Nasopharyngeal colonization at birth and the development of early-onset neonatal sepsis

Andi Dwi Bahagia Febriani, Nilam Sartika Putri, Ema Alasiry, Dasril Daud

Abstract

Background Neonatal sepsis is one of the major causes of morbidity and mortality in neonates. Exposure to maternal bacteria during pregnancy or delivery allows for colonization of the normal upper airway. Such bacteria become the major ecological species in the infant. If the colonizing bacteria invade the bloodstream, early-onset neonatal sepsis (EONS) could occur.

Objective To evaluate for an association between colonization of the newborn nasopharynx and EONS, as well as for agreement between nasopharyngeal swab culture and blood culture isolate results.

Methods This prospective cohort study was conducted in Dr. Wahidin Sudirohusodo General Hospital and Ibnu Sina Hospital, Makassar, South Sulawesi. Nasopharyngeal swab culture was taken within 2 hours of life from newborns who met the inclusion criteria, then they were followed up for signs of EONS. Blood culture was taken from subject with EONS.

Results Of the 100 newborns, 69 (69%) had nasopharyngeal bacterial colonization, of whom 5.8% (4/69) experienced EONS. Of the remaining 31 (31%) without colonization, 9.7% (3/31) experienced EONS. There was no significant difference in frequency of EONS between newborns with and without nasopharyngeal colonization. Although Gram-negative bacteria were predominant among colonized newborns, there was no significant difference to numbers of Gram-positive bacteria as a causative agent of EONS. Only one patient with EONS had the same bacterial species in both the nasopharynx and blood culture isolate.

Conclusion Newborn nasopharyngeal colonization at birth is not associated with EONS. [Paediatr Indones. 2020;60:287-92; DOI: 10.14238/pi60.6.2020.287-92].

Keywords: sepsis; early-onset neonatal sepsis; nasopharyngeal bacterial colonization; newborns
as the placenta, amnion, and chorion as well as anti-
infective factors in the amniotic fluid. Rupture of
the membranes allows microorganisms in the vaginal flora
or other bacterial pathogens to ascend to the amniotic
fluid and fetus. This may result in chorioamnionitis or
infected amniotic fluid, which may then be aspirated
by the fetus or neonate. Neonates can also be exposed
to vaginal flora as they pass through the birth canal.

Colonization mainly occurs in the skin, nasopharynx, oropharynx, conjunctiva, and umbilical
cord. Acquisition of bacteria by ascending or
through the birth canal leads to colonization of the
skin and mucosa. The upper respiratory tract is
one of the major sites of bacteria, and is colonized
rapidly. Usually, pathogenic bacteria survive in the
airway for several months before disappearing or
being replaced by other pathogenic bacteria. The
local immune response plays an important role in
preventing or reducing the duration of colonization.

If the immune response is unable to prevent invasion
of the colonizing bacteria or the barrier integrity
is disturbed, colonization may lead to invasive
disease, such as early-onset neonatal sepsis (EONS).
A previous study found a significant correlation
between bacterial colonization of the maternal birth
canal and colonization on newborn body surfaces
(umbilical, throat, ear) as well as EONS. The risk for
sepsis increased if there was superficial colonization
with pathogenic bacteria. The aims of our study were
to evaluate for a possible association between newborn
nasopharyngeal bacterial colonization immediately
after birth and EONS, with the hope of decreasing
mortality due to EONS with timely and appropriate
interventions.

Methods

This prospective cohort study was carried out in
Dr. Wahidin Sudirohusodo General Hospital, and
Ibnu Sina Hospital, Makassar, South Sulawesi, from
January to June 2016. The study population was
newborns delivered in Dr. Wahidin Sudirohusodo
General Hospital and Ibnu Sina hospital who met
the following inclusion criteria: newborns whose
mothers had no signs or risk of infection (fever during
labor, prolonged premature rupture of membrane
(PPROM), or urinary tract infection), underwent
vaginal or Caesarean section delivery, and provided
parental informed consent. Exclusion criteria were
obvious congenital malformation, asphyxia, and
need for resuscitation. Nasopharyngeal swabs were
obtained for culture studies from all eligible newborns
immediately after birth. The newborns were followed
up until 72 hours of life. Nasopharyngeal swab culture
was done according to standard operational procedure
to avoid contamination. The swabs were collected
using a Dacron-tipped swab placed in Amies transport
media. Swabs were streaked on to blood agar plates
and incubated for 18-24 hours at 37°C. Suspected
colonies were further identified by Gram stain (IMViC
for Gram- negative and mannitol salt agar for Gram-
positive cocci). Blood cultures were performed only
for subjects who developed EONS. Swabs and blood
culture were analyzed at Hasanuddin University
Medical Research Centre (HUM-RC) Laboratory.

