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## Coagulase-Negative Staphylococcal Skin Carriage among Neonatal Intensive Care Unit Personnel: from Population to Infection<sup>∇</sup>

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**Coagulase-negative staphylococci (CoNS) are a major cause of sepsis in neonatal intensive care units (NICU) worldwide. Infecting strains of these commensal bacteria may originate from NICU personnel. Therefore, we studied the characteristics of CoNS isolates from NICU personnel and compared them to those of 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 the thumbs of NICU personnel every 2 weeks during the summer 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* and *icaA* carriage as well as penicillin, oxacillin, and gentamicin resistance were significantly more prevalent in CoNS strains from NICU personnel than in community isolates. Similar trends were observed when postvacation strains were compared to prevacation strains. Furthermore, genetic analysis showed that 90% of the blood isolates were closely related to strains found on the hands of NICU 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 the 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 of up to 66% of late-onset sepsis have been reported (8, 17). The high incidence of these infections is due not only to a high rate of invasive procedures in immunocompromised patients but also to the bacterium's ability to form biofilms (10).

The biofilm-forming property of CoNS generally is considered 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 the presence of the *ica* genes has been shown to be a predictor for biofilm formation in *Staphylococcus epidermidis* (8, 9). Furthermore, we previously showed a strong association between the carriage of *icaA* and *mecA*, the gene coding for methicillin resistance.

Antibiotic resistance in CoNS, especially against  $\beta$ -lactam antibiotics, has increased over the years. The *mecA* gene is present in more than 80% of the CoNS late-onset sepsis isolates (8). The high rate of antibiotic resistance and their biofilm-forming capacities probably enable CoNS to persist in the intensive care environment by giving them a selective advantage over 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 characteristics similar to those of bloodstream isolates, like high antibiotic resistance. It has been shown previously 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 also is unknown to what extent CoNS carried by personnel differ from community strains. Since this information can give more insight into 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 the CoNS carriage of NICU personnel to see if CoNS are replaced. Finally, we compared these isolates to NICU sepsis blood isolates collected in the same period to see if NICU personnel carry the infecting strains.

### MATERIALS AND METHODS

**Subjects and setting.** This study was performed from June 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 of full-time equivalent (FTE; calculated by dividing the number of hours worked by the number of hours representing full-time employment), first date of employment, antibiotic use in the past 6 months, and vacation plans were recorded at inclusion. If a subject had gone on vacation, upon return to the NICU he or she was asked for the location of the vacation and antibiotic use during vacation. The control group from which the community strains were acquired consisted of subjects from the general population. They

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were volunteers at a central location of the nonmedical setting of the Erasmus University of Rotterdam. During 3 days in September 2005, passers-by were asked to provide samples and to fill in a questionnaire recording their gender, age, postal code, faculty, function, and antibiotic use in the past 6 months.

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

**Study design and samples.** We performed a longitudinal study of the skin carriage of CoNS among NICU personnel. All included subjects were sampled once every 2 weeks during the sample period. When a subject had gone on vacation, a sample was taken immediately after return to the NICU (postvacation sample). Postvacation samples that were taken after entrance to the NICU were excluded. Control subjects were sampled only once. Postvacation samples were compared to the last regular 2-week sample before the vacation (prevacation sample).

To remove transient flora, the subjects washed their hands with Palmolive Naturals liquid hand wash with almond milk (Colgate-Palmolive Nederland BV, Weesp, The Netherlands) for at least 30 s and dried their hands with a clean paper towel. Samples were obtained from the thumb of their dominant hand on a phenol-manitol agar plate (5% NaCl). Plates were incubated at 37°C for 2 days and subsequently at room temperature for 5 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 the absence of coagulase activity. Catalase-negative and coagulase-positive strains were excluded. CoNS isolates were stored in glycerol-containing liquid medium at -80°C until further analysis. For comparison to 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 *Staphylococcus 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 internal transcribed spacer (ITS) PCR as described before (8). Unknown ITS PCR patterns were identified with Vitek 2 (bioMérieux, Marcy l'Etoile, France). 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 identical isolates by RFEL at one time point, only one of these isolates was included for further analysis. Biofilm production analysis also was performed as previously described, with the addition of 1% glucose to the initial growth medium (8). Strains with an optical density at 595 nm ( $OD_{595}$ ) < 0.30,  $0.30 \leq OD_{595} \leq 1.0$ , and  $OD_{595} > 1.0$  were defined as biofilm-negative, weak biofilm formers, and strong biofilm formers, respectively. Biofilm production was tested on 50 randomly selected strains of the prevacation, postvacation, and control groups. The blood isolates all were tested. Susceptibility determinations for penicillin, oxacillin, gentamicin, erythromycin, clindamycin, cotrimoxazol, levofloxacin, rifampin, and vancomycin were performed by the disk diffusion methodology (13) in accordance with the guidelines and criteria of the CLSI (3). Oxacillin resistance was detected by the use of cefoxitin as an indicator antibiotic. Resistance was defined by measuring the zone diameters for the respective antibiotics, as defined by the CLSI (3). Resistance to vancomycin was monitored by growth on a vancomycin screen agar. Screen agar contained a concentration of 6 µg/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 SPSS software, version 11 (Chicago, IL). The chi-square test was used for the 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 4-month study period, 69 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

