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Original Article

Maternal Recto-Vaginal Organisms and Surface Skin Colonization in Infants

Zahra Akbarian Rad¹⁻³, Seddigheh Esmailzadeh⁴, Mohsen Haghshenas Mojaveri¹⁻³, Mojgan Bagherzadeh⁵, Mostafa Javanian^{6*}

1. Non-Communicable Pediatric Diseases Research Center, Babol University of Medical Sciences, Babol, Iran

2. Department of Neonatology, Babol University of Medical Sciences, Babol, Iran

3. Clinical Research Development Unit of Rohani Hospital, Babol University of Medical Sciences, Babol, Iran

4. Infertility and Health Reproductive Research Center, Babol University of Medical Sciences, Babol, Iran

5. Babol University of Medical Sciences, Babol, Iran

6. Infectious Diseases and Tropical Medicine Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran

ABSTRACT

Background: Early-onset sepsis is a common cause of neonatal mortality and mainly occurs due to the colonized microorganisms in the maternal recto-vaginal area. The present study aimed to evaluate the effects of maternal risk factors on recto-vaginal colonization and transmission rate of micro-flora from mothers to infants.

Methods: Upon admission, two samples were obtained from the distal third of vagina and rectum of mothers using sterile cotton swabs. Six hours after birth, sterile swab samples were collected from the external ear canal, nose, groin, and umbilicus of the infants. The samples were transferred to the laboratory on Stuart transport medium. Afterwards, the samples were transferred to standard culture media within 24 hours. Transmission rate of organisms was calculated based on the maternal and neonatal findings.

Results: In total, 13 bacterial and fungal species were detected in the samples. *Escherichia coli* was the most frequent gram-negative organism in the rectal and vaginal cultures (29.34%) with the transmission rate of 49.4% to the infants. Infants of the mothers with premature rupture of membranes (PROM) had significant *Enterobacter cloacae* colonization. In addition, frequency of *Candida albicans* was higher in the mothers with gestational hypertension (21.4%) compared to non-hypertensive women (4.9%). Infants requiring advanced cardiopulmonary resuscitation (CPR) had significantly higher *E. coli* colonization (45.1%) compared to those not needing CPR (27.6%).

Conclusion: According to the results, gram-negative bacteria (*E. coli* and *Enterobacteriaceae*) were the most frequent organisms in the maternal recto-vaginal area and body surface of the neonates in our clinical setting. Therefore, chemoprophylaxis is recommended for these organisms in prolonged PROM.

Keywords: Communication disorders, Congenital hypothyroidism, Diagnosis, Mass screening, Sensorineural hearing loss

Introduction

Microorganisms that colonize the recto-vaginal and urinary system of pregnant women may be vertically transmitted to newborns and cause neonatal sepsis in the case of prematurity and rupture of the amniotic sac (1). Despite the advancement in neonatal care, sepsis remains a potentially fatal condition. Delayed diagnosis and treatment of neonatal sepsis is associated with high morbidity and mortality (2).

Infections are the most common cause of neonatal mortality, accounting for 35% of

neonatal deaths and 75% of the deaths within the first week of life (3). Overall incidence rate of neonatal sepsis is 2-6 per 1,000 live births (4, 5). In developing countries, the incidence rate is estimated at 3.5-4.3 per 1,000 live births (5). In a study in Iran, the incidence of neonatal sepsis was reported to be 9.2% (6). Early-onset neonatal sepsis (within the first week and most commonly the first three days of life) is mainly caused by microorganisms that may be transmitted vertically from the mother to the infant before or

* Corresponding author: Mostafa Javanian, Infectious Diseases and Tropical Medicine Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran. Tel: +989111120154; Email: mjavanian@gmail.com

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during birth (4, 7).

Maternal recto-vaginal organisms play a key role in early-onset neonatal sepsis. The microorganisms involved in early-onset sepsis (EOS) may vary depending on time and region across the world. For instance, in western countries, *Streptococcus group B* is considered to be the most organism, while *Escherichia coli*, *Klebsiella*, and *Acinetobacter* have been reported to be the most frequent organisms in EOS in India. In a study in Iran, *Enterobacteriaceae* was reported as the most common pathogen in EOS (8, 9).

