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## Colonization pattern of coagulase-negative staphylococci in preterm neonates and the relation to bacteremia

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Abstract: Coagulase-negative staphylococci (CoNS) are the major cause of sepsis in extreme preterm (EPT) newborns, but data about the CoNS colonization in EPT newborns prior to invasive infection are limited.

Our aim was to describe the early establishment of the CoNS microflora in EPT newborns and to compare the colonization pattern in neonates with and without positive CoNS blood cultures. From a cohort of 46 EPT neonates, newborns with positive CoNS blood culture were identified (n = 10) and compared with matched controls. Samples for bacterial cultures were obtained repetitively from nares, perineum, and umbilicus. All CoNS isolates were characterized using the PhenePlate system for biochemical fingerprinting.

Persistent CoNS strains were found on d 2-3 after delivery in 7/20 newborns, and there was a tendency for earlier colonization in nares than in perineum or umbilicus. The CoNS blood strains were prevalent in superficial sites prior to positive blood culture (11/14 blood strains), but no single invasive pathway was identified. Most CoNS blood strains (9/14) persisted on superficial sites after antibiotic treatment.

We hypothesize that the invasive pathways in neonatal CoNS sepsis are complex and that colonization of mucosal membranes and umbilical catheters might be of equal importance.

Response to Reviewers: The comments of the reviewer have been a great help for us when revising the manuscript, and we want to express our thanks to the reviewer for constructive criticism.

As listed below, we would like to respond to the reviewer's comments.

1. The reviewer asked whether restriction of the cases to only those classified as having true sepsis (and thus exclusion of the cases most probably representing contaminants) would change the results. - In the original version of the manuscript we decided to compare only "blood culture positive" vs "bloodculture negative" in order to avoid any kind of bias according to the classification in "sepsis" and "contaminants". However, we agree with the reviewer that comparison of "sepsis" - "non-sepsis" is

of interest and has included that kind of analysis in the results (page 10 line 22 and page 10 line 48). In summary these comparisons show the same trends as for the whole study group, however the numbers of included sepsis-newborns are limited, and the differences thus not statistically significant. - Our proportion of newborns with true CoNS sepsis however (6/46) however is similar to the incidence reported from other Western centra.

2. Why do we not present data on all 46 newborns? - In the present study we choose a case-control design and consider this as relevant, since only a minor proportion of the total cohort displayed a CoNS positive blood culture. However, we are just now analysing the data from all 46 preterms compared to healthy term newborns and plan to present these data in future manuscript.

3. Why do the findings of PFGE not quite match the PhP data? - A comment on this has been included in the Discussion (page 15 line 34).

4. Results of PFGE for typing validation of pairs of colonising and blood isolates identified as identical from PhP? - data has been included in the results (page 11 line 56).

5. Missing reference in the discussion. - Error in the proof by us. - The last part of the sentence that would correspond to the missing reference was thought to be omitted already in the original manuscript. This is now corrected.

We hope that the reviewer will find the new version of the manuscript improved compared to the original version and suggest it for publication in EJCMIID.

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# Colonization pattern of coagulase-negative staphylococci in preterm neonates and the relation to bacteraemia

Running title: Preterm CoNS colonization and bacteraemia

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**ABSTRACT**

1  
2 Coagulase-negative staphylococci (CoNS) are the major cause of sepsis in extreme preterm  
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4 (EPT) newborns, but data about the CoNS colonization in EPT newborns prior to invasive  
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6 infection are limited.  
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9 Our aim was to describe the early establishment of the CoNS microflora in EPT newborns  
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11 and to compare the colonization pattern in neonates with and without positive CoNS blood  
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13 cultures.  
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16 From a cohort of 46 EPT neonates, newborns with positive CoNS blood culture were  
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18 identified (n = 10) and compared with matched controls. Samples for bacterial cultures were  
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20 obtained repetitively from nares, perineum, and umbilicus. All CoNS isolates were  
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22 characterized using the PhenePlate system for biochemical fingerprinting.  
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26 Persistent CoNS strains were found on d 2–3 after delivery in 7/20 newborns, and there was a  
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28 tendency for earlier colonization in nares than in perineum or umbilicus. The CoNS blood  
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30 strains were prevalent in superficial sites prior to positive blood culture (11/14 blood strains),  
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32 but no single invasive pathway was identified. Most CoNS blood strains (9/14) persisted on  
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34 superficial sites after antibiotic treatment.  
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38 We hypothesize that the invasive pathways in neonatal CoNS sepsis are complex and that  
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40 colonization of mucosal membranes and umbilical catheters might be of equal importance.  
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## INTRODUCTION

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2 In healthy term infants and other non-compromised individuals, coagulase-negative  
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4 staphylococci (CoNS) represent commensals with low invasive potential. However, in  
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6 immunocompromised patients and those with infections related to indwelling medical  
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8 devices, CoNS infections constitute a significant nosocomial problem. Preterm newborns,  
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10 especially extreme preterm (EPT) infants, are prone to CoNS sepsis, since they are  
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12 immunologically immature, have pervious skin and mucosal barriers, and are regularly  
13  
14 exposed to invasive procedures during neonatal intensive care (1, 2). Although the acute  
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16 mortality is low, CoNS sepsis is associated with a significant increase in acute neonatal  
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18 morbidity, and most probably contributes to the development of post-inflammatory sequelae  
19  
20 such as bronchopulmonary dysplasia and CP syndromes (3–5).  
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26 Neonatal CoNS infections represent late onset sepsis, with onset of symptoms at 7–15 days  
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28 postnatal age (6). Since persistence of single CoNS clones for several years is well  
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30 documented in neonatal intensive care units (NICUs) (7–10), as is the emergence of multi  
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32 drug resistant CoNS strains (including glycopeptides) (7, 8), there is an urgent need for  
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34 epidemiologic surveillance in the NICUs and preventive/infection control strategies against  
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36 neonatal invasive CoNS infections (11, 8).  
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41 CoNS possess a unique ability to colonize the skin and mucous membranes, and newborn  
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43 children become colonized by their first CoNS strains early after birth (12–14). In EPT  
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45 newborns, most of these early colonizing CoNS strains are acquired from the NICU  
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47 environment (12, 13). However, detailed data about the establishment of the CoNS microflora  
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49 in EPT newborns, as well as the correlation between superficial and invasive isolates of  
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51 CoNS, are limited. In order to elaborate better strategies for the prevention of neonatal CoNS  
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53 septicaemia, further insights into the colonization pattern of CoNS in EPT newborns prior to  
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55 deep infection are necessary.  
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1 Methodological difficulties constitute the major reasons for our limited knowledge of the  
2 establishment and dynamics of the normal flora of the newborn. Pulsed field gel  
3 electrophoresis (PFGE) and other conventional genetic typing methods are laborious and not  
4 applicable to the large populations of bacterial isolates found in superficial samples, whereas  
5 earlier phenotypic typing methods do not show appropriate discriminatory power (15, 16).  
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10 Phenotypical fingerprinting based on multiple biochemical characteristics might be a useful  
11 tool for comprehensive typing of larger numbers of CoNS isolates. One such biochemical  
12 fingerprinting assay is the PhenePlate system (PhP), which is well validated as a screening  
13 tool for selection of related CoNS isolates (17).  
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21 The first aim of the present study was to describe the early establishment of the CoNS  
22 microflora in EPT newborns, and the second aim was to compare the colonization patterns in  
23 bacteraemic neonates with those in preterm infants without invasive CoNS infection.  
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## MATERIAL AND METHODS

