Neonatal nasopharyngeal bacterial colonization: Prevalence, antimicrobial resistance, and concomitant early-onset sepsis

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Background

Early-onset sepsis is one of the most common causes of mortality in neonates. This study aimed to determine the prevalence rate of nasopharyngeal colonization with common bacterial agents causing early-onset neonatal sepsis and their relationship with blood culture outcomes in neonates.

Method

All neonates transferred to the neonatal intensive care unit were included in the study. Posterior pharynx secretions were swabbed and cultured. The isolated bacteria were identified by biochemical standard tests. Antibiotic Sensitivity Test was performed by the disk diffusion method using Mueller-Hinton agar.

Results

The pharyngeal specimens collected from 114 newborns were positive in 83 (72.8%) of cases. Staphylococcus epidermidis was the most common bacterium. Twelve neonates had positive blood cultures. Concurrent positive blood and pharyngeal culture were reported in 8 (7%) cases, which in 3 (37.5%) of them blood and pharyngeal cultures were similar.

Conclusion

Staphylococcus epidermidis accounted for 38.6% of bacteria cultured from pharyngeal swabs and 66.7% of bacteria cultured from blood samples, 37.5% of which were resistant to ampicillin and 100% sensitive to vancomycin. One-hundred percent of E. coli cultures from neonatal pharynges were resistant to ampicillin and about 50% of them were resistant to gentamicin, cefotaxime, and ceftriaxone.

Keywords

newborn, nasopharyngeal culture, antibiotic resistance, Staphylococcus epidermidis, Escherichia coli

1. Introduction

Early-Onset Neonatal sepsis (ENOS) is one of the most common causes of mortality in neonates[1]. The bacteria causing ENOS are generally transferred from the mother to the infant before or during labor[1]. By definition, the ENOS is conceived to occur within the first 72 h of life in infants admitted to the neonatal intensive care unit or within the first 7 days of life in term neonates[2–4]. The incidence of ENOS in neonates with very low birth weight is estimated to be 15–24 per thousand[5]. Group B Streptococci (GBS) and *Escherichia coli* (*E. coli*) are the leading causes of early-onset sepsis. Research indicates that the rectum or genital tract of 10-40% of pregnant women is colonized by GBS. There is a 30-70 percent chance of GBS transfer from colonized mothers to infants before or during childbirth, with 1 to 3% of the cases leading to severe disease[6]. Antibiotic administration during delivery conceivably reduces the incidence of GBS infections in term and preterm neonates[7]. With a decreased incidence of GBS as a major contributor to the onset of sepsis[8], the role of gram-negative bacteria,such as *E. coli* in particular, has increased[1, 9, 10]. In a study by Tameliene et al, genitalia colonization with *E.coli* reported in 7-13% of pregnant women. *E. coli* was detected from blood culture of 21% of their stillbirth fetuses[11]. In recent years, in addition to *E. coli*, *Coagulase-Negative Staphylococci* (CONS) has played a major role in the development of EONS, especially in very low birth weight infants[5]. By estimation, 9.3% and 2.5% of infants are colonized at birth with *Staphylococcous aureus* and *Methicillin-Resistant Staphylococcous Aureus* (MRSA), respectively[12]. *Coagulase-Negative Staphylococci* (CONS) is reported in the oral cavity of 88.89% of newborns at 24-53 hours of birth[13]. Some centers consider CONS as real infections[1, 10, 14]; however, others regard them as contamination[15–17].

This study aimed to determine the prevalence rate of nasopharyngeal colonization of newly born infants with common bacterial agents causing early-onset neonatal sepsis, especially *coagulase-negative Staphylococci*, and their relationship with blood culture outcomes in neonates admitted to a neonatal intensive care unit.

2. Material and Methods

2.1. Ethical Consideration

The study design was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (identifier: IR.AJUMS.REC.1396.954). All methods were performed in accordance with the relevant guidelines and regulations by including a statement in the Ethics approval and consent to participate section**.** Written informed consent was obtained from parents and assured them that the sampling was not any danger to the baby and patient information remains confidential and will not be released.