With this background in mind, the present study aimed to evaluate the most common maternal recto-vaginal organisms in a geographical and social region in Iran in order to decide the most effective empirical antibiotic therapy for neonatal sepsis. Moreover, obstetricians have been advised to select chemoprophylaxis as an effective therapy in the case of premature rupture of membranes (PROM).

Methods

This cross-sectional, descriptive-analytical study was conducted on pregnant women with the gestational age of >26 weeks, who were admitted to the maternity ward of Ayatollah Rohani Hospital (level III maternal and neonatal care) in Babol, located in the north of Iran during October 2012-2014. The women had progressive labor pain. Written informed consent was obtained from all the participants prior to the study.

Data were collected using a questionnaire containing the demographic characteristics and risk factors of the mothers, including urinary tract infections, gestational age on admission, prolonged PROM, and mode of delivery (cesarean section/natural vaginal).

Exclusion criteria were mothers not giving birth 48 hours after admission for any reason, history of antibiotic use during the past week leading to admission, and multiple pregnancies upon admission. Samples were obtained from the rectal and vaginal (distal third of vagina) areas of the mothers using sterile cotton swabs and transferred to the laboratory on Stuart transport medium.

Six hours after birth (natural vaginal/cesarean section), sterile swabs were used to collect samples from the external ear canal, nose, groin, and umbilicus of the infants, which were transferred to the laboratory on Stuart transport medium. Afterwards, the samples were

transferred to standard culture media within 24 hours. Transmission rate of each organism was calculated using the following formula:

$$TR = \frac{\text{number of culture positive mother and infant}}{\text{number of culture positive mother}}$$

Data analysis was performed in SPSS version 22 using Chi-square to assess the correlations between the variables, and P-value of less than 0.05 was considered significant.

Result

In total, 1,050 samples were collected from the mothers and infants, and 410 samples were analyzed. In terms of the mode of delivery, 71% of the women had natural vaginal delivery, and 29% had cesarean section.

Gestational age was within the range of 26-42 weeks (mean: 37±2.46 weeks). All the infants were born via singleton pregnancy, including 50.7% females and 49.3% males. Resuscitation was required by 51 infants (12.4%) at birth. The results of culture analysis indicated 13 bacterial and fungal species in the mothers and infants (Table 1). Frequency of gram-positive organisms in the mothers and neonates was 41.48% and 49.98%, respectively, and frequency of gram-negative organisms was 52.99% and 48.78%, respectively. The most common bacteria in the neonatal cultures (born via vaginal delivery and cesarean section) were *S. epidermidis* (43.3%) and *E. coli* (32.3%). However, no significant difference was observed in the frequency of bacteria between the mothers with natural delivery and cesarean section.

Frequency of group B *Streptococcus* (GBS) in the neonates born via vaginal delivery and cesarean section was 11.7% (n=34) and 1.7% (n=2), respectively (P=0.001). In 35 mothers with diabetes, the most common organisms were *S. epidermidis* and *E. coli*, and no significant difference was observed between diabetic and non-diabetic mothers in this regard. In this group, only one culture was positive for *Pseudomonas*, which was not considered statistically significant.

Among the participants, 184 cases had progressed PROM before labor pain (range: 2-120 hours; mean: 9.8±2.06 hours), and only 15 mothers experienced PROM for ≥18 hours. *Enterobacter cloacae* was the most frequent organism in the mothers with PROM (regardless of length) (50.9 versus 29.6) (P=0.002).

In the neonates of the mothers with and

Table 1. Frequency of Bacteria in Recto-Vaginal Culture of Mothers and Skin Surface Culture of Neonates

Bacteria	Recto-Vaginal Culture (%)	Neonatal Skin Culture (%)
<i>E. coli</i>	29.34	25.36
<i>E. cloacae</i>	19.03	20.01
<i>E. aerogenes</i>	4.11	2.49
<i>Pseudomonas</i>	0.25	0.45
<i>Klebsiella</i>	0.25	0.45
Group B <i>Streptococcus</i>	5.92	7.48
<i>S. aureus</i>	1.8	1.87
<i>S. epidermidis</i>	29.6	37
<i>S. saprophyticus</i>	3.6	3.32
Coagulase-Negative <i>Staphylococci</i>	0.12	0
<i>Enterococcus</i>	0.25	0.2
<i>Bacillus</i>	0.12	0
<i>C. albicans</i>	5.53	1.24

