

Colonization Dynamics of Antibiotic-Resistant Coagulase-Negative Staphylococci in Neonates

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Coagulase-negative staphylococci (CoNS) isolated in neonatal late-onset sepsis are often antibiotic resistant. We analyzed CoNS from skin and feces of neonates during hospitalization. Antibiotic resistance of skin isolates increased during hospitalization, especially in *Staphylococcus haemolyticus*. *Staphylococcus warneri* showed low antibiotic resistance. Our data suggest that different CoNS species may play distinct roles in colonization.

Coagulase-negative staphylococci (CoNS) are the most frequent cause of late-onset sepsis among newborn infants in neonatal intensive care units (NICU) worldwide. Bloodstream isolates were frequently antibiotic resistant, similar to CoNS isolates from NICU personnel and from NICU sites (1, 2). Previously, it was shown that the majority of CoNS causing sepsis among neonates can be found on the hands of NICU personnel (1). Since the incidence of antibiotic-resistant CoNS in the non-medical population is low, it is generally assumed that neonates become colonized with antibiotic-sensitive CoNS after birth. It is, however, unknown how skin and gut colonization with resistant CoNS develops during NICU hospitalization. A better understanding of CoNS colonization dynamics may assist the development of preventive strategies, for example, improvement of hygienic measures. We therefore studied CoNS colonization dynamics in neonates, focusing on the development of antibiotic resistance. We investigated skin and intestinal colonization, as well as maternal CoNS colonization after birth.

This study was performed from mid November 2006 to mid March 2007 at the NICU of Erasmus MC–Sophia Children’s Hospital, Rotterdam, The Netherlands. All infants born at this hospital with a presumed hospitalization time of at least 7 days (gestational age of ≤ 30 weeks, birth weight of $\leq 1,500$ g or other reason) were included in this study. Children who were discharged within 72 h were excluded.

We performed a longitudinal study of skin and intestinal carriage of CoNS among neonates and their mothers. Samples were taken from all infants 24, 48, and 72 h (± 4 h) and, if still hospitalized, 7, 14, and 21 days after birth. Samples were taken from the mothers only once in the first 3 days and 7, 14, and 21 days after delivery. Skin samples from infants were obtained by gently pressing the bottom of a foot on a phenol-mannitol agar (PMA) plate (5% NaCl). Intestinal samples were obtained by culturing feces on PMA plates. Samples from mothers were obtained by culture of the thumb, by pressing it on a PMA plate (1). Selection, culturing, storage, species determination by internal transcribed spacer PCR, antibiotic susceptibility determination, and statistical analysis were performed as previously described (1), with the exception that in view of the multiple tests performed, we set the limit of significance at $P = 0.01$ (two-sided), instead of the conventional $P = 0.05$.

Forty-one infants were initially included in the study. One in-

TABLE 1 General characteristics of neonates ($n = 40$)

Characteristic	Value ^a
Birth weight (g) (range; SD)	1,185 (530–1,650; 291)
Gestational age (wk) (range; SD)	28 4/7 (25–32 2/7; 1 6/7)
% males	65
% delivered by Caesarian section	33
% receiving antibiotics	
Postpartum ^b	93
During hospitalization ^c	80
Mean no. of days of hospitalization (range; SD)	21.6 (5–92; 19.4)
Mortality (%)	13

^a Data are expressed as means, unless otherwise specified.

^b Postpartum antibiotics consisted of one dose of penicillin and gentamicin given directly postpartum.

^c Antibiotics were given as needed (e.g., on the basis of suspected or proven nosocomial infection). The choice of antibiotics depended on the indication.

fant was then excluded after being discharged within 72 h. General characteristics are summarized in Table 1.

For all time points, CoNS cultures were positive for all but nine (23%) children at 24 h after birth; one was also positive at 48 h after birth.

A total of 559 isolates was analyzed after exclusion of ineligible isolates (see Table S1 in the supplemental material). Figure 1 shows antibiotic resistance, multidrug resistance, and presence of *mecA* over time. All isolates were susceptible to vancomycin.

Skin isolates showed increasing resistance to levofloxacin, gentamicin, and cefoxitin, increasing *mecA* carriage, and increasing

Received 2 November 2012 Accepted 2 November 2012

Published ahead of print 7 November 2012

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Supplemental material for this article may be found at <http://dx.doi.org/10.1128/JCM.02935-12>.