2.2. Methods

This cross-sectional, prospective study was performed in the neonatal intensive care unit of Imam Khomeini Hospital in Ahvaz, Iran, from December 2018 to April 2019. All neonates transferred to the neonatal intensive care unit for any reason were included in the study if they met the inclusion criteria. Neonates born with vaginal delivery; neonates born with cesarean section and mother's fever (axillary temperature) above 38 °C, membrane rupture ≥ 18 h, preterm rupture of membrane, leukocytosis, and odorous vaginal discharge were included. Newborns requiring resuscitation at birth, neonates with a severe anomaly at birth, and those with a hospitalization duration of longer than 60 minutes were excluded. Written informed consent was obtained from parents and assured them that the sampling was not any danger to the baby and patient information remains confidential and will not be released. Posterior pharynx secretions were swabbed, and the specimens were preserved in transport media. They were sent to the Reference Laboratory of Ahvaz University of Medical Sciences and were culture on Blood agar and MacConkey agar (Merck, Germany) and incubated at 37 °C for 18-24 h.. For blood culture, 1ml of blood was taken and inoculated in 10 ml nutrient broth and incubated at 37°C. When growth appeared, the culture was inoculated into a Blood agar and MacConkey agar (Merck, Germany). The grown bacteria were identified by Gram staining, type of hemolysis, and biochemical standard tests such as catalase, coagulase, DNase test, bacitracin and novobiocin sensitivity, Mannitol fermentation test, CAMP test, TSI, malonate, citrate, urea, SIM tests, and gas production[18]. Antibiotic Sensitivity Test was performed by the disk diffusion method (Kirby-Bauer) using Mueller-Hinton agar (Merck, Germany) at 37 °C for 18-24 h. Results were evaluated according to the Clinical Laboratory Standard Institute (CLSI) guidelines[19].

2.3. Data analysis (Statistical methods)

The quantitative variables were described as means and medians, and the standard deviation. Frequency and percentage were employed to describe the qualitative variables. The normality of data distribution was assessed using Q-Q and Kolmogorov-Smirnov tests. In cases where the variable conversion method was not possible, nonparametric tests were used. Independent t-test, Mann-Whitney test, chi-square test, and Fisher's exact test were used for data analysis. The data were analyzed using SPSS software, version 22, and the significance level was set at $P \leq 0.05$.

3. Results

Of the 114 studied neonates, 59 (51.75%) were male and 55 (48.24%) were female. The mean gestational age and birth body weight were 33.39 ± 3.48 weeks and 2085.34 ± 692.24 g, respectively. The mothers' mean age was 28.17 ± 6.13 years, and the mean number of pregnancies per woman was 2.68 ± 1.66. Moreover, 25 (21.9%) of the mothers had a history of abortion and 2 (1.8%) had a history of previous child death. Over half (57.9%) of deliveries were through the cesarean section. The pharyngeal culture was positive in 83 (72.8%) of the newborns (Table 1).

Table 1
Different bacterial isolates from the pharyngeal culture

Bacterial Name	No. (Percent)
Staphylococcus epidermidis	32(38.6)
Klebsiella	12(14.5)
Escherichia coli	10(12)
Staphylococcus aureus	9(10.8)
Streptococcus agalactiae	6(7.2)
Enterobacter	4(4.8)
Bacillus	3(3.6)
Citrobacter	2(2.4)
Lactobacillus	2(2.4)
Streptococcus viridans	2(2.4)
Acinetobacter	1(1.2)

Staphylococcus epidermidis was the most common bacterium isolated from pharyngeal samples in all weight groups (Table 2).