Table 2. Maternal/Neonatal Vertical Transmission Rate of Microorganisms

Bacteria	Positive Mothers (N)	Positive Neonates (N)	Mother (+)/ Neonate (+)	Mother (-)/ Neonate (+)	Transmission Rate (%)	P-value
<i>E. coli</i>	231	122	114	8	49.4	<0.001
<i>E. cloacae</i>	149	98	89	9	59.7	<0.001
<i>E. aerogenes</i>	32	12	12	0	37.5	<0.001
Group B <i>Streptococcus</i>	46	36	36	0	78.3	<0.001
<i>S. aureus</i>	14	9	2	7	14.3	0.002
<i>S. epidermidis</i>	227	177	141	36	62.1	<0.001
<i>S. saprophyticus</i>	29	16	6	10	20.7	<0.001
Coagulase-Negative <i>Staphylococci</i>	1	0	0	0	-	-
<i>Enterococcus</i>	2	1	1	0	-	-
<i>Pseudomonas</i>	2	2	0	2	0	-
<i>Klebsiella</i>	2	2	1	1	50	-
<i>Bacillus</i>	1	0	0	0	-	-
<i>C. albicans</i>	42	4	0	4	-	-

without PROM, the frequency of *Enterobacter cloacae* was 33.3% and 19%, respectively ($P < 0.05$). However, no significant difference was observed in the frequency of the other bacteria between the two groups. For instance, the frequency of GBS with and without PROM was 14% and 9.8% in the mothers and 12.3% and 7.1% in the neonates, respectively ($P > 0.05$).

Among 28 women with gestational hypertension, the most common bacteria were similar to diabetic mothers. Frequency of *Candida albicans* was significantly higher in hypertensive mothers (21.4%) compared to non-hypertensive mothers (4.9%) (CI=0.99-6.88; RR=2.62; $P=0.043$).

In the present study, 27 subjects had a history of urinary tract infections (UTIs) in their current pregnancy, and the most common bacteria in this group were *E. coli* (63%). No significant difference was observed in bacterial colonization between the mothers with and without UTIs.

Resuscitation at birth with bag and mask and advanced stages was performed on 51 neonates. In resuscitated infants, *E. coli* frequency was significantly higher (23 out of 51 cases; 45.1%) compared to non-resuscitated neonates (99 out of 359 cases; 27.6%).

Transmission rate was calculated to determine the risk of bacterial transmission from the mother to the newborn (Table 2), which was statistically significant for all the microorganisms, with the exception of *Candida albicans* and *Pseudomonas*. In the case of these organisms, colonization in the neonates was completely independent of the mother. On the other hand, all the infants colonized by GBS and *Enterobacteriaceae* were born to the mothers colonized by these organisms.

Discussion

According to the present study, *E. coli* and *Enterobacter cloacae* were the most common gram-negative bacteria in the maternal recto-vaginal culture. Among gram-positive bacteria, *S. epidermidis* and GBS were the most frequent organisms. In a similar study in Iran, gram-negative bacteria (e.g., *Enterobacteriaceae* and *Klebsiella*) and coagulase-negative bacteria (e.g., *Staphylococcus*) were reported to grow more frequently than other organisms (10). Furthermore, Hamed et al. reported that *E. coli* and *Enterococci* were more frequent than *S. epidermidis* and GBS in recto-vaginal colonization, which is consistent with the results of the present study (11). In the studies performed in India and

Pakistan, similar results were reported. In EOS and LOS (Late Onset Sepsis), the frequency of gram-negative bacteria (e.g., *E. coli* and *Klebsiella*) has been reported to be higher compared to gram-positive bacteria (12, 13).

In a study regarding neonatal sepsis by Stoll et al. (2006-2009), GBS, *E. coli*, and *S. aureus* were reported to colonize in 43%, 29%, and 5% of the cases, respectively. Moreover, the prevalence of GBS infection (0.41 per 1,000 live births) was reported to be higher than *E. coli* infection (0.28 per 1,000 live births) (14). Another research conducted in the United States investigated maternal colonization by methicillin-resistant and sensitive *S. aureus* indicated the colonization rate to be 0.7% and 13%, respectively, while the total frequency of *Staphylococcus* was 1.8%. Therefore, it could be inferred that in eastern countries, *E. coli* and other gram-negative bacteria are the most common maternal organisms causing neonatal sepsis (15).