Early onset of neonatal sepsis was diagnosed
if there were two or more signs suggestive of sepsis
with at least one abnormal laboratory parameter, or
one or more signs suggestive of sepsis with two or
more abnormal laboratory parameters that occurred
in ≤72 hour of life. The signs suggestive of sepsis
were: respiratory distress, seizure, reduced level of
consciousness (lethargy to unconscious), temperature
instability, reduced activity, poor feeding, irritability,
vomiting, and abdominal distension. The abnormal
laboratory parameters were leukopenia or leukocytosis,
I/T ratio ≥0.2, increased CRP or procalcitonin, and
signs of infection on blood smear or positive blood
culture.

Statistical analyses were done with IMB SPSS
Statistic version 23. All data were grouped based on
the type and purpose, and then analyzed by univariate
analysis, unpaired student T-test, Mann-Whitney
test, and Chi-square test. This study was approved
by the Ethics Committee of Hasanuddin University
Medical School.

Results

Of 100 subjects, 69 (69%) subjects with nasopharyngeal
bacterial colonization and 31 (31%) subjects without
colonization at birth were identified during the study
period. Of the 69 subjects with nasopharyngeal
colonization, there were 28 boys (40.6%) and 41 girls
(59.4%), which was not significantly different from the distribution in the group without colonization (P=0.131). Nor were there significant differences between groups in terms of birth weight, gestational age, or delivery type (Table 1).

There was no significant difference in EONS incidence between newborns with and without nasopharyngeal bacterial colonization (OR 0.574; 95%CI 0.121 to 2.736; P=0.674) (Table 2). However, Mann-Whitney test revealed significant correlations between EONS and birth weight, as well as EONS and gestational age (Table 3).

Of the 69 nasopharyngeal swab cultures, 6 subjects exhibited more than one type of bacterial species growth. However, the species in those subjects had the same Gram stain type. Gram-negative bacteria (66.7%) were more common than Gram-positive bacteria (33.3%). The most common Gram-positive and Gram-negative bacteria were Staphylococcus.epidermidis (14.67%) and Alcaligenes faecalis (17.3%), respectively. None of newborns with these colonizing bacteria developed EONS (Table 4).

Of 7 newborns with EONS, 4 were colonized and 3 were not colonized. Among colonized newborns who developed EONS, only two subjects had positive blood culture and only one of those had the same bacterial species as the colonized nasopharynx (Table 5). Among non-colonized newborns who developed EONS, only one subject had a positive blood culture.

### Table 1. Characteristics of newborns with and without nasopharyngeal bacterial colonization

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>With colonization (n=69)</th>
<th>Without colonization (n=31)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, n (%): Male/Female</td>
<td>28 (40.6)/41 (59.4)</td>
<td>18 (58.1)/13 (41.9)</td>
<td>0.131*</td>
</tr>
<tr>
<td>Birth weight, g: Median/Range</td>
<td>2970/1800-4100</td>
<td>2760/1835-3300</td>
<td>0.052**</td>
</tr>
<tr>
<td>Gestational age, weeks: Median/Range</td>
<td>38/36-41</td>
<td>38/36-40</td>
<td>0.089***</td>
</tr>
<tr>
<td>Type of delivery, n (%): Vaginal/Caesarean section</td>
<td>58 (84.1)/11 (15.9)</td>
<td>29 (93.5)/2 (6.5)</td>
<td>0.334*</td>
</tr>
</tbody>
</table>

*Chi-square test; **unpaired student T-test; ***Mann-Whitney test

### Table 2. Analysis of nasopharyngeal bacterial colonization and EONS (N=100)

<table>
<thead>
<tr>
<th>Variables</th>
<th>With colonization (n=69)</th>
<th>Without colonization (n=31)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>EONS, n (%): Yes/No</td>
<td>4 (5.8)/65 (94.2)</td>
<td>3 (9.7)/28 (90.3)</td>
<td>7 (7.0)/93 (93.0)</td>
</tr>
</tbody>
</table>