Table 2
Distribution of bacterial isolates from pharyngeal samples, based on the birth body weight

Bacteria	Weight(Percent)				Total (Percent)
	<1000(N*. 5)	1000-1499 (N.31)	1500-2499 (N. 54)	≥2500 (N.24)	
Escherichia coli	0(0)	5(29.4)	2(4.9)	3(13)	10(12)
Klebsiella	2(100)	3(17.6)	6(14.6)	1(4.3)	12(14.5)
Staphylococcus aureus	0(0)	3(17.6)	5(12.2)	1(4.3)	9(10.8)
Staphylococcus epidermidis	0(0)	3(17.6)	18(43.9)	11(47.8)	32(38.6)
Streptococcus agalactiae	0(0)	2(11.8)	2(4.9)	2(8.7)	6(7.2)
Bacillus	0(0)	0(0)	2(4.9)	1(4.3)	3(3.6)
Acinetobacter	0(0)	0(0)	1(2.4)	0(0)	1(1.2)
Citrobacter	0(0)	0(0)	1(2.4)	1(4.3)	2(2.4)
Enterobacter	0(0)	1(5.9)	2(4.9)	1(4.3)	4(4.8)
Lactobacillus	0(0)	0(0)	1(2.4)	1(4.3)	2(2.4)
Streptococcus viridans	0(0)	0(0)	1(2.4)	1(4.3)	2(2.4)
Total	2(100)	17(100)	41(100)	23(100)	83(100)

*N= Number

E. coli were resistant to ampicillin in 100% of cases and to gentamicin and amikacin in 50% of cases (Table 3).

Table 3

Antibiotic resistance pattern of bacterial isolates from pharyngeal samples.

Bacteria	*Escherichia coli %*	*Klebsiella %*	*Staphylococcus aureus %*	*Staphylococcus epidermidis %*	*Streptococcus agalactiae %*
Ampicillin	100	66.7	33.3	37.5	16.7
Vancomycin*	-	-	0	0	0
Oxacillin	-	-	11.1	15.6	0
Imipenem	20	25	22.2	18.8	0
Meropenem	10	25	6.3	6.3	0
Amikacin	40	33.3	33.3	18.8	0
Gentamicin	50	75	55.6	34.4	0
Co-trimoxazole	40	58.3	33.3	37.5	33.3
Cefotaxime	50	83.3	55.6	31.3	0
Ceftazidime	50	66.7	40.6	40.6	50
Colistin†	0	0	-	-	-

∗ Only gram positive bacteria were assessed.

† Only gram negative bacteria were assessed.

Thirteen newborns died. Neonates pharyngeal samples were positive in 84.6% of the deceased infants and 71.28% of the surviving neonates (P = 0.882) (Table 4).

Table 4

The bacterial isolates from the pharyngeal samples of dead newborns

Bacteria	**No. Cases (%)**
Negative	2(15.38)
E. coli	3(23.07)
Staphylococcus aureus	3(23.07)
Staphylococcus epidermidis	3(23.07)
Streptococcus agalactiae	2(15.3)
Total	13(100)

Twelve neonates had positive blood cultures: *Staphylococcus epidermidis* in 8 (66.7%), *E. coli* in 2 (16.6%), and *Acinetobacter* in 2 (16.6%) cases. The cultured *Staphylococcus epidermidis* was resistant to ampicillin in 100% and sensitive to vancomycin in 100%; the *E. coli* was resistant to ampicillin in 30% and sensitive to meropenem and amikacin in 100%; and the *Acinetobacter* was resistant to ampicillin in 100%, to meropenem in 50% and amikacin in 100% of cases.

Concurrent positive culture from both blood and pharyngeal were reported in 7 (6.14%) cases, Also, the bacterial isolates in 3 (37.5%) of which blood and pharyngeal cultures were similar. Out of the 114 cases, only 3 (2.6%) had similar pharyngeal and blood cultures (Tables 5).

Table 5
Similarity and differentiation between bacterial isolates from pharyngeal and blood samples.

Blood isolates	Pharyngeal isolates	NO. Cases (%)
Staphylococcus epidermidis	*Staphylococcus epidermidis*	1(8.33)
Staphylococcus epidermidis	*Klebciella*	2(16.66)
Staphylococcus epidermidis	*Citrobacter*	1(8.33)
Staphylococcus epidermidis	Negative	4(33.33)
E. coli	*E. coli*	2(16.66)
Acinetobacter	*Staphylococcus aureus*	1(8.33)
Acinetobacter	Negative	1(8.33)
Total		12(100)

Two of the 12 neonates with positive blood cultures died; one was positive for *E. coli* and the other for *Acinetobacter*. The neonate with positive blood culture for *E. coli* also reported a positive pharyngeal culture for the bacterium. However, their strains were not determined.

