

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

by Andi Dwi Bahagia Febriani

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

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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/pi60.4.2020.287-92].

Keywords: sepsis; early-onset neonatal sepsis; nasopharyngeal bacterial colonization; newborns

The World Health Organization (WHO) identified three major causes of neonatal mortality: infections including pneumonia, tetanus, and diarrhea (36%), preterm birth (28%), and asphyxia (23%).^{1,2} The World Bank reported that the mortality rate of newborn babies in Indonesia was 16 per 1,000 live births in 2013. The incidence of sepsis in developing countries is still high (1.8-18/1000) compared to developed countries (1-5/1000).³

Although the infection can be caused by viruses, fungi and parasites, bacterial infection is the most important in neonatal sepsis.⁴ Exposure can occur in utero, during labor, or after birth. If it occurs in utero or during childbirth, it can lead to early-onset sepsis. Intrauterine fetuses are relatively safe from infection because they are protected by various organs, such

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as the placenta, amnion, and chorion as well as anti-infective factors in the amniotic fluid.⁵ 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 fetus³² neonate. Neonates can also be exposed to vaginal flora as they pass through the birth canal.⁶

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.⁷ Usually, pathogenic bacteria survive in the airway for several months before disappearing¹⁶ r being replaced by other pathogenic bacteria. The local immune response plays an important role in preventing or reducing the duration of colonization.⁸ If the immune response is unable to prevent invasion of the colonizing bacteria or the barrier integrity is disturbed, c⁶onization may lead to invasive disease, such as early-onse⁶ neonatal sepsis (EONS). A previous study found a significant correlation between bacterial colonization of the maternal birth canal and colonization on newborn bo⁶ surfaces (umbilical, throat, ear) as well as EONS. The risk for sepsis increased if there was superficial colonization with pathogenic bacteria.⁹ 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

¹ 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. ³⁴ 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 operat²³al procedure to avoid contamination. The swabs were collected using a ¹⁸ cron-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 on⁴t 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.⁵

Statistical analyses were done with IMB SPSS *Statistic version 23*. A¹ data were grouped based on the type and purpose, and then analyzed by univariate analysis, unpaired studen¹⁸-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, 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

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

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

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

Variables	With colonization (n=69)	Without colonization (n=31)	Total
EONS, n (%)			
Yes	4 (5.8)	3 (9.7)	7 (7.0)
No	65 (94.2)	28 (90.3)	93 (93.0)

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)

Variables	EONS (n = 7)	Not EONS (n = 93)	P value
Birth weight			0.046
Median	2,500	2,890	
Range	1,900-3,200	1,800-4,100	
Gestational age			0.032
Median	37	38	
Range	36-38	36-41	

Mann-Whitney test

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

Type of bacteria	EONS	Not EONS	Total
Gram-positive, n (%)			
<i>Bacillus sp</i>	0	4	4 (5.3)
<i>Staphylococcus aureus</i>	1	9	10 (13.4)
<i>Staphylococcus epidermidis</i>	0	11	11 (14.7)
Gram-negative, n (%)			
<i>Klebsiella pneumoniae</i>	1	8	9 (12)
<i>Enterobacter cloacae</i>	0	1	1 (1.3)
<i>Enterobacter agglomerans</i>	0	6	6 (8)
<i>Enterobacter aerogenes</i>	1	2	3 (4)
<i>Enterobacter hafniae</i>	1	3	4 (5.3)
<i>Proteus mirabilis</i>	0	1	1 (1.3)
<i>Providencia alcalifaciens</i>	0	3	3 (4)
<i>Providencia alcaligenes</i>	0	2	2 (2.7)
<i>Pseudomonas aeruginosa</i>	0	3	3 (4)
<i>Alcaligenes faecalis</i>	0	13	13 (17.3)
<i>Acinetobacter calcoaceticus</i>	0	5	5 (6.7)

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

Number of cases	Nasopharyngeal swab	Blood culture
1	<i>Enterobacter hafniae</i> (Gram-negative)	<i>E. coli</i> (Gram-negative)
1	<i>Enterobacter aerogenes</i> (Gram-negative)	-
1	<i>S. aureus</i> (Gram-positive)	<i>S. aureus</i> (Gram-positive)
1	<i>Klebsiella pneumoniae</i> (Gram-negative)	-

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.¹⁰

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 rectal, vaginal 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.³ Our findings also suggest that while *Alcaligenes faecalis* was common in nasopharynx colonization, it did not cause neonatal sepsis. Similarly, a previous study found that the majority of isolates from the newborn nasopharynx were Gram-negative.¹⁶ In contrast, 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 pneumoniae* colonization (Gram-negative).⁹ Another previous meta-analysis of studies in Western countries found bacterial colonization mostly by GBS and *E. coli*.¹²

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,¹⁷ 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.^{14,16} 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.¹ LBW infants have 10 times increased risk of EONS compared to normal birth weight infants.¹⁸ A study also showed that birth weight and gestational age were associated with EONS due to GBS.¹⁴ Group B Streptococcus and Gram-negative bacteria, especially *E. coli*, are the main causes of EONS. Although *E. coli* and *Klebsiella* were found in newborns from mothers with the same colonizing species, *Klebsiella* was more common in blood culture of infants with EONS.⁹ 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.⁶ An Indonesian study showed that *Acinetobacter calcoaceticus* was the most common bacterial cause of neonatal sepsis,¹⁷ while another study found that neonatal sepsis was mostly caused by *Pseudomonas sp.* and *Klebsiella sp.*¹⁹ 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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