JCM Accepts, published online ahead of print on 7 November 2012
J. Clin. Microbiol. doi:10.1128/JCM.02935-12

Colonization dynamics of antibiotic resistant coagulase-negative staphylococci in neonates

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28 Keywords: antibiotics, neonatal intensive care unit, staphylococcus epidermidis, mecA,

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30 Abbreviated title: Neonatal colonization dynamics of antibiotic resistant coagulase-

31 negative staphylococci

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33 Running title: antibiotic resistant CoNS in neonates

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35 **ABSTRACT**

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

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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 (3, 6). Previously, it was shown that the majority of CoNS causing sepsis among neonates can be found on the hands of NICU personnel (3). 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 admission. A better understanding of CoNS colonization dynamics may assist to the development of future 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 inborn infants with a presumed admission time of at least seven days (gestational age ≤ 30 weeks, birthweight ≤ 1500g or other reason) were included in this study. Children who were discharged within 72 hours were excluded.

We performed a longitudinal study of skin and intestinal carriage of CoNS among neonates and their mothers. All infants were sampled 24, 48 and 72 hours (+/- 4 hours) and, if still admitted, 7, 14 and 21 days after birth. Their mothers were sampled only once in the first 3 days, and after 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 their thumb (3). Selection, culturing, storage, species determination by internal transcribed spacer PCR, antibiotic susceptibility determination and statistical analysis were performed as previously described (3), 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 included in the study. One infant was excluded after being discharged within 72 hours. General characteristics are summarized in Table 1.

For all time points, CoNS cultures were positive for all children except for nine (23%) children at 24 hours after birth of which one also at 48 hours after birth.

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

Skin isolates showed increasing antibiotic resistance for levofloxacin, gentamicin, cefoxitin, increasing *mecA* carriage and increasing 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 compared to other isolates.

S. epidermidis was the most prevalent species among skin (33%) and intestinal (53%) isolates (Figure 2). Compared to intestinal isolates, *S. warneri* was more prevalent among skin isolates (23% vs. 9%, P=0.002). *S. haemolyticus* prevalence increased significantly over time among skin isolates (9%, T=24 hours vs. 25%, T=21 days, P=0.002)

(Figure S1). Other species did not change significantly over time. Compared to other species, *S. warneri* isolates were significantly less levofloxacin (3% vs. 32%, $P<0.001$), co-trimoxazole (3% vs. 24%, $P<0.001$), erythromycin (34% vs 58%, $P<0.001$) and multidrug (52% vs. 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 (Figure S2).

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 increases rapidly in the first week of admission. Staphylococci are among the first gut colonizers (1). In 1982 Wade et. al. proposed that gut may be the primary source for infecting CoNS (7). 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 the gut is an unhealthy environment for *S. warneri*. As suggested before, *S. warneri* is probably a relatively harmless species in neonatal sepsis (3), in contrast to *S. haemolyticus* (2, 4, 5). *S. haemolyticus* proved to be a good gut colonizer. During admission, skin prevalence and antibiotic resistance of *S. haemolyticus* increases. 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 only sampled the bottom of one foot of the children and no other skin parts. We doubt that microbial colonization of feet and other exposed skin differ at this age, as there is no weight pressure on the feet yet. Another possible flaw is that we only picked 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 visible in each sample, we assume that analysis of all strains not change our result 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 admission on 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 preventive strategies, for example by stimulation of less virulent CoNS species.

ACKNOWLEDGEMENTS

The authors would like to thank all participating parents and infants for their friendly cooperation. Furthermore, the authors would like to 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.

REFERENCES

1. **Adlerberth, I., E. Lindberg, N. Aberg, B. Hesselmar, R. Saalman, I. L. Strannegard, and A. E. Wold.** 2006. Reduced enterobacterial and increased staphylococcal colonization of the infantile bowel: an effect of hygienic lifestyle? Pediatr Res **59:**96-101.
2. **Foka, A., V. Chini, E. Petinaki, F. Kolonitsiou, E. D. Anastassiou, G. Dimitracopoulos, and I. Spiliopoulou.** 2006. Clonality of slime-producing methicillin-resistant coagulase-negative staphylococci disseminated in the neonatal intensive care unit of a university hospital. Clin Microbiol Infect **12:**1230-1233.
3. **Hira, V., M. Sluijter, W. H. Goessens, A. Ott, R. de Groot, P. W. Hermans, and R. F. Kornelisse.** 2010. Coagulase-negative staphylococcal skin carriage among neonatal intensive care unit personnel: from population to infection. J Clin Microbiol **48:**3876-3881.
4. **Klingenberg, C., A. Ronnestad, A. S. Anderson, T. G. Abrahamsen, J. Zorman, A. Villaruz, T. Flaegstad, M. Otto, and J. E. Sollid.** 2007. Persistent strains of coagulase-negative staphylococci in a neonatal intensive care unit: virulence factors and invasiveness. Clin Microbiol Infect **13:**1100-1111.
5. **Low, D. E., B. K. Schmidt, H. M. Kirpalani, R. Moodie, B. Kreiswirth, A. Matlow, and E. L. Ford-Jones.** 1992. An endemic strain of Staphylococcus haemolyticus colonizing and causing bacteremia in neonatal intensive care unit patients. Pediatrics **89:**696-700.

