

Colonization of rectovaginal *Escherichia coli* and group B streptococci in mothers and on infants' body surface and their related risk factors

Original Article

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Abstract:

Background: Microorganisms that cause early neonatal sepsis are usually already colonized rectovaginal area in mothers. The most common of these organisms is group B streptococci (GBS) and intestinal gram-negative bacteria mostly *Escherichia coli* (*E.coli*). The use of prophylactic antibiotics against GBS has increased in recent years. This study aimed to determine the current situation and frequency of *E.coli* and GBS colonization in mothers and their infant.

Methods: All pregnant women with gestational age ≥ 26 weeks, progressive labor pain and no history of using antibiotic were entered into the current study. A sterile cotton swab culturing from distal third of vaginal and rectum of mothers, and six hours after delivery from external ear canal, nose, groin and umbilicus of infant has been taken. All samples were transferred to the laboratory in Stuart's media, and then cultured to standard media within 24 hours and the main two organisms in neonatal sepsis (*E.coli* and GBS) were isolated from mothers' and infants' cultures.

Results: *E.coli* and GBS were 56.3% and 11.2% respectively in rectovaginal culture, and 29.8% and 8.8% in infants' body surface culture. There was a significant difference in rectovaginal GBS colonization between term (13.6%) and preterm (3.2%) ($P=0.005$), while the frequency of positive *E.coli* culture was 52.8% in term deliveries and 68.1% in preterm ones, showing a significant difference ($P=0.009$).

Conclusions: Since *E.coli* is more common in preterm delivery in this geographical region, in cases of amniotic membrane rupture, mothers should be adequately protected with prophylactic antibiotics against neonatal sepsis.

Keywords: Maternal colonization, Neonatal sepsis, *Escherichia coli*, Group B Streptococci, Prophylactic antibiotic

Citation:

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Introduction:

Bacteria can reach and infect the fetus or neonate in many ways such as through mother's blood flow (maternal bacteremia), vagina, cervix or fecal contamination of the birth canal in both ruptured and intact amniotic sac cases, thereby causing amnionitis, intrauterine pneumonitis and preterm childbirth. Infection can ultimately occur through aspiration of birth canal contents or neonatal mucosal surface colonization during passage through birth canal leading to pneumonia, bacteremia, and neonatal sepsis [1-3]. Neonatal sepsis refers to a systemic bacterial infection in infants younger than 28 days, confirmed in at least one positive blood culture [4]. Neonatal sepsis is a major factor in neonatal morbidity and mortality, with higher mortality rate in preterm and very

low birth weight (VLBW) infants [5]. Neonatal sepsis can be divided into early onset sepsis (EOS) and late onset sepsis (LOS) groups [4]. There are different definitions for EOS, depending on the age at onset. Bacteremia and bacterial meningitis in less than 72 hour-old infants admitted to Neonatal Intensive Care Unit or in non-admitted infants aged less than 7 days are both grouped as EOS [6-8].

Organisms that cause EOS are usually already colonized in mothers' rectovaginal area and can infect amniotic fluid, placenta, cervix or vagina. These pathogens are likely to ascend during amniotic sac rupture or before the onset of childbirth and cause intra-amniotic infection [9].

Mode of childbirth affects neonatal body surface colonization. In vaginal childbirth, infant is directly in contact with colonized bacteria in birth canal. However, in cesarean section, infant has no direct contact with colonized bacteria in the birth canal, and is more affected by colonization of bacteria on mother's skin and environmental bacteria. In other words, bacteria are mainly transferred to the infant vertically in vaginal delivery and horizontally in cesarean [10].

Bacterial agents of neonatal sepsis have changed over time. Before antibiotics were introduced in the 1930s and 40s, Group A Streptococci were the most common cause of neonatal sepsis, but after the widespread use of antibiotics, gram-negative bacilli became the most common cause. Staphylococcus aureus and Escherichia coli (E.coli) were common in the 50s and 60s. In the 70s, Group B Streptococci were identified as the main pathogen for neonatal sepsis.

Although administration of prophylactic antibiotic to mothers with Group B Streptococci (GBS) colonization during childbirth has reduced neonatal sepsis caused by this pathogen, GBS and E.coli are still the first and second most common pathogens causing sepsis in neonatal centers across North America and Europe [1].

According to the World Health Organization report, the prevalence of GBS colonization in pregnant women varies between 5% and 40% in different countries; this is about 26% in America, and 22.76%, 6%, and 4.8% in Iran according to three different studies [10-12].

However, the prevalence of colonization varies according to age, parity, race, concomitant vaginal infection, genetic factors, socioeconomic status and recent intercourse. The vertical GBS transfer between mother and infant varies from 29% to 85% (mean 51%), and is affected by severity of colonization of birth canal. Neonatal colonization may occur during

birth or through bacterial ascend (even with intact amniotic sac) [10].

