and *S. simulans* from children with clinical and laboratory evidence of sepsis and pneumonia.

Of 54 CoNS-infected newborns assessed in our study, 79.6% were premature, 33.3% of which were extremely premature (GA < 31 weeks), and 50.0% had birthweight < 1,500 g, with median weight equal to 1,495 g. From a comparative standpoint, similar results have been obtained by other researchers.4,8,24

There are several factors that contribute towards the enhanced susceptibility of preterm newborns, especially those with very low birthweight, to infection. The immaturity of the immune system, which consists of deficient phagocytosis, opsonization by antibodies and complement functions, also contributes to the severity of infection.25

Table 3 - Newborns’ laboratory data

<table>
<thead>
<tr>
<th>Laboratory data</th>
<th>Infected n</th>
<th>Infected %</th>
<th>Noninfected n</th>
<th>Noninfected %</th>
<th>Total n</th>
<th>Total %</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucocytosis</td>
<td>14</td>
<td>25.9</td>
<td>7</td>
<td>13.2</td>
<td>21</td>
<td>19.6</td>
<td>ns</td>
</tr>
<tr>
<td>Leucopenia</td>
<td>12</td>
<td>22.2</td>
<td>6</td>
<td>11.3</td>
<td>18</td>
<td>16.8</td>
<td>ns</td>
</tr>
<tr>
<td>Neutrophilia</td>
<td>24</td>
<td>44.4</td>
<td>9</td>
<td>17.0</td>
<td>33</td>
<td>30.8</td>
<td>0.0014</td>
</tr>
<tr>
<td>Left shunt</td>
<td>29</td>
<td>53.7</td>
<td>21</td>
<td>39.6</td>
<td>50</td>
<td>46.7</td>
<td>ns</td>
</tr>
<tr>
<td>Platelet reduction</td>
<td>4</td>
<td>7.4</td>
<td>2</td>
<td>3.8</td>
<td>6</td>
<td>5.6</td>
<td>ns</td>
</tr>
<tr>
<td>Positive CRP</td>
<td>27</td>
<td>50.0</td>
<td>25</td>
<td>47.2</td>
<td>52</td>
<td>48.6</td>
<td>ns</td>
</tr>
<tr>
<td>Total NB</td>
<td>54</td>
<td>50.5</td>
<td>53</td>
<td>49.5</td>
<td>07</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

CRP: C-reactive protein, NB: newborn, ns: no significance (P>0.05)
Table 4 - Logistic regression model

<table>
<thead>
<tr>
<th>NBs' data (95%)</th>
<th>P value</th>
<th>Odds Ratio</th>
<th>Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preterm</td>
<td>0.5409</td>
<td>1.669</td>
<td>0.323</td>
</tr>
<tr>
<td>Weight &lt; 1,500 g</td>
<td>0.0085*</td>
<td>5.988</td>
<td>1.579</td>
</tr>
<tr>
<td>ICU admission</td>
<td>0.0666</td>
<td>12.04</td>
<td>0.843</td>
</tr>
<tr>
<td>Catheterization</td>
<td>0.4630</td>
<td>2.159</td>
<td>0.276</td>
</tr>
<tr>
<td>Catheter complication</td>
<td>0.3393</td>
<td>6.535</td>
<td>0.138</td>
</tr>
<tr>
<td>Mechanical ventilation</td>
<td>0.1044</td>
<td>3.386</td>
<td>0.777</td>
</tr>
<tr>
<td>Parenteral nutrition</td>
<td>0.6691</td>
<td>1.475</td>
<td>0.248</td>
</tr>
<tr>
<td>Non-removal of foreign bodies</td>
<td>0.0257*</td>
<td>4.405</td>
<td>1.197</td>
</tr>
<tr>
<td>Two or more foreign bodies</td>
<td>0.1612</td>
<td>5.524</td>
<td>0.506</td>
</tr>
<tr>
<td>Leucocytosis</td>
<td>0.5396</td>
<td>1.650</td>
<td>0.333</td>
</tr>
<tr>
<td>Leucopenia</td>
<td>0.4019</td>
<td>2.020</td>
<td>0.389</td>
</tr>
<tr>
<td>Neutrophilia</td>
<td>0.0700</td>
<td>3.412</td>
<td>0.904</td>
</tr>
<tr>
<td>Left shunt</td>
<td>0.3316</td>
<td>1.926</td>
<td>0.513</td>
</tr>
<tr>
<td>Previous antibiotic therapy</td>
<td>0.0096*</td>
<td>5.376</td>
<td>1.508</td>
</tr>
</tbody>
</table>

NB: newborn.  * 5% level of significance.

The median age of the 54 CoNS-infected newborns was 10 days, which is significantly different from that of the infection-free group (four days). These data denote the peculiar characteristic of these microorganisms, that is, their involvement in the etiology of late-onset episodes of nosocomial infection. Only two (4.4%) of the CoNS-infected babies were less than two days old, and one of them showed membrane rupture greater than 24 hours, as risk factor. Similar results have been obtained by Hensey et al., who reported an incidence rate of only 12% for CoNS infection in the first 48 hours of life, while all infections caused by Streptococcus B, Streptococcus viridans and Haemophilus influenzae occurred within this period and were associated with prolonged membrane rupture.

Among the risk factors for infection, the use of a catheter was significantly more frequent in CoNS-infected newborns. This finding is in agreement with the data obtained by other researchers who have drawn attention to the importance of this procedure. Vascular catheters are sources of infection, since they are easily colonized by skin microorganisms found around the insertion site, especially by CoNS, which are prevalent as normal flora of the skin and mucous membranes.