### *Patients*

Preterm newborns <31 w gestational age, born at the delivery ward at Örebro University hospital and admitted to the NICU between April 2004 and November 2006, were eligible for the study. Samples for bacterial cultures from nares, umbilicus, and perineum were obtained using sterile, dry cotton swabs at 24 h and on day 2, d 5–6, d 7–8, and d 14 after delivery.

Samples for blood cultures were initiated when clinically indicated. For sepsis evaluation, 0.5–1 ml of blood was obtained by peripheral venous puncture or by a newly inserted vascular umbilical catheter.

During the study period, 46 newborns were consecutively enrolled. From this cohort, all newborns  $\leq 28$  days of age with blood cultures yielding CoNS and no other pathogen were retrospectively identified and selected for further evaluation. For each case, a control newborn matched for birth date (first priority) and gestational age (second priority) and with blood cultures negative for CoNS was selected from the same original study population. Data concerning invasive infections and antibiotic treatment during the first 28 d of postnatal age were collected.

Neonatal sepsis was defined as a positive blood culture in combination with  $\geq 3$  clinical signs consistent with infection and a peak C-reactive protein  $\geq 20$  mg/L within 72 h from onset of symptoms. Patients that were transferred to another hospital or died within 28 d of age were excluded.

The NICU at Örebro University Hospital provides full neonatal intensive care, except for major neonatal surgery. Immediate postnatal rescue measurements are performed with attention to sterile techniques. All NICU staff members are instructed to use 70% alcohol gel for hand disinfection and to wear plastic gloves when handling individual patients.



### *Bacterial samples*

1  
2 Bacterial samples from superficial sites were cultured overnight on blood agar medium  
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4 (4.25% Columbia II Agar [BBL, Becton Dickinson, Baltimore, MD, USA], 0.3% Agar No. 2  
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6 [LAB M Ltd., Bury, UK], and 5% bovine blood). All the growth on the agar plates was  
7  
8 sampled and dissolved in preservation medium (yeast extract; DIFCO Laboratories, Sparks,  
9  
10 MD, USA) and horse serum added trypticase soy broth (TSB; BBL, Sparks MD, USA), and  
11  
12 stored at -70°C pending further analysis.  
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17 Samples for blood cultures were immediately administered into BACTEC Peds bottles  
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19 (Becton Dickinson, Franklin Lakes, NJ, USA). A non-radiometric BACTEC 9240 system was  
20  
21 used to detect bacteria and fungi.  
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25 Minimum inhibitory concentration (MIC) determinations were performed using the Etest (AB  
26  
27 Biodisk, Solna, Sweden).  
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30 The species of the blood isolates of CoNS were identified biochemically by the ID32 Staph  
31  
32 (bioMérieux, Marcy l'Etoile, France) and in some cases confirmed by sequencing the *rpoB*  
33  
34 gene, as previously described (18).  
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### *PhenePlate system*

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41 Bacterial samples from nares, umbilicus, and perineum were subcultured overnight at 37°C  
42  
43 on blood agar medium. Ten CoNS colonies from each sample were randomly chosen and  
44  
45 subsequently biochemically fingerprinted using the PhP system modified for typing of CoNS  
46  
47 (PhPlate AB, Stockholm, Sweden), as previously described (17). In summary, the system  
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49 consists of 96-well microtitre plates, each with four sets of 22 different reagents. Colour  
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51 indicators visualize changes in pH as a result of bacterial metabolization of these substrates.  
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56 The absorbance value for each reaction is measured repeatedly and the mean value is  
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58 calculated for each reaction. The results for different strains are compared in pairs, and the  
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1 correlation coefficients are clustered using the unweighted pair group method of arithmetic  
2 mean (UPGMA). In the present study the absorbance was measured after 16, 40, and 64 hours  
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4 at 620 nm in accordance with the manufacturer's instructions, and isolates with a correlation  
5  
6 coefficient  $\geq 0.975$  were regarded as belonging to the same PhP type (19). All data processing,  
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8 including optical readings and calculations of correlation coefficients as well as clustering  
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10 dendrograms, was performed with PhenePlate software.  
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### 16 *PFGE*

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19 Characterization of the CoNS isolates was performed by PFGE of chromosomal *SmaI* digests,  
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21 prepared with the GenePath group I reagent kit (Bio-Rad Laboratories, Hercules, CA), and  
22  
23 PFGE patterns were obtained with a contour-clamped homogenous electric field apparatus  
24  
25 (GenePath System; Bio-Rad) as previously described (9). Differences in band patterns were  
26  
27 processed and analyzed with Molecular Analyst Fingerprinting software (version 1.6; Bio-  
28  
29 Rad Laboratories, Hercules, CA, USA) using the Dice coefficient, and represented by  
30  
31 unweighted pair grouping by mathematical averaging (UPGMA) with 1% band tolerance and  
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33 0.5% optimization, but finally documented by visual examination and interpreted as  
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35 recommended by Tenover *et al.* (20).  
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### 44 *Ethics*

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46 The regional committee for medical research ethics approved the study, and parental informed  
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48 consent was required for participation.  
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*Statistics*

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2 The Mann-Whitney U test and Student's unpaired *t* test were used for two-group assessments  
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4 of differences in continuous variables. Fisher's exact test was used to estimate differences in  
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6 proportions.  
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9 The phenotypic diversity of the CoNS isolates was calculated as Simpson's Diversity Index  
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11 ( $D_i$ ) using the PhenePlate software. A high  $D_i$  indicates that the analyzed isolates are evenly  
12  
13 distributed among the different bacterial phenotypes. The  $D_i$  was calculated both for all  
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15 isolates obtained from each patient and for selected isolates; specifically, only one of each set  
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17 of identical isolates (with a correlation coefficient  $\geq 0.975$ ) found at each sampling site and  
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19 point of time was included.  
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## RESULTS

### *Patients*

Ten preterm newborns with blood cultures positive for CoNS (patients A–J), representing 11 episodes, were identified during the study period, and they were matched with 10 controls.