E.coli (active aerobic gram-negative bacilli of enterobacteriaceae family) is the second most common pathogens causing 24% of EOS cases in which 81% sepsis was related to the preterm neonates [5]. The increasing use of prophylactic antibiotics during childbirth in recent years has occurred along with the increase in sepsis induced by E.coli and other gram-negative pathogens. Recent advances in increasing survival of preterm and VLBW infants also appears to cause the increase in E.coli-related sepsis [1].

It seems that both the most common rectovaginal organisms in each area and gestational age should be considered before administering prophylactic antibiotics. Thus, it was decided to develop a better understanding of the current situation and frequency of E.coli and GBS as the most common bacteria to have the right choice of prophylactic antibiotics.

Methods:

Participants in the present descriptive analytical study included all pregnant women with gestational age more than 26 weeks admitted to the maternity ward of Ayatollah Rohani Hospital in Babol between October 2012 and October 2014 with progressive labor pains. Pregnant women with history of antibiotic use in the last week leading to admission and lack of delivery during 48 hours hospitalization were excluded from the present study. Informed consents were obtained from all participating women.

Data were collected using a questionnaire containing mother's demographic details and risk factors including urinary tract infection, gestational age at admission, PROM, and mode of childbirth (cesarean or vaginal). If infant was not available for sampling, mother's sample would be deleted.

At admission, two rectal and two vaginal (distal third of vagina) sterile swab samples were taken from each mother, which were transferred to the laboratory in Stuart's transport media. Six hours after birth (vaginal or cesarean), sterile swab samples were taken by neonatal ward nurse from infants' external ear canal, nose, groin, and umbilicus, which were transferred in Stuart's transport media to microbiology laboratory. All samples were transferred to standard culture media within 24 hours.

Isolated bacteria were then reported by a microbiologist. Data were analyzed in SPSS-22 using Chi-square to assess the relationship between variables.

Frequency of the two main organisms involved in neonatal sepsis (E.coli and GBS) was also found. Significance level was considered $P < 0.05$.

Results:

A total of 410 pairs of mother-infant samples were ultimately examined. Mean age of mothers was 25.7 ± 5.55 years, of whom, 70.73% of mothers were primiparous and 29.27% multiparous. The majority of women (71%) had vaginal childbirths and only 29% had cesarean deliveries. Mean gestational age was 37 ± 2.46 weeks, ranging from 26 to 42 weeks. Ninety-

four babies (23%) were under 37 weeks gestation of age and 316 (77%) were ≥ 37 weeks and term. Frequency of E.coli was 56.3% in maternal rectovaginal culture (231 cases of 410) against 11.2% in GBS (46 cases of 410). In surface cultures isolated from infants, frequency of E.coli was 29.8% against 8.8% GBS. Frequency of E.coli and GBS in term and preterm deliveries, PROM, History of UTI and mode of delivery are shown in table 1. Thirty-six infants of 46 culture positive mothers had surface colonization of GBS. Vertical transfer was 78.3% for GBS and 49.4% for E.coli.

Table -1: Frequency of E.coli and GBS in mother and infant, term and preterm deliveries, PROM, History of UTI and mode of delivery

Variable		GBS Frequency(%)	P- value	E.coli Frequency(%)	P- value
frequency based on gestational age in mothers	Term(n=316)	43(13.6%)	0.005	167(52.8%)	0.009
	Preterm(n=94)	3(3.2%)		64(68.1%)	
frequency based on gestational age in infants	Term(n=316)	36(11.4%)	0.001	84(26.6%)	0.01
	Preterm(n=94)	0		38(40.4%)	
PROM	Yes(n=183)	24(13.1%)	0.27	104(56.8%)	0.85
	No(n=227)	22(9.7%)		127(55.9%)	
History of UTI	Yes(n=27)	4(14.8%)	0.54	17(63%)	0.47
	No(n=383)	42(11%)		214(55.9%)	
Mode of delivery in mothers	Cesarean(n=119)	5(4.2%)	0.004	71(59.7%)	0.39
	Vaginal delivery(n=291)	41(14.1%)		160(55%)	
Mode of delivery in infants	Cesarean(n=119)	2(1.7%)	0.001	28(23.5%)	0.081
	Vaginal delivery(n=291)	34(11.7%)		94(32.3%)	

Discussion:

The frequency of E.coli was 56.3% in rectovaginal culture against 11.2% for GBS in the present study with vertical transmission rate 49.4% and 78.3% respectively. The prevalence of Group B Streptococcus colonization is different in various parts of Iran. In a study conducted by Hamed et al., the prevalence of GBS was 6% in mothers and 5% in infants, with 80% vertical transfer, [12]. This was reported 5.2% in Tabriz [13]. A similar study in Shiraz reported colonization of 9.1% with vertical transfer of 60% [14]. Therefore, in the present study, the frequency of GBS was similar to that in Shiraz and vertical transmission rate was the same as the study of Hamed et al.

