Coagulase-negative staphylococcal skin carriage among NICU personnel: from population to infection

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Running title: CoNS skin carriage among NICU personnel
Coagulase-negative staphylococci (CoNS) are worldwide a major cause of sepsis on neonatal intensive care units (NICU). Infecting strains of these commensal bacteria may originate from NICU personnel. Therefore, we studied characteristics of CoNS isolates from NICU personnel and compared those with isolates from the general population and from sepsis patients. Furthermore, we studied the epidemiological effect on CoNS carriage of NICU personnel after a period of absence. In our study we isolated CoNS from thumbs of NICU personnel every two weeks during the summer period of 2005 and sampled personnel returning from vacation and a control group from the general population. Furthermore, we collected sepsis isolates from this period. Isolates were tested for antibiotic resistance, mecA and icaA carriage, biofilm production and genetic relatedness. We found that mecA, icaA and penicillin, oxacillin and gentamicin resistance were significantly more prevalent in CoNS strains from personnel than in community isolates. Similar trends were also observed if post-vacation strains were compared with pre-vacation strains. Furthermore, genetic analysis showed that 90% of the blood isolates were closely related to strains found on the hands of personnel. Our findings revealed that CoNS carried by NICU personnel differ from those in the general population. Hospital strains are replaced by community CoNS after a period of absence. NICU personnel are a likely cause for cross-contamination of virulent CoNS that originate from the NICU to patients.
Coagulase-negative staphylococci (CoNS) are the most frequent cause of late-onset sepsis among newborn infants in neonatal intensive care units (NICU) worldwide. Incidences up to 66% of late onset sepsis have been reported (8, 16). The high incidence of these infections is not only due to a high rate of invasive procedures in immune compromised patients, but also with the bacteria’s ability to form biofilms (10).

The biofilm forming property of CoNS is generally considered as their most important virulence factor. Biofilm formation is mediated by several factors, such as surface proteins and the polysaccharide intercellular adhesin (PIA). PIA is regulated by the ica operon, and presence of the ica genes have shown to be predictors for biofilm formation in *S. epidermidis* (8-9). Furthermore, we previously showed a strong association between carriage of *icaA* and *mecA*, the gene coding for methicillin resistance.

Antibiotic resistance in CoNS, especially against β-lactam antibiotics, has increased over the years. The *mecA* gene is present in over 80% of the CoNS late-onset sepsis isolates (8). The high rate of antibiotic resistance and their biofilm forming capacities probably enables CoNS to persist in the intensive care environment by giving them a selective advantage compared to other, more susceptible, species.

Since CoNS are commensal skin bacteria, it is generally hypothesized that infecting strains originate from NICU personnel. This theory is supported by the fact that NICU personnel carry CoNS that have similar characteristics to bloodstream isolates, like high antibiotic resistance. It has previously been shown that new graduate NICU nurses acquire antibiotic resistant staphylococci over time (2). It is however unknown if this colonization persists after a period of absence from the NICU. It is also unknown to which extent CoNS
carried by personnel differ from community strains. Since this information can give more insight in the origins of infecting CoNS strains and the dynamics of CoNS carriage, we studied different characteristics, i.e. antibiotic resistance and biofilm forming properties of CoNS isolated from NICU personnel and community strains. Furthermore we studied the effect of a period of absence from the NICU on CoNS carriage of NICU personnel to see if CoNS are replaced. Finally, we compared these isolates with NICU sepsis blood isolates collected in the same period, to see if NICU personnel carry the infecting strains.
MATERIAL & METHODS

Subjects and Setting.

This study was performed from June 2005 to September 2005 at the NICU of Erasmus MC – Sophia Children’s Hospital, Rotterdam, The Netherlands. This NICU consists of three wards with nine level III beds each. All permanently attached doctors and nurses of the NICU were eligible for inclusion. Gender, age, percentage full-time equivalent (FTE), first date of employment, antibiotic usage in the past six months and vacation plans were recorded at inclusion. If a subject had gone on vacation, they were asked for the location of their vacation and antibiotic usage during vacation upon return to the NICU. The control group from which the community strains were acquired consisted of subjects from the general population. They were volunteers at central location of the non-medical setting of the Erasmus University Rotterdam. During three days in September 2005, by-passers were asked to be sampled and to fill in a questionnaire, recording their gender, age, postal code, faculty, function and antibiotic usage in the past six months.