Two pairs of cases and controls were twins. The demographic characteristics are given in Table 1. One patient (patient G) contributed with two episodes. Six of the eleven episodes met the criteria for CoNS sepsis.

One of the controls was diagnosed with *E. coli* bacteraemia at delivery. No positive blood cultures were found among the other nine controls.

All 10 blood culture–positive newborns and 6 of the controls received parenteral antibiotics (Table 1). Cefotaxime + gentamicin was administered to 14 newborns (10 cases and 4 controls) at one or more episodes. Vancomycin was administered to 8 of the 9 cases with meticillin-resistant CoNS isolates in blood cultures (Fig. 1) and to one of the controls.

All newborns had an umbilical arterial or venous catheter inserted soon after birth, and in 4 of the 10 blood culture–positive patients the umbilical catheter was still present at the time of a positive CoNS blood culture. The duration of indwelling umbilical catheters did not differ between the patients and the controls (median 5 d, range 2 d – 8 d).

### *Consecutive CoNS colonization by location in cases and controls*

Seven of the 20 investigated preterm newborns were already colonized by 1–6 different CoNS isolates at < 24 h after delivery, one newborn in the umbilicus and the others in the nares or perineum. Except in one newborn, none of these initially recovered CoNS strains persisted beyond 24 h of age. Soon thereafter, however, a more persistent CoNS microflora was established. Fourteen of the 18 newborns that were sampled on d 2–3 were colonized in the

nares (n = 10) and/or in the umbilicus (n = 5) or perineum (n = 7), and in 13 of these 18 newborns one or more of these strains could still be recovered on d 14. On d 5–7 most newborns were colonized both in the nares (19/20) and in the umbilicus (17/20).

The numbers of isolates eligible for further investigation varied among the included newborns, since some cultures from superficial sites (especially those sampled at the first event) yielded fewer than the 10 colonies of CoNS per sample that were stipulated for PhP, and some displayed no growth of bacteria at all. From the blood culture–positive group of newborns, a median of 105 isolates per case (range 62–151) were eligible for further investigation, compared with a median of 85 isolates in the controls (range 6–110, p = 0.034). The same tendency, though not statistically significant, was found when the comparison was restricted to the cases classified as representing true sepsis (n = 6) and their corresponding controls; From the sepsis cases a median of 101 isolates per case were retrieved (range 62 – 100) compared to a median of 85 isolates from the sepsis controls (n = 6, range 75 – 110, p = 0.34). In total, 1916 CoNS isolates were characterized by PhP.

#### *Diversity in CoNS colonization in cases and controls*

A representative clustering dendrogram based on the PhP characteristics of all CoNS isolates retrieved from one individual is depicted in Fig. 2. The  $D_i$  for the cases (mean 0.88, SD 0.062) was significantly higher than for the controls (mean 0.79, SD 0.012, p = 0.04) when all isolates for each individual newborn were included in the analysis (n = 1896 isolates). Also the  $D_i$  for group of cases representing sepsis (mean 0.88, SD 0.047, n = 6) tended to be higher compared to the sepsis controls (mean 0.78, SD 0.129), however that difference was not statistically significant (p = 0.125). When only one isolate representing the same site and the same point of time for isolation within the same phenotypic cluster was selected for further assessment (n = 674 isolates in 20 infants), there were no significant differences in  $D_i$  or in

1 numbers of phenotypic clusters and single isolates between the two groups of newborns  
2 (Table 2).  
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### 6 *Blood cultures*

7 Fifteen blood isolates of CoNS representing 11 episodes were recovered from patients A–J.  
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9 Four patients each contributed two isolates from the same blood culture. One sepsis isolate  
10 (patient G, episode 1) could not be recultivated. Of the remaining 14 CoNS isolates, 11 were  
11 identified as *S. epidermidis*, one as *S. lugdunensis*, and two as *S. capitis*. All but two of the  
12 blood isolates were meticillin-resistant, and all isolates were sensitive to vancomycin (Table  
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24 The blood isolates were compared according to phenotype (PhP) and also characterized  
25 genotypically (PFGE), as illustrated in Fig. 3. Some differences were found between the  
26 phenotypic and genotypic results, reflecting the different properties of the two methods. The  
27 majority of the *S. epidermidis* strains appeared in two clusters phenotypically, though with a  
28 correlation coefficient not reaching  $\geq 0.975$ . Three pairs of CoNS strains were genetically  
29 closely related according to the PFGE patterns. The species *S. lugdunensis* and *S. capitis*  
30 differed both phenotypically and genotypically from the *S. epidermidis* (Fig. 3).  
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### 44 *Superficial colonization of CoNS in relation to invasiveness and antibiotic treatment*

45 The invasive blood isolates were prevalent among the colonizing flora in the neonates prior to  
46 positive blood cultures. In 11/14 of the blood isolates, skin or mucosal isolates belonging to  
47 the same phenotypic cluster were also found on superficial sites of the same patient, and the  
48 colonization rate did not differ between nares (n = 6 strains), perineum (n = 6 strains), and  
49 umbilicus (n = 4 strains). In addition, the PFGE patterns were indistinguishable in all but one  
50 of the cases when comparing the superficial isolates with blood isolates that clustered  
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1 together, Three blood isolates did not cluster with any colonizing isolates obtained from their  
2 corresponding patients and thus were regarded as single isolates. Two of these isolates  
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4 (patients B1: *S. lugdunensis* and J1: *S. epidermidis*) originated from blood cultures yielding  
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6 two strains of CoNS of different species, and one isolate (patient F) originated from a blood  
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8 culture obtained at 24 h after delivery. The colonization patterns of the individual patients in  
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10 relation to positive blood culture and antibiotic exposure are shown in Fig. 1.  
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14 In the majority of the patients, isolates belonging to the same phenotypic cluster as the blood  
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16 isolates still persisted on superficial sites after antibiotic treatment (9/14 isolates, Fig. 1).  
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19 Some of the CoNS blood strains were also prevalent among the superficial CoNS microflora  
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21 in the blood culture–negative controls. For example, superficial isolates clustering together  
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23 with the blood isolates 06B-861, 06B-105, and 06B-080 were found in at least half of the  
24  
25 control patients.  
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### 28 29 30 31 *CoNS colonization following Caesarean section or vaginal delivery*