Chi-square test; OR=0.574; 95%CI 0.121 to 2.736; P=0.674

### Table 3. Correlations between EONS and birth weight or gestational age (N=100)

<table>
<thead>
<tr>
<th>Variables</th>
<th>EONS (n = 7)</th>
<th>Not EONS (n = 93)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight: Median/Range</td>
<td>2,500/1,900-3,200</td>
<td>2,890/1,800-4,100</td>
<td>0.046</td>
</tr>
<tr>
<td>Gestational age: Median/Range</td>
<td>37/36-38</td>
<td>38/36-41</td>
<td>0.032</td>
</tr>
</tbody>
</table>

Mann-Whitney test
Table 4. Distribution of bacteria isolated from culture-positive nasopharyngeal swabs

<table>
<thead>
<tr>
<th>Type of bacteria</th>
<th>EONS</th>
<th>Not EONS</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-positive, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus</em> sp</td>
<td>0</td>
<td>4</td>
<td>4 (5.3)</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>1</td>
<td>9</td>
<td>10 (13.4)</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>0</td>
<td>11</td>
<td>11 (14.7)</td>
</tr>
<tr>
<td>Gram-negative, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Klebsiella pneumonia</em></td>
<td>1</td>
<td>8</td>
<td>9 (12)</td>
</tr>
<tr>
<td><em>Enterobacter cloacae</em></td>
<td>0</td>
<td>1</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td><em>Enterobacter agglomerans</em></td>
<td>0</td>
<td>6</td>
<td>6 (8)</td>
</tr>
<tr>
<td><em>Enterobacter aerogenes</em></td>
<td>1</td>
<td>2</td>
<td>3 (4)</td>
</tr>
<tr>
<td><em>Enterobacter hafniae</em></td>
<td>1</td>
<td>3</td>
<td>4 (5.3)</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>0</td>
<td>1</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td><em>Providencia alcalifaciens</em></td>
<td>0</td>
<td>3</td>
<td>3 (4)</td>
</tr>
<tr>
<td><em>Providencia alcaligenes</em></td>
<td>0</td>
<td>2</td>
<td>2 (2.7)</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>0</td>
<td>3</td>
<td>3 (4)</td>
</tr>
<tr>
<td><em>Acinetobacter calcoaceticus</em></td>
<td>0</td>
<td>5</td>
<td>5 (6.7)</td>
</tr>
</tbody>
</table>

Table 5. Distribution of bacteria from nasopharynx and blood cultures in newborns with EONS

<table>
<thead>
<tr>
<th>Number of cases</th>
<th>Nasopharyngeal swab</th>
<th>Blood culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Enterobacter hafniae</em> (Gram-negative)</td>
<td><em>E. coli</em> (Gram-negative)</td>
</tr>
<tr>
<td>1</td>
<td><em>Enterobacter aerogenes</em> (Gram-negative)</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td><em>S. aureus</em> (Gram-positive)</td>
<td><em>S. aureus</em> (Gram-positive)</td>
</tr>
<tr>
<td>1</td>
<td><em>Klebsiella pneumonia</em> (Gram-negative)</td>
<td>-</td>
</tr>
</tbody>
</table>

Discussion

This study shows a high prevalence of nasopharyngeal bacterial colonization in newborns at birth, but a low frequency of early-onset sepsis that was not different from uncolonized newborns. Of 69 neonates with bacterial colonization, only 4 neonates experienced EONS. This finding indicates that other factors may influence the incidence of EONS, including bacterial virulence, the number of bacteria (colony forming unit), and the infant immune response.

Information about the frequency of nasopharyngeal bacterial colonization in newborns is limited. A previous study stated that babies born to *Staphylococcus aureus*-carrier mothers were at risk of bacterial colonization in the nose, ears, umbilical cord, and rectum.11 A meta-analysis of 39 studies found that approximately 30-45% of newborns were surface colonized if they were born to colonized mothers.12

Early-onset neonatal sepsis is generally caused by microorganisms obtained from the mother before or during birth.9 Microorganisms that cause sepsis usually colonize the rectovaginal or genitourinary tract of pregnant women, leading to contamination of the amniotic fluid, placenta, cervix, and vagina. Type of delivery can also affect the transmission of bacteria from mother to newborn. During vaginal birth, the newborn is exposed to maternal bacteria which can colonize the skin and mucosa. This microbiota may suppress colonization by pathogenic bacteria that cause sepsis. Other factors that could affect colonization in newborns are breastfeeding, gestational age, and birth weight.13