All subjects signed a written consent form. This study was approved by the Medical Ethical Committee of Erasmus University Medical Center, Rotterdam.

Study Design and Samples.

We performed a longitudinal study of skin carriage of CoNS among NICU personnel. All included subjects were sampled once every two weeks during the sample period. When a subject had gone on vacation, a sample was taken immediately at first return to the NICU (post-vacation sample). Post-vacation samples that were taken after entrance to the NICU were excluded. Control subjects were only sampled once. Post-
vacation samples were compared with the last regular two-week sample before their vacation (pre-vacation sample).

To remove transient flora, the subjects washed their hands with Palmolive Naturals Liquid handwash with Almond Milk (Colgate-Palmolive Nederland BV, Weesp, The Netherlands) for at least 30 seconds, and dried their hands with a clean paper towel. Samples were obtained by the thumb of their dominant hand on a phenol-manitol agar plate (5% NaCl). Plates were incubated at 37°C for two days and subsequently at room temperature for five days. A maximum of three visually different colonies were picked and regrown on tryptic soy agar plates. For control samples from the general population, only one colony was picked. All colonies were tested for catalase and absence of coagulase activity. Catalase-negative and coagulase-positive strains were excluded. CoNS isolates were stored in glycerol-containing liquid media at -80°C until further analysis. For comparison with clinical isolates, all CoNS sepsis isolates from the study period were retrieved from the microbiology laboratory. A CoNS sepsis isolate was defined as described before (8).

Bacterial Analysis.

Bacterial DNA was isolated using the cetyl trimethylammonium bromide purification method as described before [3]. We performed a multiplex PCR detecting the S. aureus specific nuc gene, the mecA gene, the icaA gene, and the staphylococcal 16S RNA based on the multiplex PCR designed by Zhang et al (19). 16S RNA negative and nuc positive samples were excluded from the study. Species identification was done by ITS PCR as described before (8). Unknown ITS PCR patterns were identified with VITEK 2.
DNA fingerprinting by restriction fragment end labeling (RFEL) was performed as previously described (8). Strains with at least 88% genetic similarity were considered genetically related. When a subject showed RFEL-identical isolates at one timepoint, only one of these isolates was included for further analysis. Biofilm production analysis was also performed as previously described with addition of 1% glucose to the initial growth medium (8). Strains with OD$_{595}$ < 0.30, 0.30 $\leq$ OD$_{595}$ $\leq$ 1.0, and OD$_{595}$ > 1.0 were defined as biofilm negative, weak and strong biofilm formers, respectively. Biofilm production was tested on 50 randomly selected strains of the pre-vacation, post-vacation and control groups. The blood isolates were all tested.

Susceptibility determinations for penicillin, oxacillin, gentamycin, erythromycin, clindamycin, co-trimoxazol, levofloxacillin, rifampicin and vancomycin was performed by the disk diffusion methodology (17) in accordance with the guidelines and criteria of the Clinical and Laboratory Standards Institute (CLSI). Oxacillin resistance was detected by use of cefoxitin as indicator antibiotic. Resistance was defined by measuring the zone diameters for the respective antibiotics, as defined by the CLSI (3). Resistance for vancomycin was monitored by growth on vancomycin screen agar. Screen agar contained a concentration of 6 $\mu$g/ml. Intermediate resistance was excluded from the analysis.

Multiresistance was defined as resistance for three or more antibiotics. We also calculated the mean number of antibiotics for which each group was resistant.

**Statistical Analysis**

Statistics were performed with the Statistical Package of Social Sciences (SPSS) software, version 11 (Chicago, Illinois, USA). The Chi-square test was used for univariate
significance testing of categorical variables. Differences between groups in other variables were analyzed by the nonparametric (two-tailed) Mann-Whitney U test. $P$ values of $<0.05$ were considered significant.
RESULTS

Characteristics of patients and isolates

During the four-month study period, sixty-nine personnel members were included in the study. Fifty-seven went on vacation in the study period, eight of them twice. General characteristics of the subjects are described in Table 1. Approximately one third of the regular samples showed no growth. Of the post-vacation samples two (3%) showed no growth. After exclusion of non-eligible isolates due to sampling after entrance to the NICU or non CoNS growth, 30 individuals who went on vacation were included for analysis. Two went on vacation twice. This resulted in 51 pre-vacation isolates and 80 post-vacation isolates. We included 207 controls, of whom all samples showed bacterial growth. One-hundred eighty-six isolates were CoNS. Characteristics of these subjects are shown in Table 1. These characteristics were tested for relations with the determined bacterial characteristics. No statistically significant relations were found between the different groups. We retrieved 29 CoNS blood culture isolates of neonates with a CoNS sepsis during the same sample period. Characteristics of these infants can be found in Table 1.