6. **Szewczyk, E. M., A. Piotrowski, and M. Rozalska.** 2000. Predominant staphylococci in the intensive care unit of a paediatric hospital. J Hosp Infect **45:**145-154.

7. **Wade, J. C., S. C. Schimpff, K. A. Newman, and P. H. Wiernik.** 1982. Staphylococcus epidermidis: an increasing cause of infection in patients with granulocytopenia. Ann Intern Med **97:**503-508.

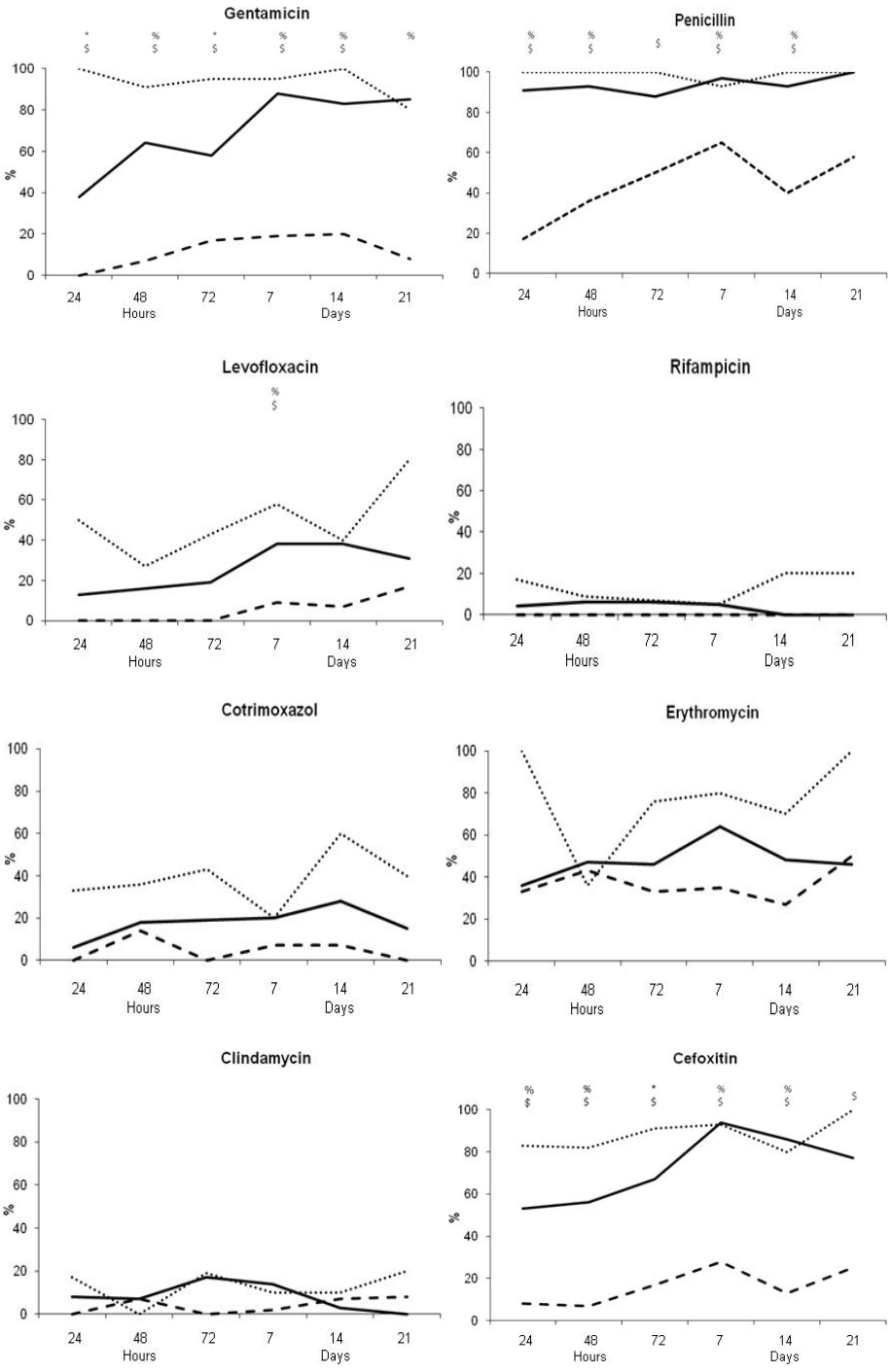
Table 1. General characteristics of included neonates