In other studies conducted in Iran, the isolation of GBS was 16.2% and 12.44%, which was higher than the current study and this difference may be owing to the regional differences [15-16]. Yet, studies conducted in Tehran have reported frequency of GBS 4.8% in one

study and 22.76% in another, and maybe, the difference is related to the large sample size in the second study [10,11].

Vaginal colonization of GBS was reported 26% in America, 23% in Tanzania, 14.6% in Hong Kong, and 15.3% in Lithuania, which was higher than that in the present study, and regional and demographic differences are the causes of this difference in the frequency of GBS [17-19].

In the present study, vertical transfer of GBS was 78.3%, but this was reported lower (38.9%) in a meta-analysis. This meta-analysis study which included 31 studies conducted in different parts of the world, this difference can be due to a low rate of vertical transfer [20].

In the present study, 77% of mothers had term and 23% had preterm childbirths. The most common bacteria in rectovaginal colonization in mothers with term childbirths were Staphylococcus epidermidis and followed by E.coli. Rectovaginal colonization with

GBS was significantly higher in mothers with term childbirth compared to preterm (13.6% V 3.2%). The most common organism isolated in rectovaginal culture in mothers with preterm childbirths was E.coli (68.1% V 52.8%), which was significantly higher compared to mothers with term childbirths.

In a study by Krohn et al., vaginal colonization with E.coli was significantly related to preterm childbirth and LBW [21]. In another study performed in America, vaginal colonization with E.coli and Klebsiella was considered a risk factor for preterm childbirth [22]. The results of these studies are similar to this study.

In the present study, no preterm infant was born with GBS colonization. This seems to be due to the protocol-based administration of prophylactic antibiotic in mothers presenting with progressive labor pains in gestational age of less than 37 weeks, which prevented colonization of preterm infants with GBS. In preterm infants, E.coli was the most common microorganism found in surface colonization. The significant relationship between E.coli colonization and preterm infants indicates the possible effect of this microorganism on preterm birth of these infants. The present study's results in relation to colonization of term and preterm infants are in line with other studies. Stoll et al. reported GBS in term infants and E.coli in preterm infants as the most common pathogen causing sepsis, and that the majority of deaths occurred in infants infected with E.coli [23]. In another study conducted by Pubolo et al. on 18 years of data on neonatal sepsis, the most common pathogens were GBS in all cases of sepsis, and E.coli in VLBW infants [24].

In the present study, colonization with E.coli and GBS did not increase the odds of urinary tract infection. Unfortunately, urinary culture results of these mothers were not available, but according to previous studies, frequency of bacteria causing UTI in pregnant mothers was not different from normal women. E.coli being the most common cause (70%-85%) and group B Streptococci were found in 2%-7% to 10% of organisms isolated from UTI of pregnant mothers [25-26-27].

In the present study, no significant relationship was found between preterm premature rupture of membranes (PPROM) and colonization with either of the two main organisms. A study conducted by Javanmanesh et al. also showed no relationship between PPRM and colonization of GBS; yet, this relationship was significant according to another study conducted in Iran by Jahromi [11-14]. The lack of a

significant relationship between PROM and colonization with GBS may be due to the administration of antibiotics for mothers that had not given birth six hours after amniotic sac rupture based on the hospital protocol. It is worth mentioning that mothers with rupture of membrane and without delivery after 2 hours at admission were placed in ROM group.

Since E.coli is the most common organism in preterm childbirths in this region, mothers and infants should be adequately protected against neonatal sepsis.

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References:

1. Ferrieri P, Wallen LD. Neonatal Bacterial Sepsis. In: Gleason CA, Devaskar SU, editors. *Neonatal diseases of the newborn*. 1. 9th ed; 2012. p. 538-50.
2. Mukhopadhyay S, Puopolo KM. Risk Assessment in Neonatal Early Onset Sepsis. *Seminars in Perinatology* 2012; 36(6): 408-15.
3. Polin RA, Papile LA, Baley JE, et al. Management of neonates with suspected or proven early-onset bacterial sepsis. *Pediatrics* 2012; 129(5):1006-15.
4. Karambin M, Zarkesh M. Enterobacter, the most common pathogen of neonatal septicemia in Rasht, Iran. *Iran J Pediatr* 2011; 21(1): 83-7.
5. Simonsen KA, Anderson-Berry AL, Delair SF, Davies HD. Early-onset neonatal sepsis. *Clinical microbiology reviews* 2014; 27(1): 21-47.
6. Edwards MS, Gonik B. Preventing the broad spectrum of perinatal morbidity and mortality through group B streptococcal vaccination. *Vaccine* 2013; 31: D66-71.
7. Hornik CP, Fort P, Clark RH, et al. Early and late onset sepsis in very-low-birth-weight infants from a large

group of neonatal intensive care units. Early human development 2012; 88: S69-74.