Our findings were similar to a study which reported no association between group B Streptococcus (GBS) colonization and sepsis in newborns. Thus, nasopharyngeal bacterial colonization of the newborn is not a risk factor for EONS, so it is not necessary to perform nasopharyngeal swab culture for diagnosing or for giving antibiotics to prevent sepsis.14 In contrast, a previous study showed a significant correlation between colonization of the infant body surface (by nasopharyngeal and ear swab, as well as gastric aspiration) and blood culture with EONS.9 In addition, another study stated that nasopharyngeal cultures positive for GBS in newborns can be used as an indicator for diagnosis and management of presumed GBS sepsis.15
Variations on bacterial colonization in nasopharynx represent local bacterial colonization. Bacteria found in the nasopharynx of our subjects were opportunistic, with a predominance of Gram-negative bacteria, including *Alcaligenes faecalis*. Similarly, a study showed evidence of *Alcaligenes faecalis* in the vagina of pregnant women and colonization of newborn body surfaces. Our findings also suggest that while *Alcaligenes faecalis* was common in nasopharynx colonization, it did not cause neonatal sepsis. Similarly, another study showed colonization of pathogenic bacteria on neonatal body surfaces. They stated that 37% of subjects had *E. coli* colonization (Gram-negative) and 24% had *Klebsiella pneumonia* colonization (Gram-negative). Another previous meta-analysis of studies in Western countries found bacterial colonization mostly by GBS and *E. coli*. Several of our subjects with EONS had negative blood cultures. Blood culture is the gold standard for the diagnosis of neonatal sepsis. However, its positivity rate is low and is affected by the inoculation volume of the blood specimen, prenatal antibiotic use, level of bacteremia, laboratory capabilities, or if the cause of sepsis is not bacterial. The different bacterial types in the nasopharynx and blood cultures may have been due to the blood culture species already existing in the nasopharynx, but not detected by nasopharyngeal swab due to other colonizing species. Or the bacteria that caused sepsis may have not come from colonization of the nasopharynx. It was also difficult to assess the lack of agreement between colonizing bacteria in the nasopharynx with blood culture results, since only half of the cases (2/4) were blood-culture positive.

Some factors that influence the occurrence of EONS include vaginal bacterial colonization of pregnant women, premature rupture of the membranes, gestational age, birth weight, and APGAR score. We also noted that gestational age and birth weight were risk factors for EONS, but neither were associated with nasopharyngeal bacterial colonization of newborns. Hence, neither variable was a confounding factor in the relationship between bacterial nasopharynx colonization and EONS.

Physical barriers, such as skin, mucous membranes, antibacterial factors, or factors that prevent bacterial adhesion mature at 32-34 weeks of gestational age and accelerate after birth. Thus, IgA levels produced by the mucosal protection layer are low in very low birth weight (VLBW) infants. LBW infants have 10 times increased risk of EONS compared to normal birth weight infants. A study also showed that birth weight and gestational age were associated with EONS due to GBS. Group B Streptococcus and Gram-negative bacteria, especially *E. coli* and *Klebsiella* were found in newborns from mothers with the same colonizing species, *Klebsiella* was more common in blood cultures of infants with EONS. A previous study concluded that Gram-negative bacteria were more common in babies, but Gram-positive bacteria were more common in the baby's blood as a cause of sepsis. An Indonesian study showed that *Acinetobacter calcoaceticus* was the most common bacterial cause of neonatal sepsis, while another study found that neonatal sepsis was mostly caused by *Pseudomonas sp.* and *Klebsiella sp.* The sepsis etiologies in our study were *E. coli* and *S. aureus*.

Limitations of the study were not counting the bacterial colonies, which could influence the occurrence of sepsis, and that 50% of our EONS cases had negative blood cultures.

In conclusion, EONS mostly occurred in subjects without nasopharyngeal colonization and the species isolated from blood culture were different from that of the nasopharynx. Therefore, newborn nasopharyngeal colonization at birth is not associated with EONS. Nasopharyngeal bacterial colonization may be an important source of infection, but routine nasopharyngeal swab culture examination would not be clinically helpful.

**Conflict of Interest**

None declared.

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References