In the present study, the use of parenteral nutrition was more frequent in infected newborns. The importance of parenteral nutrition as risk factor is justified not only by the necessity of vascular catheters and their manipulation, but also by their composition. The administration of parenteral nutrition, especially of lipids, through these catheters, can serve as a culture medium that promotes quick bacterial proliferation.

The frequency of orotracheal intubation was also significantly higher in the group of CoNS-infected newborns, which is similar to the findings of Stoll et al. The presence of a tracheal cannula acts as a foreign body, compromising the integrity of mucous barriers and favoring microbial colonization.

In addition, the presence of two or more foreign bodies and their non-removal were significantly higher in infected newborns. Several authors have underscored the importance of quickly removing these foreign bodies and of using a treatment with antimicrobial drugs. If these foreign bodies are not removed, infection could be aggravated by the production of an exopolysaccharide slime by some CoNS, which allows these microorganisms to remain in the foreign body by interfering with the host’s immune response and by reducing the efficacy of antimicrobial drugs. Our study confirms this situation, since three newborns with foreign bodies colonized by CoNS died in spite of appropriate treatment with antibiotics.

The hemogram revealed a significantly higher frequency of neutrophilia in the group of infected newborns in comparison with the group of infection-free newborns. C-reactive protein has been useful for the diagnosis and follow-up of the response to the treatment of these infections. In our study, no significant difference was observed between infected and infection-free newborns. This could be
explained by the inclusion of several newborns with infection caused by microorganisms other than CoNS in the group of infection-free newborns.

The results obtained by Schmidt et al.29 have shown that 64% of newborns infected by CoNS had positive C reactive protein and only 20% and 8% of newborns in the suspected and control groups, respectively, tested positive for the protein. However, 88% of newborns with sepsis caused by other pathogens had positive results.

The previous use of antibiotics for the treatment of newborns infected by CoNS is coherent with the data presented in the literature.27 The previous use of antibiotics can suppress the normal flora and select resistant microorganisms, thus increasing not only the risk of infection, but also the severity of infection, in addition to reducing treatment efficacy.

The results obtained through the logistic regression analysis showed that the factors which remarkably predispose to CoNS infection were birthweight less than 1,500g (OR=5.98), non-removal of foreign bodies (OR=4.40), and previous treatment with antibiotics (OR=5.38), indicating a fourfold or fivefold risk of infection by CoNS, importance of these microorganisms as significant etiologic agents when isolated from newborns under these conditions.

The death rate of newborns classified as CoNS-infected in our study was 37.0%, and CoNS were associated with 13% of the deaths of newborns; 3.7% of these deaths were possibly associated with infection caused by CoNS. The death rate associated with CoNS observed in our study is similar to that found by other authors (7 to 14.3%) in industrialized countries.8 In-hospital mortality rate was 37.6%; however, it is compatible with the population and figures reported by other authors.30 Several factors interfere with mortality, some of which are inherent to the population, such as restricted immune response of very low-weight newborns, which can be aggravated by a previous pathology associated with the difficulty in establishing an early diagnosis and with the non-removal of foreign bodies colonized by these microorganisms.

Our results showed a frequency of 26 episodes of sepsis confirmed by laboratory exams. In 15 (51.7%) of these episodes, the newborns presented catheter or cannula colonized by these microorganisms. Similar results were obtained by Noel & Edelson31 at the neonatal ICU of the New York Hospital, where among 23 episodes involving 38 patients, 57% occurred in newborns with vascular catheters that were contaminated or suspected of contamination.

The presence of sepsis associated with necrotizing enterocolitis (NEC) caused by CoNS has also been described,31 showing that these microorganisms can initially colonize the gastrointestinal tract by means of endotracheal or nasogastric intubation, and secondarily cause sepsis when the integrity of the intestinal barrier is compromised by ischemia.24 Of the 54 newborns infected by CoNS, 14 (25.9%) had episodes of NEC, six of which (42.8%) were associated with sepsis confirmed by lab exams. On top of that, four of these newborns with NEC and sepsis had been submitted to catheterization or cannulation, a fact that is similar to the results reported by Noel & Edelson.31

Our results showed a frequency of 75.9% of pulmonary infections associated with CoNS, which is higher than that observed by other authors.8 Such difference may arise from predisposing factors for pulmonary infection that are specifically related to preterm newborns, among which are respiratory problems that impair oxygenation and gas exchanges and the use of mechanical ventilation, which causes injury to the organ and makes it more susceptible to infection.24 S. epidermidis was found to be associated with 82.9% of the episodes of pneumonia, which is similar to the data obtained by Hall et al.8

In our study, 60 of 117 isolated strains of CoNS were considered clinically significant, including 35 strains isolated from blood cultures, 21 from foreign bodies and four from secretions, revealing a statistical difference in relation to the biological material of origin, with a higher frequency of clinically significant strains isolated from blood cultures and contaminants isolated from secretions. However, no statistically difference was observed for strains isolated from foreign bodies.

These results show that CoNS are important nosocomial pathogens; therefore, when isolated from blood and foreign bodies of very low-weight preterm newborns (< 1,500g), they should not be ignored, but classified as contaminants. Also, a careful examination of clinical and laboratory data is necessary in order to determine the clinical relevance of the isolated strains.

References


Correspondence:
Dra. Maria de Lourdes Ribeiro de Souza da Cunha
Dep. de Microbiologia e Imunologia
Instituto de Biociências - UNESP – Rubião Júnior
Caixa Postal 510 – CEP 18618-000
Botucatu, SP, Brazil
Phone: +55 14 6802.6058 – Fax: +55 14 6821.3744
E-mail: cunhamlr@ibb.unesp.br