TABLE 1. General characteristics of personnel, controls, and patients

Parameter	Finding <sup>a</sup>
Personnel ( <i>n</i> )	69
Male (%)	19
Age	39 (34–44)
Yr of employment	6.4 (3.8–13.1)
Nurse (%)	77
FTE >0.60 (%)	74
Antibiotic use (%)	19
Controls ( <i>n</i> )	186
Male (%)	48
Age (yr)	21 (19–23)
Living in Rotterdam (%)	59.7
Student (%)	93.5
Hospital contact in the last 6 months (%)	23.7
Antibiotic use (%)	13
Patients ( <i>n</i> )	15
Male (%)	47
Gestational age (wk, days)	29, 4 (27–35, 5)
Birth wt (g)	975 (725–1,820)
Days of admission	32 (11–59)

<sup>a</sup> Data are expressed as medians (interquartile ranges) unless specified otherwise.

samples showed no growth. Of the postvacation samples, two (3%) showed no growth. After the exclusion of noneligible 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 prevacation isolates and 80 postvacation isolates. We included 207 controls, of whom all samples showed bacterial growth. One hundred eighty-six isolates were CoNS. The 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 Fig. 1. The prevacation, postvacation, and control groups consisted largely of *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, and *Staphylococcus warneri*. The blood isolate group consisted of a significantly larger proportion of *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 Fig. 2. The presence of both *mecA* and *icaA* is highest in the blood isolate group, followed by the prevacation, postvacation, and control groups. Most of these differences are significant (Fig. 2). This is in contrast to biofilm formation, which was lowest among the blood isolates and significantly lower than that for both personnel isolates ( $P < 0.05$ ).

Resistance against antibiotics was determined for the prevacation, postvacation, and control specimens (Table 2). The

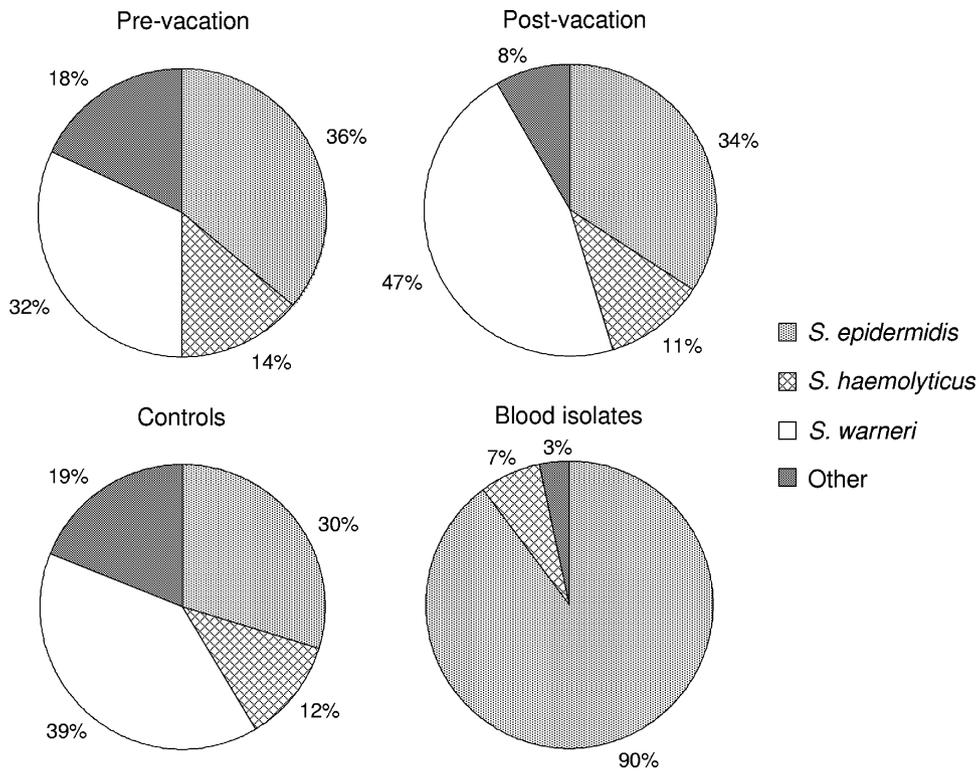


FIG. 1. Bacterial species distribution among the different study populations.