8. Schuchat A. Neonatal group B streptococcal disease-screening and prevention. New Eng J Med 2000; 343(3): 209-10.
9. Neu J, Rushing J. Cesarean versus vaginal delivery: long-term infant outcomes and the hygiene hypothesis. Clinics in perinatol 2011; 38(2): 321-31.
10. Shirazi M, Abbariki E, Hafizi A, et al. The prevalence of group B Streptococcus colonization in Iranian pregnant women and its subsequent outcome. Inter J fertility & sterility 2014; 7(4): 267.
11. Javanmanesh F, Eshraghi N. Prevalence of positive recto-vaginal culture for Group B streptococcus in pregnant women at 35-37 weeks of gestation. Med J Islamic Republic of Iran (MJIRI) 2013; 27(1): 7-11.
12. Hamed A, Akhlaghi F, Seyedi SJ, Kharazmi A. Evaluation of group B Streptococci colonization rate in pregnant women and their newborn. Acta Medica Iranica. 2012; 50(12): 805.
13. Nahaei MR, Ghandchilar N, Bilan N, Ghahramani P. Maternal carriage and neonatal colonization of Streptococcus agalactiae in Tabriz, Northwest Iran. Iran J Med Sci 2015; 32(3): 177-81.
14. Jahromi BN, Poorarian S, Poorbarfehee S. The prevalence and adverse effects of group B streptococcal colonization during pregnancy. Arch Iran Med 2008; 11(6): 654-7.
15. Moghaddam MN. Recto-Vaginal colonization of Group B Streptococcus in pregnant women referred to a hospital in Iran and its effect on Lactobacillus normal flora. J Biol Sci 2010; 10: 166-9.
16. Nasri K, Chehrei A, Manavi MS. Evaluation of vaginal group B streptococcal culture results after digital vaginal examination and its pattern of antibiotic resistance in pregnant women. Iran J Reprod Med 2013; 11(12): 999.
17. Joachim A, Matee MI, Massawe FA, Lyamuya EF. Maternal and neonatal colonisation of group B streptococcus at Muhimbili National Hospital in Dar es Salaam, Tanzania: prevalence, risk factors and antimicrobial resistance. BMC Public Health 2009; 9: 437.
18. Barcaite E, Bartusevicius A, Tameliene R, et al. Group B streptococcus and Escherichia coli colonization in pregnant women and neonates in Lithuania. Inter J Gynecol&Obstet 2012; 117(1): 69-73.
19. Chung JKO, Ling TKW, Tam DCC. The Prevalence of Group B Streptococcus Colonization in Pregnant Women in Hong Kong. J Hong Kong Inst Med Lab Sci 2011-2012; 13(1&2). Available at: <http://www.hkimls.org/j2012-2.pdf>
20. Chan GJ, Lee AC, Baqui AH, et al. Prevalence of early-onset neonatal infection among newborns of mothers with bacterial infection or colonization: a systematic review and meta-analysis. BMC Infect Dis 2015; 15(118). Available at: <http://old.biomedcentral.com/content/pdf/s12879-015-0813-3.pdf>.
21. Krohn MA, Thwin SS, Rabe LK, et al. Vaginal colonization by Escherichia coli as a risk factor for very low birth weight delivery and other perinatal complications. J Infect Dis 1997; 175(3): 606-10.
22. Carey JC, Klebanoff MA. Is a change in the vaginal flora associated with an increased risk of preterm birth? Am J obstet gynecol 2005; 192(4): 1341-6.
23. Stoll BJ, Hansen NI, Sánchez PJ, et al. Early onset neonatal sepsis: the burden of group B Streptococcal and E. coli disease continues. Pediatr 2011; 127(5): 817-26.
24. Puopolo KM, Eichenwald EC. No change in the incidence of ampicillin-resistant, neonatal, early-onset sepsis over 18 years. Pediatr 2010; 125(5): 1031-8.
25. Matuszkiewicz-Rowińska J, Małyszko J, Wieliczko M. Urinary tract infections in pregnancy: old and new unresolved diagnostic and therapeutic problems. Arch Med Sci 2015; 11(1): 67-77.
26. Sharma P, Thapa L. Acute pyelonephritis in pregnancy: a retrospective study. Austr New Zealand J Obstet Gynaecol 2007; 47(4): 313-5.
27. Hill JB, Sheffield JS, McIntire DD, Wendel Jr GD. Acute pyelonephritis in pregnancy. Obstet Gynecol 2005; 105(1): 18-23.