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doi:10.1128/JCM.02935-12

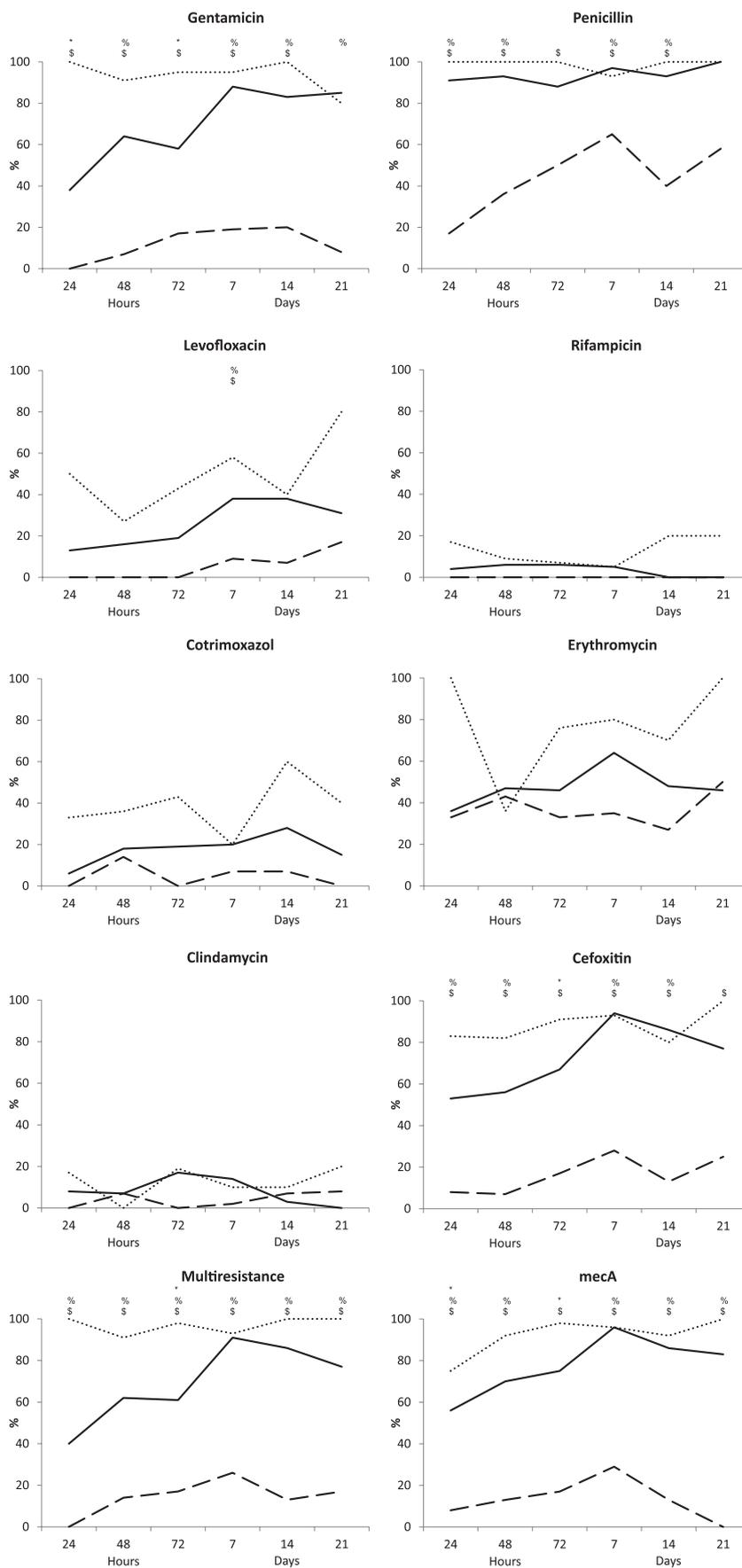


FIG 1 Rates of resistant isolates for each tested antibiotic at different time points. Solid lines, neonatal skin isolates; dotted lines, neonatal intestine isolates; dashed lines, maternal isolates. Statistically significant differences are marked by asterisks for differences between skin and gut isolates, percent signs for differences between skin and maternal isolates, and dollar signs for differences between gut and maternal isolates.