Species identification

Species identification was performed on all included specimens. The distribution of different species among the four groups is shown in Figure 2. The pre-vacation, post-vacation and control groups consisted largely of *S. epidermidis*, *S. haemolyticus* and *S. warneri*. The blood isolate group consisted of significantly more *S. epidermidis* than the other groups (P<0.001). There were no significant differences in species proportions for the other groups.
Antibiotic resistance and biofilm formation

The incidence of mecA and icaA and biofilm forming ability in the four groups is shown in Figure 2. Presence of both mecA and icaA is highest in the blood isolate group, followed by the pre-vacation, post-vacation and control groups. Most of these differences are significant (Figure 2). This is in contrast to biofilm formation, which was lowest among the blood isolates and significantly lower than both personnel isolates ($P<0.05$).

Resistance against antibiotics was determined for the pre-vacation, post-vacation and control specimens (Table 2). The pre-vacation isolates, which can be regarded as the normal NICU personnel skin flora, showed a high overall incidence of resistance for most antibiotics. The incidence of antibiotic resistance of the post-vacation isolates is generally lower than those of the pre-vacation strains and higher than those of the controls. This difference between the pre-vacation and community isolates is significant for oxacillin, gentamicin and penicillin resistance, as well as for multiresistance (all $P<0.001$). For these antibiotics, the post-vacation isolates were significantly more often resistant than the community isolates. When we compared the pre-vacation with the post-vacation strains, only gentamicin resistance was significantly higher in the pre-vacation isolates ($P=0.001$).

We also calculated the average number of resistances per isolate for each group. These numbers differed significantly as well.

To determine the relation between the duration of absence and antibiotic resistance, we analyzed the mean number of days of absence for every antibiotic in the post-vacation strains. No association was observed between antibiotic resistance or mecA carriage and a longer period of absence.
Genetic diversity

The isolated strains showed highly diverse RFEL patterns (data not shown), although several closely related isolates on the hands of different subjects on different sample points were found. For 10 subjects, we analyzed isolates from all timepoints. In eight subjects, closely related strains could be found in at least two sample periods. When comparing RFEL patterns of all pre-vacation and post-vacation strains, we found that only seven subjects (23%) had strongly related CoNS before and after vacation. Among the blood isolates three large groups of closely related strains, comprising a total of 21 strains (72%), were found (data not shown). We also compared the blood isolates with the pre-vacation, post-vacation and longitudinal isolates. Of the 29 blood isolates from the sample period, 26 (90%) were closely related to skin isolates of NICU personnel. Figure 3 shows examples of different related and unrelated RFEL patterns.
DISCUSSION

In this study, we have evaluated various characteristics of CoNS isolated from the hands of NICU personnel. We compared them with community CoNS isolates, studied changes that occur after a period of absence of one to several weeks and compared the personnel skin isolates with sepsis blood isolates. To our knowledge, this is the first study to show that NICU personnel who leave the NICU for a short (vacation) period carry less antibiotic resistant CoNS than before their absence. Two studies have been published in which staphylococcal colonization of inexperienced (student) nurses were compared with experienced (student) nurses (2, 7). Both studies have demonstrated that the experienced group carries more antibiotic resistant strains than inexperienced group and that this difference diminishes after several months. This finding suggests that hospital personnel acquire hospital associated strains over time. Our study shows that the reverse is also true: hospital personnel can loose their hospital associated strains after a short period of absence from the hospital environment. Although the difference is only significant for gentamicin resistance, the general trend suggests a change in CoNS colonization. These findings are supported by the results of the RFEL analysis confirming that CoNS colonization changes, as only in 7 out of 30 subjects pre-vacation strains and post-vacation strains are related.