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33 Delivery mode was not associated with any differences in CoNS colonization. Seventy  
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35 percent of the included newborns were delivered by acute Caesarean section (CS); of these,  
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37 7/14 infants were colonized by CoNS at 24 h postnatal age compared to 1/6 of the vaginally  
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39 delivered newborns ( $p = 0.32$ ). CoNS strains that still persisted on d 14 were recovered on day  
40  
41 2–3 in 9/12 newborns delivered by CS and in 4/6 delivered vaginally ( $p = 0.99$ ). Six newborns  
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43 (5 in the CS group and one vaginally delivered) were born after preterm rupture of the  
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45 membranes. The CoNS colonization patterns in these six newborns were similar to those in all  
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51 the other newborns (data not shown).  
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## DISCUSSION

In the present study, we found phenotyping by biochemical fingerprinting (PhP) to be a useful tool for screening of related isolates among a vast number of CoNS isolates. This made it possible to investigate the establishment of the colonizing CoNS microflora in EPT newborns with a higher discriminatory power than most earlier methods could offer (15, 16). In two-thirds of the investigated preterm newborns, colonizing CoNS strains that persisted during the first postnatal weeks were already retrieved on postnatal day 2–3. These findings are in contrast to earlier reports in which the early CoNS microflora was found to be transient and characterized by a considerable day-to-day variation (12–14, 21). However, data regarding CoNS colonization in preterm newborns are limited, and most studies were conducted in the early 1990s. Since then, neonatal intensive care has dramatically changed; early enteral feeding, decreased incidence of severe respiratory distress, and consistent education of all NICU staff members in hand disinfection are examples of achievements that most probably affect the CoNS microflora in NICU treated newborns nowadays.

In comparison to infants with negative CoNS cultures, blood culture–positive newborns showed a higher number of CoNS isolates on colonizing sites as well as a tendency to be colonized earlier after delivery. This might reflect increased transmission of CoNS strains from NICU staff members as a result of more frequent and more acute handling of the sicker newborns, who are at greater risk for later acquiring CoNS bacteraemia. The majority of the blood strains were prevalent in the superficial microflora on several sites before being retrieved from the blood, but no single predominating invasive pathway could be identified. This indicates that several loci may serve as the entry in cases of neonatal CoNS bacteraemia. We found indications for earlier CoNS colonization in the nares than in the perineum or in the umbilicus, and 80% of the cases were colonized in the nares prior to, or at the same time as,



1 acquisition of positive CoNS blood cultures. Relatively stable CoNS reservoirs in the nares  
2 and faeces of preterm newborns have been reported previously (13). In adult  
3  
4 immunocompromised patients, the possibility of mucosal colonization as an entry for invasive  
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6 CoNS infections has been reviewed by Costal *et al.* (22). Mucosal barriers in preterm  
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8 newborns are generally penetrable, but the epithelium of the upper respiratory tract is, in  
9  
10 addition, regularly traumatized by the insertion of gastric sounds, CPAP adapters, air jet  
11  
12 beams, and nasotracheal tubes, as well as by airway suction procedures. The numbers of  
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14 patients in the present study are too small to draw any firm conclusions, but it may be worth  
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16 considering that CoNS bacteraemia in newborns might be associated with various invasive  
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18 entries.  
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24 Most investigations of pathogenic mechanisms in CoNS infections so far have focused on  
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26 bacterial interactions with fibrinogen-coated polystyrene surfaces, for example, the bacterial  
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28 formation of biofilm or expression of adhesion molecules, as well as other factors of  
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30 importance in infections associated with indwelling medical devices (23, 24). These  
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32 pathogenic mechanisms are probably important in the pathogenesis of neonatal infections, but  
33  
34 no specific virulence factors that discriminate between invasive and colonizing neonatal  
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36 CoNS isolates have been identified (7, 25, 26). In EPT newborns, bacterial interactions with  
37  
38 skin and mucosal membranes might be as important as colonization of umbilical catheters,  
39  
40 and hence a broad approach is necessary when investigating the pathogenesis in neonatal  
41  
42 CoNS infections.  
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48 We did not find any differences in CoNS colonization patterns associated with delivery mode  
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50 or preterm rupture of the membranes, and thus no indications of maternal transferral of  
51  
52 persisting or invasive CoNS strains, which is in accordance with earlier reports (12, 13). It is  
53  
54 well documented that EPT newborns in the NICU environment are predominantly colonized  
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56 by nosocomial strains of CoNS, and that these strains may persist in the NICU for years,  
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1 which emphasizes the concern of nosocomial spread of multiresistant bacterial strains in the  
2 neonatal wards (11, 8, 7).  
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4 In the present study, CoNS strains phenotypically indistinguishable from CoNS blood strains  
5 persisted on superficial sites after treatment with relevant antibiotics, and several of the  
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invasive CoNS strains were also prevalent in the colonizing microflora in the blood culture–  
negative newborns. This might indicate the presence of bacterial virulence factors that  
increase the ability of nosocomial CoNS strains to colonize and persist on the skin and  
mucous membranes.

In one of the patients, the blood culture yielded both a strain of *S. epidermidis* and an isolate  
of *S. lugdunensis*. Except for one case report (27), *S. lugdunensis* is not known to cause  
neonatal bacteraemia, though this species appears to be more virulent than most other CoNS  
species (28). It was not possible to determine if this finding of *S. lugdunensis* represents true  
sepsis or a contaminant transferred by the NICU staff. However, *S. lugdunensis* was not found  
in any of the superficial skin cultures from any of the newborns.

Since PhP reveals phenotypic and PFGE genotypic polymorphism, it is not reasonable to  
expect a 100% concordance between these two typing methods. In an earlier study, we  
evaluated PhP for long-term characterization of CoNS blood isolate, and found the method  
less discriminatory than PFGE (9). In the present study, multiple CoNS isolates that were  
retrieved from each single individual during a limited time period were compared. For this  
type of investigation, PhP has shown a satisfactory capacity for identification of  
phenotypically related CoNS isolates (17), but for long-term surveillance, robust genetic  
typing methods that could be carried out on large bacterial populations are warranted.