prevacation 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 postvacation isolates generally is lower than those of the prevacation strains and higher than those of the

controls. This difference between the prevacation and community isolates is significant for oxacillin, gentamicin, and penicillin resistance, as well as for multiresistance (all  $P < 0.001$ ). For these antibiotics, the postvacation isolates were resistant significantly more often than the community isolates. When we

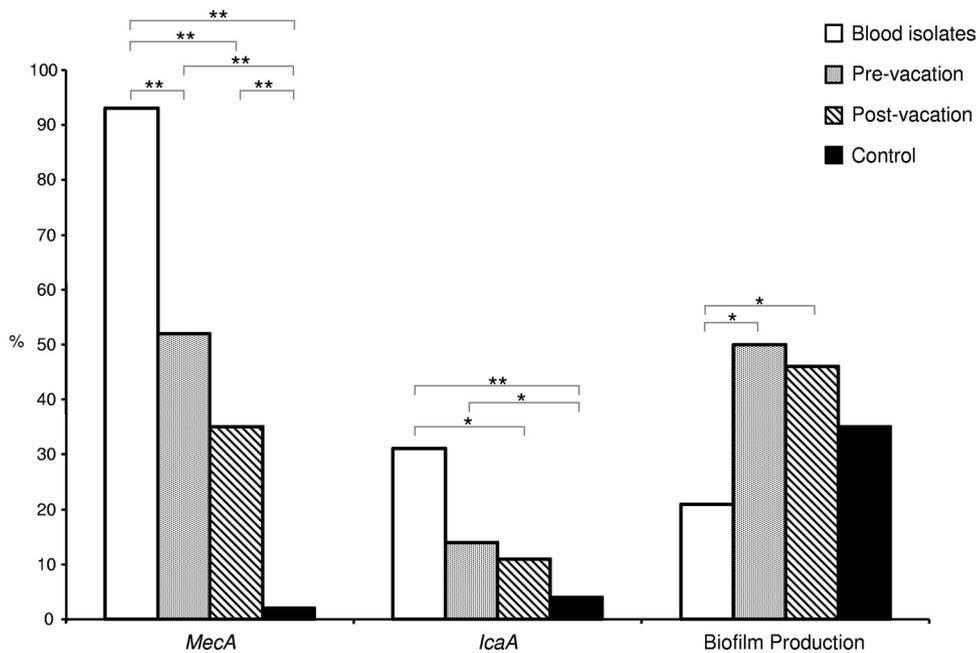


FIG. 2. Incidence of *mecA*- and *icaA*-containing and biofilm-producing strains among the different study populations. \*,  $P < 0.05$ ; \*\*,  $P < 0.001$ .

TABLE 2. Antibiotic resistance proportions (in percentages) of the different groups

Drug	Prevacation (n = 51)	Postvacation (n = 80)	Control (n = 186)
Penicillin	80 <sup>b</sup>	68 <sup>c</sup>	51 <sup>b,c</sup>
Oxacillin	55 <sup>b</sup>	43 <sup>c</sup>	8 <sup>b,c</sup>
Gentamicin	32 <sup>a,b</sup>	8 <sup>a,c</sup>	1 <sup>b,c</sup>
Erythromycin	26	26	29
Clindamycin	4	3	4
Cotrimoxazole	9	3	4
Levofloxacin	2	1	1
Rifampin	2	1	1
Vancomycin	0	0	0
Multiple (<2)	31 <sup>b</sup>	20 <sup>c</sup>	6 <sup>b,c</sup>
Mean no. of resistance <sup>d</sup>	2.1 <sup>a,b</sup>	1.5 <sup>a,c</sup>	1.0 <sup>b,c</sup>

<sup>a</sup> Significant difference between prevacation and postvacation strains.

<sup>b</sup> Significant difference between prevacation and control strains.

<sup>c</sup> Significant difference between postvacation and control strains.

<sup>d</sup> Values represent the number of antibiotics to which each isolate in a group (prevacation, postvacation, or control) is resistant divided by the number of isolates in the group.

compared the prevacation to the postvacation strains, only gentamicin resistance was significantly higher in the prevacation isolates ( $P = 0.001$ ). We also calculated the average number of types of resistance per isolate for each group. These numbers differed significantly as well.