The most striking result in our study is the high incidence of blood isolate related strains on the hands of NICU personnel. Since we only analyzed three morphologically different strains from each thumb instead of all strains, the true incidence is probably even higher. This strongly suggests that virulent CoNS are indeed spread by personnel, as several authors have suggested before (11, 13). As was previously described, appropriate
hand hygiene among NICU personnel is important for the reduction of sepsis among neonates (14).

Because of restricted antibiotic policies in The Netherlands (18), we expected low antibiotic resistance among community isolates. However, half of the samples were still resistant to penicillin, although this was significantly less than the samples from personnel. The significant difference in oxacillin is most likely due to frequent use of the \( \beta \)-lactam antibiotics flucloxacillin in our NICU. The difference with gentamicin resistance is attributable to aminoglycoside-modifying enzymes which are usually plasmid or transposon encoded. (12) Acquisition and loss of resistance may therefore occur much faster than with other antibiotics. Interestingly, a quarter of the control strains were resistant to erythromycin, which did not differ from the personnel strains.

The differences between the personnel and control groups can likely be ascribed to the intensive care environment, where there is a high use of antibiotics. Resistant strains are selected and reside in the unit, where personnel get colonized with these strains. The high amount of \( mecA \) positive strains among the blood isolates suggest an antibiotic selection factor, whereas the high amount of \( icaA \) positive strains suggests a biofilm selection factor. It should be noted that a positive association between \( mecA \) and \( icaA \) carriage has been described. (8)

Another interesting result is the low number of pre-vacation strains. One-third of the regular samples (among which the pre-vacation samples) showed no growth, which is much higher than the post-vacation and control samples. We believe this difference is due to the extensive use of hand alcohol among NICU personnel, which lowers the number of transferable CoNS. However, the high incidence of antibiotic resistance and \( mecA \) and \( icaA \)
positive strains in the pre-vacation samples may also imply a selection of these strains by the inadequate use of hand alcohol. It is known that low doses of alcohol enhance biofilm formation in CoNS in vitro (4). Therefore, hand rinsing should be thoroughly done as well.

Species identification has been done by ITS PCR, which has proven to be a reliable tool. (8, 15) Although the species distribution in the pre-vacation, post-vacation and control groups are comparable, the blood isolate group contains much more *S. epidermidis*. This is consistent with other studies, where *S. epidermidis* is described as the most frequently isolated staphylococcal species (1, 8-9). Surprisingly, the blood isolate group contains significantly less biofilm producing strains as well, even though it does contain significantly more *icaA* positive strains. In contrast, previous studies show that *S. epidermidis* produces more biofilm than other species (6, 9). Our results may be explained by the short period of four months, in which our strains were isolated. Most isolates in this period belong to three closely related groups, which coincidentally show low biofilm production. Hence, it may as well be that analysis over a longer period shows a positive association between *S. epidermidis* and biofilm production. A study of the CoNS isolates from our NICU in 2003 does show this association (8).

Another notable result is the high incidence of *S. warneri* on the hands of both NICU personnel and the control group. Two studies from 2007 also describe a high incidence of *S. warneri* on the hands of NICU nurses. (1, 5) Both studies note that previous studies have not described the predominance of *S. warneri* on the hands of hospital personnel. Cimiotti et. al. have suggested that time, geographic region or specific work settings may play an important role. We have shown that latter is not the case: the incidence of *S. warneri* in our non-medical control group is as high as in our medical...
Despite the high skin incidence of *S. warneri*, there were no *S. warneri* strains among the blood isolates, suggesting that this species is relatively harmless in neonatal sepsis.

There are some flaws in our study that need to be considered. Most importantly, we only analyzed three morphologically different strains from each thumb, instead of all strains. Especially in our RFEL analysis, this may have led to an underestimation of recurring strains, as these strains may simply not have been picked. For comparison of the tested bacterial characteristics, however, we suspect that picking three strains leads to an underestimation in significant differences between pre-vacation and post-vacation strains, as the incidence of these characteristics was higher in most individual cases in the pre-vacation group.

Another flaw concerns our control group. Although we regard our control group as being “the general population”, this is not entirely correct. Because we sampled at a university, our control group consists of mostly young students. The difference between the control group and the NICU personnel is not only in age, but also in gender as the NICU personnel consists mostly of women. However, we assume these factors have no or limited influence on the studied microbial characteristics.