In summary, we report early acquisition of nosocomial CoNS strains in the colonizing  
microflora of preterm newborns, and note that these bacterial strains are able to persist  
regardless of antibiotic treatment. The invasive pathways in neonatal CoNS bacteraemia are

1 probably complex, and this should be taken into consideration in studies both of bacterial  
2 virulence factors and of when to carry out preventive strategies against invasive CoNS  
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4 infections in preterm newborns.  
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12  
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**REFERENCES**

1. Stoll BJ, Hansen N, Fanaroff AA, Wright LL, Carlo WA, Ehrenkranz RA, Lemons JA, Donovan EF, Stark AR, Tyson JE, Oh W, Bauer CR, Korones SB, Shankaran S, Laptook AR, Stevenson DK, Papile LA, Poole WK (2002). Late-onset sepsis in very low birth weight neonates: the experience of the NICHD Neonatal Research Network. *Pediatrics* 110:285-291
2. Fanaroff AA, Stoll BJ, Wright LL, Carlo WA, Ehrenkrans RA, Stark AR, Bauer CR, Donovan EF, Korones SB, Laptook AR, Lemons JA, Oh W, Papile LA, Shankaran S, Stevenson DK, Tyson JE, Poole WK; NICHD Neonatal Research Network (2007). Trends in neonatal morbidity and mortality for very low birthweight infants. *Am J Obstet Gynecol* 196:147.e1–8
3. Liljedahl M, Bodin L, Schollin J (2004). Coagulase-negative staphylococcal sepsis as a predictor of bronchopulmonary dysplasia. *Acta Paediatr* 93:211–215
4. Speer CP (2003). Inflammation and bronchopulmonary dysplasia. *Semin Neonatol* 8:29–38
5. Wilson-Costello D, Borawski E, Friedman MA, Redline R, Fanaroff AA, Hack M (1998). Perinatal correlates of cerebral palsy and other neurologic impairment among very low birth weight children. *Pediatrics* 102:315–322
6. Isaacs D (2003). A ten year, multicentre study of coagulase negative staphylococcal infections in Australian neonatal units. *Arch Dis Child Fetal Neonatal Ed* 88:F89–F93
7. Klingenberg C, Rønnestad A, Anderson AS, Abrahamsen TG, Zorman J, Villaruz A, Flægstad T, Otto M, Eriscon Sollid JE (2007). Persistent strains of coagulase-negative staphylococci in a neonatal intensive care unit: virulence factors and invasiveness. *Clin Microbiol Infect* 13:1100–1111

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8. Villari P, Saturno C, Iacuzio L (2000). Molecular epidemiology of *Staphylococcus epidermidis* in a neonatal intensive care unit over a three-year period. *J Clin Microbiol* 38:1740–1746
9. Björkqvist M, Söderquist B, Törnqvist E, Sjöberg L, Fredlund H, Kühn I, Colque-Navarro P, Schollin J (2002). Phenotypic and genotypic characterisation of blood isolates of coagulase-negative staphylococci in the newborn. *APMIS* 110:332–339
10. Vermont CL, Hartwig NG, Fleer A, de Man P, Verbrugh H, van den Anker J, de Groot R, van Belkum A (1998). Persistence of clones of coagulase-negative staphylococci among premature neonates in neonatal intensive care units: two-center study of bacterial genotyping and patient risk factors. *J Clin Microbiol* 36:2485–2490
11. Center KJ, Reboli AC, Hubler R, Rodgers GL, Long SS (2003). Decreased vancomycin susceptibility of coagulase-negative staphylococci in a neonatal intensive care unit: evidence of spread of *Staphylococcus warneri*. *J Clin Microbiol* 41:4660–4665
12. Keyworth N, Millar MR, Holland KT (1992). Development of cutaneous microflora in premature neonates. *Arch Dis Child* 67:797–801
13. Eastick K, Leeming JP, Bennett D, Millar MR (1996). Reservoirs of coagulase negative staphylococci in preterm infants. *Arch Dis Child Fetal Neonatal Ed* 74:F99–104
14. D’Angio CT, McGowan KL, Baumgart S, St Geme J, Harris MC (1989). Surface colonization with coagulase-negative staphylococci in premature neonates. *J Pediatr* 114:1029–1034
15. Sloos JH, Dijkshoorn L, Vogel L, van Boven CPA (2000). Performance of phenotypic and genotypic methods to determine the clinical relevance of serial blood

isolates of staphylococcus epidermidis in patients with septicemia. *J Clin Microbiol* 38:2488–2493

16. Spare MK, Tebbs SE, Lang S, Lambert PA, Worthington T, Lipkin GW, Elliott TS (2003). Genotypic and phenotypic properties of coagulase-negative staphylococci causing dialysis catheter-related sepsis. *J Hosp Infect* 54:272–278
17. Nilsson-Augustinsson Å, Koskela A, Öhman L, Söderquist B (2007). Characterization of coagulase-negative staphylococci isolated from patients with hip prostheses: use of phenotypic and genotypic analyses, including tests for the presence of the *ica* operon. *Eur J Clin Microbiol Infect Dis* 26:255–265
18. Hellmark B, Söderquist B, Unemo M (2009). Simultaneous species identification and detection of rifampicin resistance in staphylococci by sequencing of the *rpoB* gene. *Eur J Clin Microbiol Infect Dis* 28:183–190
19. Jung K, Brauner A, Kühn I. Typing of Coagulase-negative staphylococci from peritonitis in CAPD-patients by the PhP-CS system and REA (1995). *APMIS* 103:679–685
20. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, Swaminathan B (1995). Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 33:2233–2239
21. Hall SL, Riddell SW, Barnes WG, Meng L, Hall RT (1990). Evaluation of coagulase-negative staphylococcal isolates from serial nasopharyngeal cultures of premature infants. *Diagn Microbiol Infect Dis* 13:17–23
22. Costa SF, Miceli MH, Anaissie EJ (2004). Mucosa or skin as source of coagulase-negative staphylococcal bacteraemia? *Lancet Infect Dis* 4:278–286