To determine the relationship between the duration of absence and antibiotic resistance, we analyzed the mean number of days of absence for every antibiotic in the postvacation 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 at different sample time points were found. For 10 subject, we analyzed isolates from all time points. In eight subjects, closely related strains could be found in at least two sample periods. In comparisons of RFEL patterns of all prevacation and postvacation 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 to the prevacation, postvacation, 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 to community CoNS isolates, studied changes that occur after a period of absence of one to several weeks, and compared the personnel skin isolates to 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 fewer antibiotic-resistant CoNS than they did before their absence. Two studies have been published in which the staphylococcal colonization of inexperienced (student) nurses

were compared to those of experienced (student) nurses (2, 7). Both studies have demonstrated that the experienced group carries more antibiotic-resistant strains than the 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 lose 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 were prevacation strains and postvacation strains related.

The most striking result in our study is the high incidence of

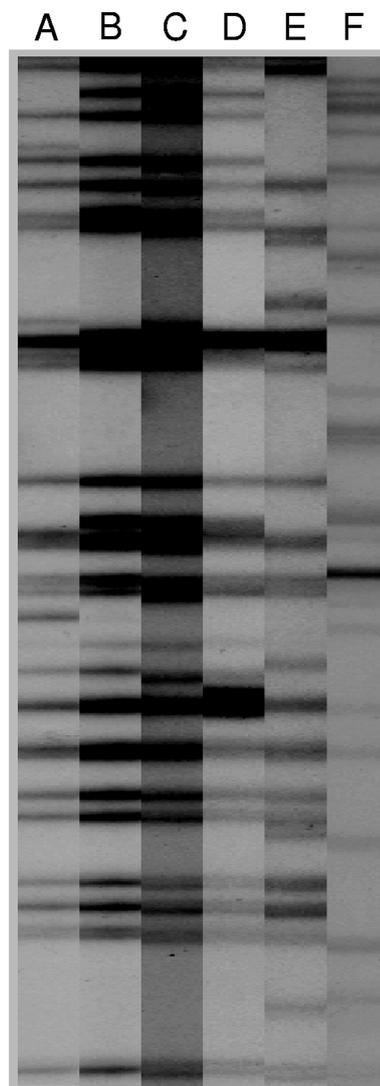


FIG. 3. Example of several RFEL gel patterns. (A) *S. epidermidis* ATCC 12228 control strain; (B) *S. epidermidis* blood isolate; (C) *S. epidermidis* prevacation isolate identical to that shown in panel B; (D) *S. epidermidis* prevacation isolate closely related to that shown in panel B; (E) unrelated *S. epidermidis* postvacation isolate; (F) unrelated *S. warneri* postvacation isolate.

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, 14). As was previously described, appropriate hand hygiene among NICU personnel is important for the reduction of sepsis among neonates (15).

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 that of the samples from personnel. The significant difference in oxacillin most likely is due to the frequent use of the  $\beta$ -lactam antibiotic flucloxacillin in our NICU. The difference with gentamicin resistance is attributable to aminoglycoside-modifying enzymes, which usually are plasmid or transposon encoded (12). The acquisition and loss of resistance therefore may occur much faster than that with other antibiotics. Interestingly, a quarter of the control strains were resistant to erythromycin, as was the case with the personnel strains.

The differences between the personnel and control groups likely can 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 large number of *mecA*-positive strains among the blood isolates suggest an antibiotic selection factor, whereas the large number 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 prevacation strains. One-third of the regular samples (including the prevacation samples) showed no growth, which is a much higher proportion than that for the postvacation 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 prevacation samples also may 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 done thoroughly as well.

Species identification has been done by ITS PCR, which has proven to be a reliable tool (8, 16). Although the species distribution in the prevacation, postvacation, and control groups are comparable, the blood isolate group contains a much larger proportion of *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 fewer 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 4 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 produc-

tion. 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 play an important role (1). We have shown that the latter is not the case: the incidence of *S. warneri* in our nonmedical control group is as high as that in our medical group. Despite the high incidence of *S. warneri* in skin samples, 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 simply may not have been picked. For the comparison of the tested bacterial characteristics, however, we suspect that picking three strains leads to an underestimation in significant differences between prevacation and postvacation strains, as the incidence of these characteristics was higher in most individual cases in the prevacation 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 took our samples at a university, our control group consists mostly of young adult 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 carry more  $\beta$ -lactam- and gentamicin-resistant, multiresistant, and *mecA*- and *icaA*-positive CoNS than community strains. Personnel also carry fewer 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, as this surely reduces the transfer of CoNS.

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