Number of patients	40
Birthweight (grams) (range; sd)	1185 (530-1650; 291)
Gestational age (weeks) (range; sd)	28 4/7 (25-32 2/7; 1 6/7)
Male (%)	65
Ceasarian section (%)	33
Postpartum antibiotics[1] (%)	93
Antibiotics during admission[2] (%)	80
Hospitalisation (days) (range)	21.6 (5-92; 19.4)
Mortality (%)	13

Data are expressed as mean, unless specified otherwise. sd: standard deviation

[1]Postpartum antibiotics consisted of one gift of penicillin and gentamicin directly postpartum.

[2]Antibiotics were given on indication (e.g. suspected or proven nosocomial infection). The choice of antibiotics depended on the indication.



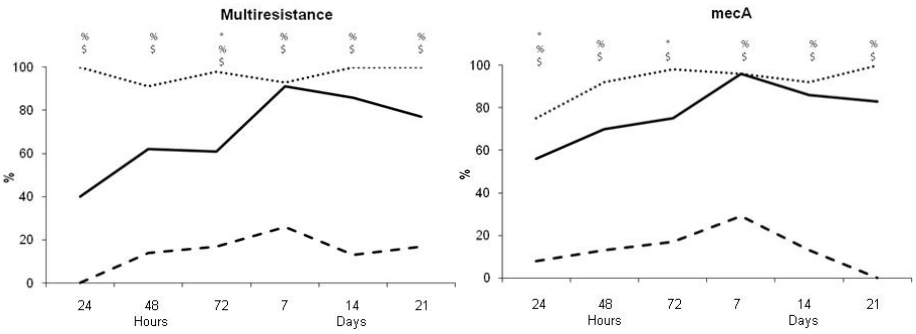


Figure 1. Rates of resistant isolates (%) for each tested antibiotic at different time points. The solid line (—) depicts neonatal skin isolates, the dotted line (···) depicts neonatal intestine isolates and the dashed line (---) depicts maternal isolates. Statistically significant differences are marked by * for difference between skin and gut isolates, by % for difference between skin and maternal isolates, and by $ for difference between gut and maternal isolates.

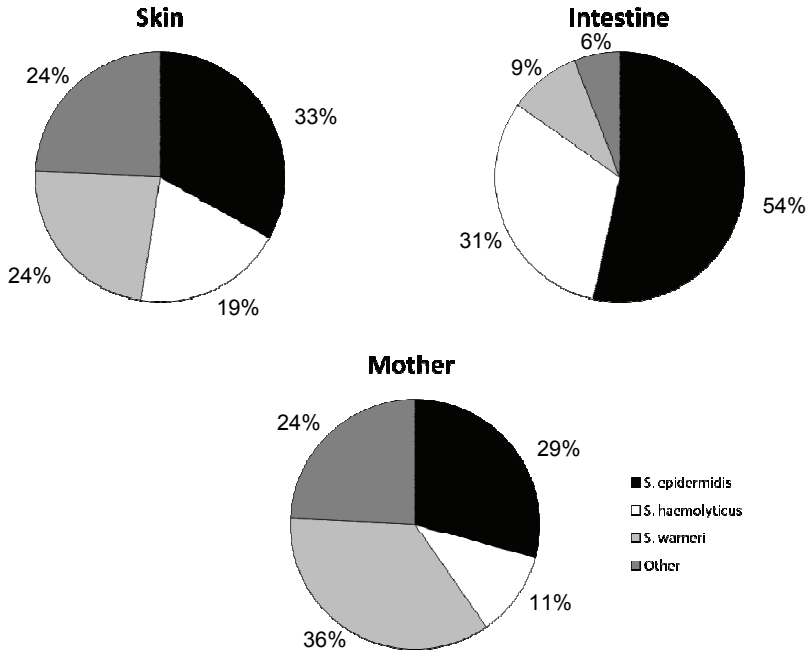


Figure 2. Bacterial species distribution among the different study groups.