The pharyngeal culture was positive in 34 (64.2%) neonates with membrane rupture, 23 (79.3%) in infants with rupture < 18 hours, and 26 (81.3%) in infants with rupture ≥ 18 hours. The risks of positive pharyngeal culture in neonates with ruptures < 18 h and ≥ 18 h were 2.14 and 2.42 times, respectively. Also, the risk of positive pharyngeal culture was 1.13 higher in neonates with the membrane rupture ≥ 18 h compared to < 18 h (P = 0.151). Multiple logistic regression showed a 2.28 higher chance of positive pharyngeal culture in cases with membrane rupture than those without membrane rupture, with the correlation being statistically significant (P = 0.053). In terms of free oxygen intake, the chance of receiving oxygen in the negative pharyngeal culture group was 1.7 times higher than in the positive pharyngeal culture group, although the difference was not statistically significant (P = 0.302). The use of nasal NCPAP was 1.86 times more in the negative pharyngeal culture group than in the positive pharyngeal culture, although the difference was not statistically significant (P = 0.157). The chance of using mechanical ventilation in the positive pharyngeal culture group was 1.74 times higher than that of the negative pharyngeal culture, but the difference was not statistically significant (P = O.426). Other variables such as sex, weight, gestational age, type of delivery, mother's age, number of pregnancies, and white blood cell count of patients were not significantly different between positive and negative pharyngeal culture groups.

4. Discussion

Our study shows that there is not a significant association between pharyngeal and blood bacterial culture (p= 0.73). Several large neonatal centers in the US used to culture specimens taken from different neonatal surfaces at birth, on the assumption that neonatal colonization is a precursor of neonatal sepsis and that positive bacterial culture is a cause of invasive infection[20–22]. Numerous studies have shown that bacterial culture from the axilla, groin, ear canal, and stomach secretions has poor predictive value for the diagnosis of early-onset sepsis and is therefore of limited value[23–25]. However, some studies have demonstrated that nasopharyngeal culture has the highest positive predictive value among cultures of other body

surfaces[25, 26]. One study showed that the probability of clinical sepsis in colonized infants was significantly higher than in non-colonized infants[27].

In our study, out of the 114 neonates admitted to the neonatal intensive care unit, 6 (7.2%) were colonized with GBS. Although, no cases of positive GBS blood cultures were found in the infants. While the colonization of pregnant women with GBS is of a substantial prevalence in Iran[28], neonatal sepsis with GBS is uncommon, and gram-negative bacteria stands as the leading cause of neonatal infection[29]. The use of CDC guidelines since 1996 concerning the use of antibiotic prophylaxis in mothers colonized with GBS has led to a remarkable reduction in neonatal infections with these bacteria[30].

In this study, *Staphylococcus epidermidis* was isolated from 38.6% of pharengeal swabs and 66.7% of blood samples of neonates. Out of these isolates, 37.5% were resistant to ampicillin and 100% sensitive to vancomycin. The prevalence of coagulase-negative Staphylococci in the development of early-onset sepsis ranges from 22.5 to 72.7%[27–29]. This may be partly due to contamination during sampling for culture or due to the widespread presence of this group of bacteria on human skin. Although *Staphylococcus epidermidis* is a bacterium with low pathogenicity in individuals with normal immune systems, it can cause invasive infections in infants, especially infants with low birth weight.

In our study, ten neonates were colonized with *E. coli* which this bacterium was also isolated from blood samples 2 (20%) cases of them. Tameliene et al.'s study, the transmission rate from colonized mothers with *E. coli* to their neonates was 21.3%. In their study, blood cultures of neonates were not assessed Tameliene *et al.* (2012) showed that the transmission rate from colonized mothers with *E. coli* to their neonates was 21.3%. In this study, blood cultures of neonates were not assessed[11].