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23. Vuong C, Otto M (2002). *Staphylococcus epidermidis* infections. *Microbes Infect* 4:481–489.
24. von Eiff C, Peters G, Heilmann C (2002). Pathogenesis of infections due to coagulase-negative staphylococci. *Lancet Infect Dis* 2:677–685
25. de Silva GD, Kantzanou M, Justice A, Massey RC, Wilkinson AR, Day NP, Peacock SJ (2002). The *ica* operon and biofilm production in coagulase-negative *Staphylococci* associated with carriage and disease in a neonatal intensive care unit. *J Clin Microbiol* 40:382–388.
26. Bradford R, Abdul Manan R, Daley AJ, Pearce C, Ramalingam A, D’Mello D, Mueller Y, Uahwatanasakul W, Qu Y, Grando D, Garland S, Deighton M (2006). Coagulase-negative staphylococci in very-low-birth-weight infants: inability of genetic markers to distinguish invasive strains from blood culture contaminants. *Eur J Clin Microbiol Infect Dis* 25:283–290
27. Tee WSN, Soh SY, Lin R, Loo LH (2003). *Staphylococcus lugdunensis* carrying the *mecA* gene causes catheter-associated bloodstream infection in premature neonate. *J Clin Microbiol* 41:519–520
28. Frank KL, del Pozo JL, Patel R (2008). From clinical microbiology to infection pathogenesis: how daring to be different works for *Staphylococcus lugdunensis*. *Clin Microbiol Rev* 21:111–133

## ABBREVIATIONS

1  
2  
3 CoNS = Coagulase-negative staphylococci

4  
5 EPT = extreme preterm

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7 PFGE = pulsed field gel electrophoresis

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9 PhP = PhenePlate system

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11 MIC = minimum inhibitory concentration

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13 UPGMA = unweighted pair grouping by mathematical averaging

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15  $D_i$  = diversity index

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**Table 1.** Preterm newborns with blood cultures yielding CoNS (cases) and blood culture–negative controls.

	Cases (n = 10)	Controls (n = 10)	p <sup>*</sup>
	Median (range)	Median (range)	
Gestational age	29 w + 3 d (24 w + 3d – 30w + 3d)	29 w + 3 d (24 w + 1 d – 30 w + 4 d)	0.88 n.s.
Birth weight	1067 g (590 g – 1495 g)	1282 g (600 g – 1570 g)	0.66 n.s.
Postnatal age at start of first antibiotic treatment	3.5 d (0 d – 10 d)	1.0 d (0 d – 5 d)	0.16 n.s.
Duration of antibiotic treatment <14 d after delivery	9.0 d (4 d – 14 d)	4.5 d (0 d – 13 d)	0.10 n.s.
Duration of antibiotic treatment ≤28 d after delivery	10 d (6 d – 19 d)	4.5 d (0 d – 23 d)	0.02

\*Mann-Whitney U-test

n.s. = not significant

**Table 2.** Diversity in colonizing CoNS isolates from nares, perineum, and umbilicus in preterm newborns with positive CoNS blood culture and in controls. In cases of multiplicates within an individual phenotypic cluster of isolates representing the same locus and point of time for isolation, only one of these isolates is selected for the analysis.

	Cases n = 10 mean (SD)	Controls n = 10 mean (SD)	p <sup>§</sup>
Numbers of isolates included	37.0 (11.40)	30.6 (8.35)	0.16
Diversity index	0.907 (0.033)	0.902 (0.037)	0.739
Numbers of PhP clusters	6.7 (2.16)	5.8 (2.70)	0.42
Numbers of single isolates	10.8 (3.39)	9.0 (4.19)	0.30

<sup>§</sup> unpaired *t* test

**Table 3.** CoNS blood isolates from preterm newborns. S = sensitive and R = resistant

Patient	Strain	Species	Antibiotic resistance		
			<i>Meticillin</i>	<i>Gentamicin</i>	<i>Vancomycin</i>
A	04B475	<i>S. epi</i>	R	R	S
•B	06B860	<i>S. lugdunensis</i>	S	S	S
	06B861	<i>S. epi</i>	R	S	S
C	05B780G	<i>S. epi</i>	R	R	S
	05B780V	<i>S. capitis</i>	R	R	S
•D	06B195	<i>S. epi</i>	R	S	S
•E	06B105	<i>S. epi</i>	S	S	S
F	04B360	<i>S. epi</i>	R	S	S
G*	04B671	<i>S. epi</i>	R	R	S
•H	06B081	<i>S. epi</i>	R	S	S
	06B080	<i>S. epi</i>	R	S	S
•I	06B558	<i>S. epi</i>	R	S	S
J	06B709	<i>S. epi</i>	R	R	S
	06B716	<i>S. capitis</i>	R	R	S

Minimum inhibitory concentration (MIC) cut-off values for the bacterial isolates to be classified as sensitive to the different antibiotics: Meticillin MIC <1 mg/L; Gentamicin MIC <0.25mg/L; Vancomycin MIC <4 mg/L; • indicates a sepsis patient; *S. epi* = *S. epidermidis*

\* second episode



## FIGURE LEGENDS

**Figure 1.** Relation between blood isolates of CoNS, superficial colonization by the same bacterial strain, and antibiotic treatment in CoNS blood culture–positive preterm newborns (n=10). ● = sepsis patient

**U** = umbilicus, **P** = perineum and **N** = nares. n.d = superficial bacterial culture not obtained.

↑ indicates the occurrence of positive CoNS blood culture (↑) = blood isolate not recultivated.