In the present study, frequency of GBS was higher in the infants born via vaginal delivery (11.7%) compared to those born via cesarean section and (1.7%). However, mode of delivery had no effect on the colonization of other organisms. According to a study performed in the north of Iran, there was no significant correlation between the mode of delivery and incidence of neonatal sepsis (16). On the other hand, the findings of Stonii on the colonization of infants admitted to the neonatal intensive care unit due to suspected sepsis showed that *E. coli* and *C. albicans* colonization were affected by the mode of delivery and gestational age, while colonization by *Klebsiella pneumoniae*, *E. cloacae*, and other *Candida* species mostly occurred in the infants undergoing medical interventions in the hospital (17). It is notable that the mentioned studies emphasized on the association of neonatal sepsis and mode of delivery rather than surface colonization.

In the current research, no significant correlation was observed between maternal diabetes and recto-vaginal colonization. Conversely, Akhlaghi et al. reported the significant rectal colonization of GBS in diabetic mothers compared to non-diabetic mothers; however, vaginal GBS colonization (16%) was not considered significant (18). The results of two other studies are in congruence with the current research regarding the lack of a significant correlation between diabetes and GBS colonization (19, 20). According to the present study, *Enterobacter cloacae* was the second most

frequent organism in the infants of the mothers with PROM following *S. epidermidis*. Mean duration of PROM in the present study was approximately 10 hours, and it seems that PROM duration of less than 18 hours could induce bacterial colonization, increasing the risk of neonatal sepsis. Unlike several other studies, our findings indicated that the duration of PROM (<18 hours) may affect colonizing pathogenic bacteria (e.g., *Enterobacter cloacae*), requiring prophylaxis in order to prevent EOS (21, 22). In the current research, no significant difference was observed in GBS colonization between the mothers with and without PROM, which could be due to the few number of the subjects with PROM duration of more than 18 hours (15 cases).

Among 28 mothers with gestational hypertension, *Candida albicans* was significantly colonized. In a study by Mulla et al. (2012), vaginal GBS colonization had a suppressive effect on preeclampsia. However, such effect was not confirmed in another study by Mulla et al. (2015) (23, 24). Unfortunately, data is scarce regarding the correlation of *C. albicans* and gestational hypertension.

In the present study, *E. coli* was the most frequent bacteria in the mothers with UTIs, while no significant difference was observed between the subjects with and without UTIs. Several studies have denoted that UTIs during pregnancy increase the risk of prematurity and low birth weight (25, 26). However, our findings are not consistent with these results, which could be due to the low number of the mothers with a history of UTIs (n=27) or patients receiving therapy before childbirth.

In recent years, the incidence of neonatal sepsis caused by gram-negative bacteria has increased, especially in low-birth-weight newborns (27, 28). In the present study, we did not examine the organisms involved in neonatal sepsis; however, gram-negative bacteria (mostly *E. coli* and *Enterobacter cloacae*) were more frequent than other organisms. In an earlier report by Stoll et al., the incidence of EOS decreased, and gram-negative bacteria (especially *E. coli*) accounted for 60.7% of the cases with sepsis. Furthermore, the mentioned report suggested that PROM and prematurity are the two major risk factors for neonatal sepsis (29).

According to two studies performed in Iran, *Enterobacteriaceae* was the most common pathogen in EOS, LOS, and hospital sepsis in neonates (10, 16). In the present study, *Enterobacter cloacae* was the second most

frequent gram-negative species in all the cultures; it should be noted that no follow-up was carried out for neonatal sepsis. A significant correlation was also observed between maternal recto-vaginal organisms and surface colonization in infants. The highest transmission rates belonged to GBS, *Enterobacter cloacae*, and *E. coli*, respectively. These findings are in line with the study results in India, which denoted that *E. coli* and *Klebsiella* had the highest frequency in neonatal sepsis (13).

In the present study, the infants requiring resuscitation at birth were mostly colonized by *E. coli*. It seems that recto-vaginal colonization by *E. coli* is a risk factor for complications at birth.

Conclusion

According to the results, gram-negative bacteria (e.g., *E. coli* and *Enterobacter cloacae*) were the most frequent bacteria to colonize in the maternal recto-vaginal area and skin surface of their infants in our clinical setting. In the cases with the PROM duration of less than 18 hours, chemoprophylaxis is recommended against gram-negative *Enterobacter cloacae*. Selecting the most effective antibiotic therapy in EOS is of paramount importance.

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Conflicts of interests

None declared.

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