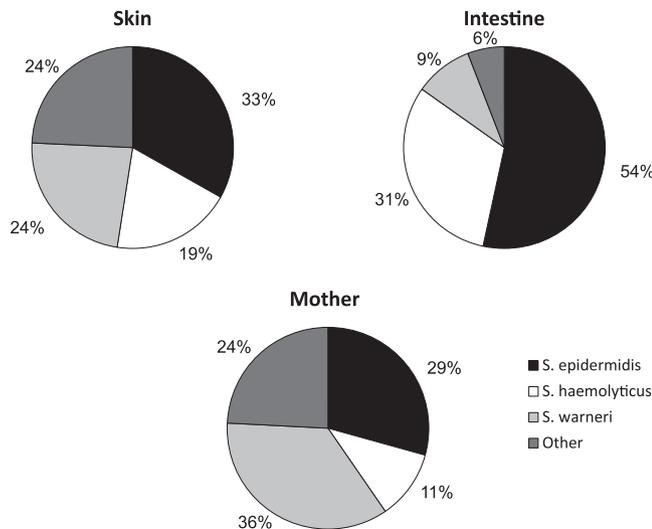


FIG 2 Bacterial species distribution among study groups.

multidrug resistance over time (all $P < 0.001$). Intestinal and maternal isolates did not show significant changes over time.

Intestinal CoNS isolates were more antibiotic resistant and *mecA* positive than other isolates. Maternal isolates showed less antibiotic resistance and *mecA* carriage than other isolates.

Staphylococcus epidermidis was the most prevalent species among skin (33%) and intestinal (53%) isolates (Fig. 2). Compared to intestinal isolates, *Staphylococcus warneri* was more prevalent among skin isolates (23% versus 9%; $P = 0.002$). *Staphylococcus haemolyticus* prevalence increased significantly over time among skin isolates (9% at 24 h versus 25% at 21 days; $P = 0.002$) (see Fig. S1 in the supplemental material). Other species did not change significantly over time. Compared to other species, *S. warneri* isolates were significantly less resistant to levofloxacin (3% versus 32%; $P < 0.001$), cotrimoxazole (3% versus 24%; $P < 0.001$), and erythromycin (34% versus 58%; $P < 0.001$) and less multidrug resistant (52% versus 67%, $P = 0.004$) resistant. Levofloxacin ($P = 0.004$), gentamicin ($P = 0.002$), cefoxitin ($P < 0.001$), and multidrug ($P = 0.004$) resistance and *mecA* carriage ($P < 0.001$) increased over time among *S. haemolyticus* skin isolates (see Fig. S2 in the supplemental material).

To our knowledge, this is the first study to show the dynamics of antibiotic resistance in CoNS on the skin and in the gut of neonates. As expected, antibiotic resistance in CoNS on the skin of neonates was low right after birth but increased rapidly in the first week of hospitalization. Staphylococci are among the first gut colonizers (3). In 1982, Wade et al. proposed that gut may be the primary source for infecting CoNS (4). In our study, antibiotic resistance in fecal isolates is very high from the beginning, probably due to postpartum antibiotics. *S. warneri*, which was the least resistant species, was significantly less prevalent among gut CoNS. It is unknown whether this is an effect of antibiotics or whether the gut is an unhealthy environment for *S. warneri*. As suggested before, *S. warneri* is probably a relatively harmless species in neonatal

sepsis (1), in contrast to *S. haemolyticus* (5–7). *S. haemolyticus* proved to be a good gut colonizer. During hospitalization, skin prevalence and antibiotic resistance of *S. haemolyticus* increased. Further studies on the role of *S. haemolyticus* in intestinal colonization and sepsis of neonates are therefore necessary.

There are potential flaws in our study. Out of practicality, we sampled only the bottom of one foot of each child and no other skin. We doubt that microbial colonization of feet and that of other exposed areas of skin differ at this age, as there is no weight pressure on the feet yet. Another possible flaw is that we picked only three colonies from each cultured sample. In theory, this could result in either an under- or overestimation of resistant isolates. As there were usually no more than three different strains detected in each sample, we assume that an analysis of all strains would not change our results significantly.

In summary, we showed that neonates are colonized with resistant CoNS right after birth, especially in the gut. Resistant skin isolates, especially *S. haemolyticus*, become more prevalent during hospitalization in the NICU, while prevalence of the antibiotic-sensitive *S. warneri* decreases, implying important resistance differences among CoNS species. Our data contribute to an increased understanding of CoNS colonization dynamics and possibly to the development of preventive strategies, for example by stimulation of less virulent CoNS species.

ACKNOWLEDGMENTS

We thank all participating parents and infants for their friendly cooperation. Furthermore, we thank Wim Hop for statistical advice and Joyce van der Weijde and Remco van Zundert for their excellent technical assistance.

There were no conflicts of interest.

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