Bars indicate antibiotic treatment:  = bensylpenicillin + garamicin,

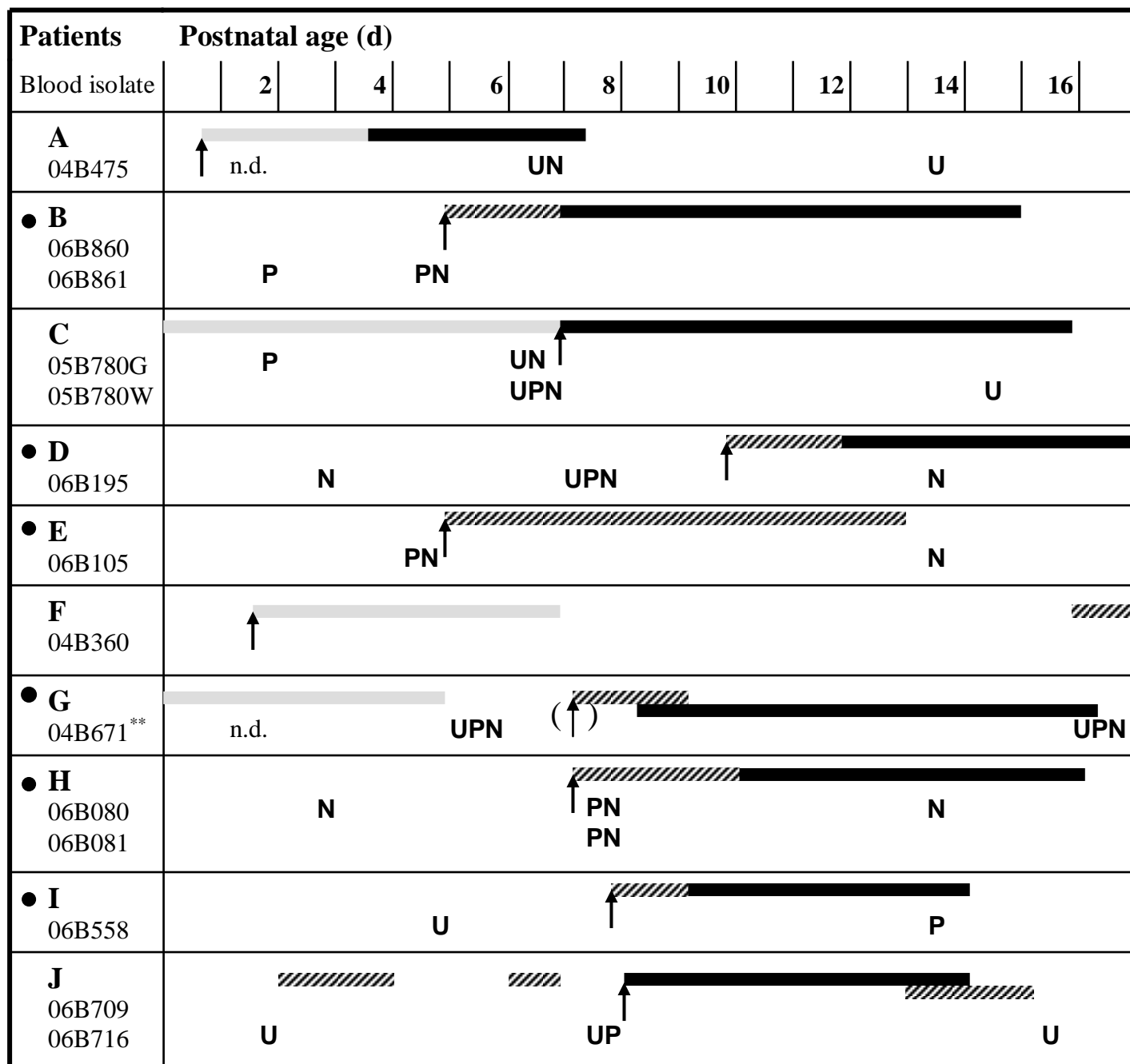
 = cefotaxim + garamicin, and  = vancomycin.

**Figure 2.** A representative clustering dendrogram based on the PhP characteristics of all CoNS isolates retrieved from one individual preterm newborn. N = nares and P = perineum. No positive cultures from the umbilicus were obtained in this patient.

### Figure 3.

Phenotypic and genotypic characteristics of CoNS blood isolates (n = 14) from preterm newborns (n = 10). The upper clustering dendrogram describes the phenotypic relationship between the bacterial isolates based on the PhP characteristics. The lower dendrogram describes the genotypic characteristics of the same isolates based on PFGE as visualized on the corresponding agarose gel.