Antibiotic susceptibility test in our study showed that 100 *E. coli* isolates from neonatal pharynges were resistant to ampicillin and about 50% of the cases were resistant to gentamicin, cefotaxime, and ceftriaxone. Tameliene *et al.* (2012) in a similar study showed that 3.77% of *E. coli* isolates were resistant to ampicillin while 100% of them were sensitive to gentamicin and cefotaxime[31].

In some of studies, *E. coli* resistance rate to gentamicin in neonatal sepsis was 3%, while resistance rate to ampicillin was between 30 and 85 percent[32–34]. The studies in other parts of Iran also showed, resistance rate to ampicillin was 100% and to gentamicin was 0 to 33%[31, 35]. A study (2011) at our center reported that 100% *E. coli* isolates were resistance to ampicillin and 82% to gentamicin[32].

Indiscriminate use of ampicillin and gentamicin probably underlies the entry of resistant *E. coli* into the country. Although in our study, *E. coli* was isolated from two cases of blood and pharyngeal cultures, their serotypes were not determined. It cannot be established, therefore, that the neonatal sepsis originated from the pharyngeal colonizing *E. coli*

The rate of positive pharyngeal cultures in neonates with membrane rupture of longer than 18 h was higher than newborns without membrane rupture or neonates with membrane rupture less than 18 h. Although the difference was not statistically significant, it showed that a longer membrane rupture increases the chances of developing colonization. There was no significant difference in mortality rate between the colonized and non-colonized infants in this study. There was no significant difference between colonized and non-colonized groups in terms of the clinical course and the need for mechanical ventilation. The low number of subjects to assess the incidence rate of sepsis associated with nasopharyngeal colonization with *E. coli* and the non-assessment of their serotype were among the limitations of this study.

5. Conclusion

The results of this study showed that many babies are colonized at birth, but their colonization is not significantly associated with sepsis, mortality, blood cultures, or the need for medical care. Neonatal screening at birth for colonization with bacteria does not seem to be conducive to the treatment or the course of the disease.

Empirically, the treatment of neonates with suspected sepsis initiates with gentamicin and ampicillin administration. The high resistance of *E. coli* which was the most common bacterium after *Staphylococcus epidermidis and Klebsiella* to ampicillin and, to a degree, to gentamicin is a serious threat in the treatment of neonates with sepsis in our center. Besides, *Staphylococcus epidermidis* was the most common isolate from blood and pharynx samples which showed high resistance to ampicillin and gentamicin. This suggests the possibility of revision in empiric antibiotic selection for the treatment of infants with suspected early-onset sepsis.

6. List of abbreviations

Early-Onset Neonatal sepsis (ENOS)

Group B Streptococci (GBS)

Escherichia coli (E. coli)

Coagulase-Negative Staphylococci (CONS)

Methicillin-Resistant Staphylococcous Aureus (MRSA)

Nasal continuous positive airway pressure (NCPAP)

7. Declarations

7.1. Ethics approval and consent to participate

The study design was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (identifier: IR.AJUMS.REC.1396.954). All methods were performed in accordance with the relevant guidelines and regulations by including a statement in the Ethics approval and consent to participate section**.** Written informed consent was obtained from

parents and assured them that the sampling was not any danger to the baby and patient information remains confidential and will not be released.

7.2. Consent for publication

Not applicable

7.3. Availability of data and materials

The datasets generated and/or analysed during the current study are not publicly available due to the confidentiality of patients information and the authors commitment not to publish but are available from the corresponding author on reasonable request.

7.4. Competing interests

The authors declare that they have no competing interests

7.5. Funding

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7.6. Authors' contributions

Masoud Dehdashtian, Mojtaba Moosavian, Meysamreza Boghrati wrote the main manuscript text and Mania Arshadi did the laboratory procedures and Arash Malekian, Mohammad Reza Aramesh prepared the tables. All authors reviewed the manuscript.

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