In this study, we demonstrated that NICU personnel carries more β-lactam and gentamicin resistant, multiresistant and *mecA* and *icaA* positive CoNS than community strains. Personnel also carry less antibiotic resistant CoNS after a period of absence. Furthermore, almost all blood isolates from the sample period were related to isolates from the hands of personnel. These findings demonstrate that virulent CoNS are acquired on the NICU and personnel are likely to be an important cause for cross-contamination with these
CoNS. In agreement with many others, we therefore stress the importance of good hand hygiene, sure this reduces the transfer of CoNS.
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There were no conflicts of interest.

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REFERENCES


### Table 1

General characteristics of personnel, controls and patients

<table>
<thead>
<tr>
<th>Personnel</th>
<th>n=69</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (%)</td>
<td>19</td>
</tr>
<tr>
<td>Age</td>
<td>39 (34 - 44)</td>
</tr>
<tr>
<td>Years of employment</td>
<td>6.4 (3.8 - 13.1)</td>
</tr>
<tr>
<td>Nurse (%)</td>
<td>77</td>
</tr>
<tr>
<td>FTE &gt;0.60 (%)</td>
<td>74</td>
</tr>
<tr>
<td>Antibiotic Usage (%)</td>
<td>19</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Controls</th>
<th>n=186</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (%)</td>
<td>48</td>
</tr>
<tr>
<td>Age</td>
<td>21 (19 - 23)</td>
</tr>
<tr>
<td>Living in Rotterdam (%)</td>
<td>59.7</td>
</tr>
<tr>
<td>Student (%)</td>
<td>93.5</td>
</tr>
<tr>
<td>Hospital contact in the last 6 months (%)</td>
<td>23.7</td>
</tr>
<tr>
<td>Antibiotic Usage (%)</td>
<td>13</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patients</th>
<th>n=15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (%)</td>
<td>47</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>29 4/7 (27 - 35 5/7)</td>
</tr>
<tr>
<td>Birth weight (grams)</td>
<td>975 (725 - 1820)</td>
</tr>
<tr>
<td>Days of admission</td>
<td>32 (11 - 59)</td>
</tr>
</tbody>
</table>

**NOTE.** Data are expressed as median (interquartile range), unless specified otherwise.
Table 2

Antibiotic resistance proportions (%) of the different groups.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Pre-vacation</th>
<th>Post-vacation</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=51</td>
<td>n=80</td>
<td>n=186</td>
</tr>
<tr>
<td>Penicillin</td>
<td>80&lt;sup&gt;b&lt;/sup&gt;</td>
<td>68&lt;sup&gt;c&lt;/sup&gt;</td>
<td>51&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>43&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>32&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>8&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>1&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>26</td>
<td>26</td>
<td>29</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>4</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Co-trimoxazol</td>
<td>9</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Levofoxacin</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Multiresistance</td>
<td>31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean # of resistance</td>
<td>2.1&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.5&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>1.0&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Significant difference between pre-vacation and post-vacation strains

<sup>b</sup>Significant difference between pre-vacation and control strains

<sup>c</sup>Significant difference between post-vacation and control strains
FIGURE LEGENDS

**Figure 1.** Bacterial species distribution among the different study populations.

**Figure 2.** Incidence of mecA and icaA containing and biofilm producing strains among the different study populations. *P<0.05 and **P<0.001.

**Figure 3.** Example of several RFEL gel patterns. (A) *S. epidermidis* ATCC 12228 control strain; (B) *S. epidermidis* blood isolate; (C) *S. epidermidis* pre-vacation isolate identical to B; (D) *S. epidermidis* pre-vacation isolate closely related to B; (E) unrelated *S. epidermidis* post-vacation isolate; (F) unrelated *S. warneri* post-vacation isolate.
Figure 1

Pre-vacation
- 36%
- 14%
- 32%
- 18%

Post-vacation
- 34%
- 11%
- 47%
- 8%

Controls
- 30%
- 12%
- 39%
- 19%

Blood isolates
- S. epidermidis
- S. haemolyticus
- S. warneri
- Other
- 90%
- 7%
- 3%

□ S. epidermidis
□ S. haemolyticus
□ S. warneri
□ Other
Figure 2

**MecA**

**IcaA**

**Biofilm Production**

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Blood isolates

Pre-vacation

Post-vacation

Control

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* Significant difference

** Highly significant difference

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Legend:

- Black: Control
- Red: Pre-vacation
- Blue: Post-vacation
- White: Blood isolates

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