Figure 1



\*\* Retrieved on d 23

Figure 2

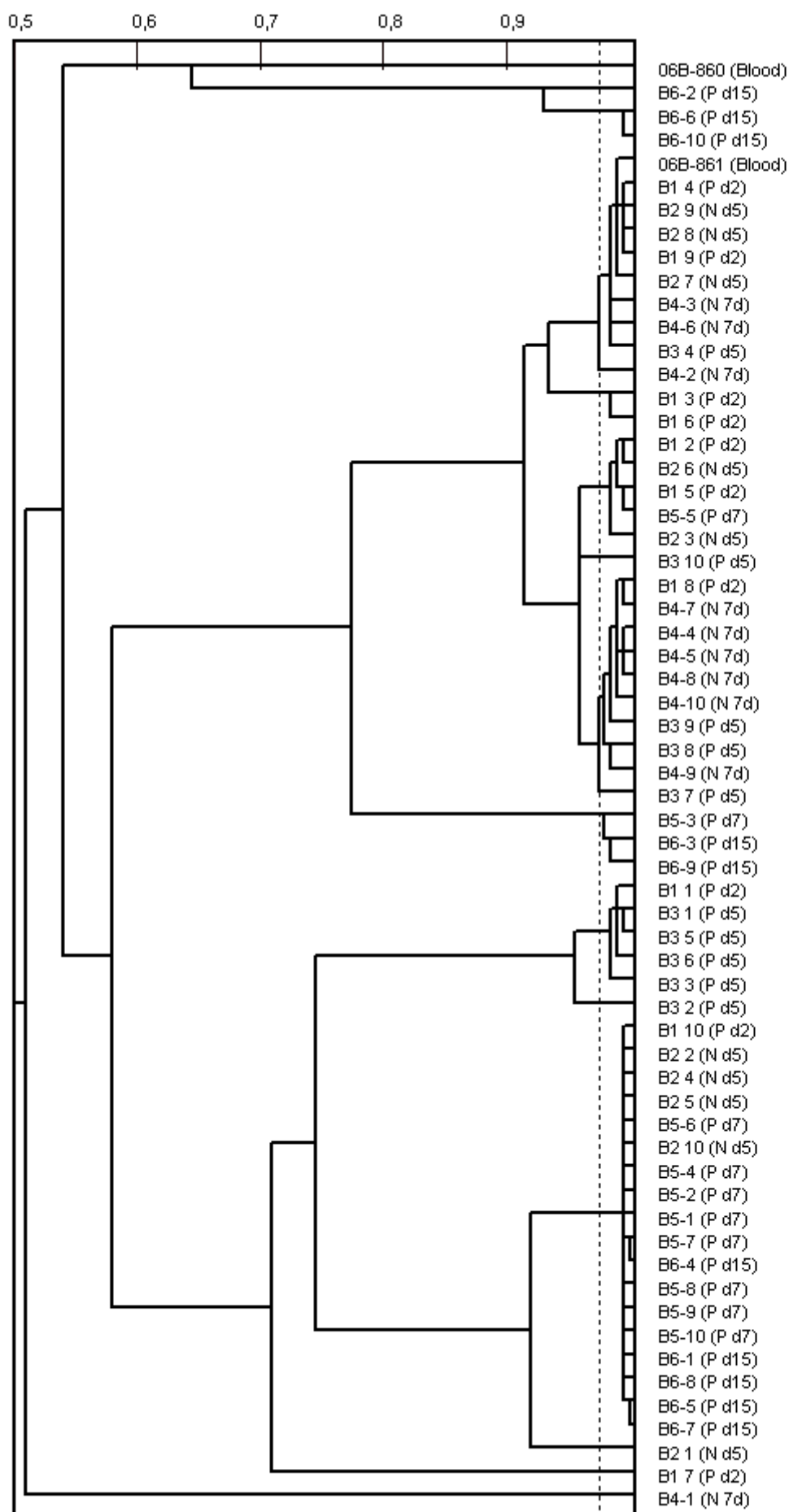
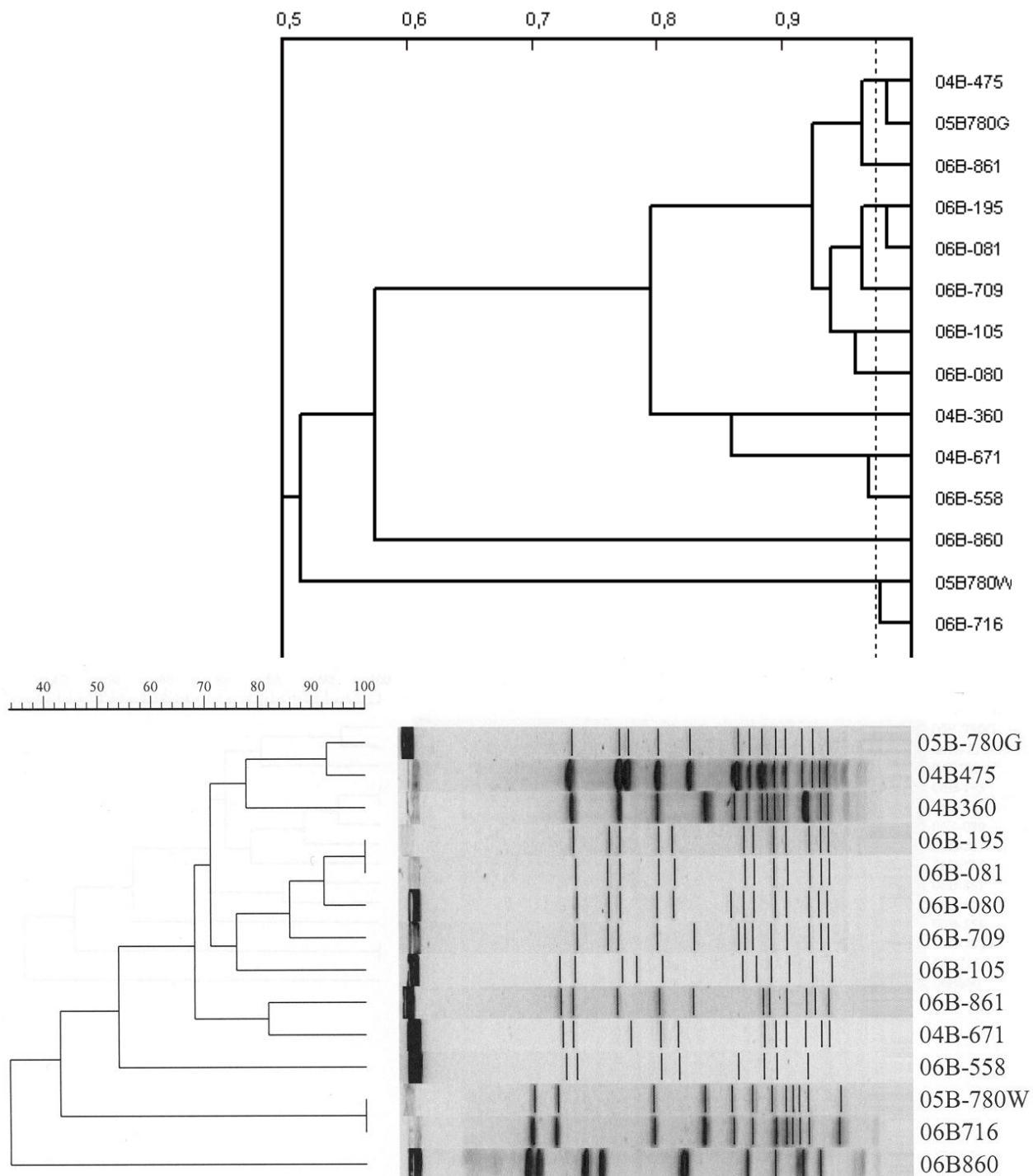


Figure 3



The comments of the reviewer have been a great help for us when revising the manuscript, and we want to express our thanks to the reviewer for constructive criticism.

As listed below, we would like to respond to the reviewer's comments.

1. The reviewer asked whether restriction of the cases to only those classified as having true sepsis (and thus exclusion of the cases most probably representing contaminants) would change the results. - In the original version of the manuscript we decided to compare only "blood culture positive" vs "bloodculture negative" in order to avoid any kind of bias according to the classification in "sepsis" and "contaminants". However, we agree with the reviewer that comparison of "sepsis" - "non-sepsis" is of interest and has included that kind of analysis in the results (page 10 line 22 and page 10 line 48). In summary these comparisons show the same trends as for the whole study group, however the numbers of included sepsis-newborns are limited, and the differences thus not statistically significant. - Our proportion of newborns with true CoNS sepsis however (6/46) however is similar to the incidence reported from other Western centra.

2. Why do we not present data on all 46 newborns? - In the present study we choose a case-control design and consider this as relevant, since only a minor proportion of the total cohort displayed a CoNS positive blood culture. However, we are just now analysing the data from all 46 preterms compared to healthy term newborns and plan to present these data in future manuscript.

3. Why do the findings of PFGE not quite match the PhP data? - A comment on this has been included in the Discussion (page 15 line 34).

4. Results of PFGE for typing validation of pairs of colonising and blood isolates identified as identical from PhP? - data has been included in the results (page 11 line 56).

5. Missing reference in the discussion. - Error in the proof by us. - The last part of the sentence that would correspond to the missing reference was thought to be omitted already in the original manuscript. This is now corrected.

We hope that the reviewer will find the new version of the manuscript improved compared to the original version and suggest it for publication in EJCMIID.