Original Article

Colonization pattern of Gram positive organisms causing neonatal sepsis in pregnant women

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Abstract

Background: Neonatal sepsis is one of the major causes of mortality and morbidity in neonates. Early onset neonatal sepsis (EONS) is commonly caused by mother’s vaginal and rectal organisms before or during the delivery process.

Objective: The aim of this study was to determine the colonization pattern of common gram positive organisms responsible for neonatal sepsis, in women of 35-37 weeks of pregnancy.

Methodology: A cross sectional analytical study was conducted from July 2018 to June 2019 at the department of Microbiology of Sir Salimullah Medical College, Dhaka. Total 107 of each vaginal and vaginorectal samples was collected from pregnant women at 35-37 weeks of pregnancy attending at the out-patient department of the Obstetrics and Gynaecology unit of Sir Salimullah Medical College & Mitford Hospital, Dhaka. Vaginal and vaginorectal samples were tested by standard culture technique using Todd–Hewitt broth, blood agar, High chrome UTI agar media. Data was collected by a questionnaire and results were analyzed by statistical package for the social sciences (SPSS) program.

Results: In this study, among 107 vaginal swab samples Staphylococcus aureus were the predominant isolate (51.40%), followed by Enterococci (20.56%), Coagulase negative Staphylococcus (14.95%) and Group B Streptococcus (5.61%). Similarly among 107 vaginorectal swab samples commonest isolates were Staphylococcus aureus (56.1%), followed by Enterococci (52.34%), Coagulase negative Staphylococcus (11.21%) and Group B Streptococcus (6.54%).

Conclusion: The organisms from the vaginal and vaginorectal swabs were essentially the same. Staphylococcus aureus and Enterococci were the most abundant organisms that can cause neonatal sepsis, isolated from the vaginal and vaginorectal swab samples.

Introduction

Neonatal sepsis is one of the important cause of mortality and morbidity of newborns and is more common in developing countries compared with developed countries¹. In developing countries the incidence of sepsis is higher (1.8-18/1000) than the incidence in developed countries (1-5/1000)².

In Bangladesh neonatal mortality rate (NMR) is 27/1000 live birth and among this neonatal sepsis contributes to about 36% of total neonatal death. Neonatal sepsis has been classified as either early onset sepsis (EOS), presenting at 0-7 days of age, or late onset sepsis (LOS), occurring at 7-28 days of age³. EOS is due to vertical transmission during labor or birth. Maternal rectovaginal organisms play an important role in the incidence of early neonatal sepsis. LOS is due to vertical, horizontal or nosocomial infection⁴.⁵.

Several bacterial species are known to colonize both the gastrointestinal and the reproductive tract, and the rectum has been suggested to play an important role as a source or reservoir for organisms that colonize the vagina⁶. Among gram positive bacteria Group B Streptococcus (Strepto-
Colonization pattern of Gram positive organisms causing neonatal sepsis ... floras of the mother during the delivery. *Enterococcus* spp., are the most important cause of nosocomial infections and are the second most common cause of urinary tract infections (UTI) in hospitals. It also causes endocarditis, postpartum endometritis, abortion and neonatal sepsis. It was estimated that 10% cases of neonatal bacteremia and sepsicaemia are caused by *Enterococci*. This infection can be acquired from the vagina of the mother or as cross infection from the hospital.

**Materials and Methods**

Department of Microbiology, Sir Salimullah Medical College, Dhaka. Study population included pregnant women of 35-37 weeks attending at the out-patient department of the Obstetrics and Gynaecology unit of Sir Salimullah Medical College & Mitford Hospital, Dhaka. Those excluded from the study were: Pregnant woman of <35 weeks and >37 weeks of gestation, rupture of membrane at the time of sample collection, pregnant women with per vaginal bleeding at the time of sample collection, patient under antimicrobial therapy or history of intake of antibiotics during the past 2 weeks, preexisting medical disorders complicating pregnancy, women with urinary tract infection or vaginal infection in the current pregnancy and patients who did not give consent for this study. In this study, 214 samples (one sample from the vagina and one sample from the vaginorectal) were taken from 107 women at 35-37 weeks of pregnancy. A questionnaire and a check list were the tools of data collection. Informed written consent was taken from each patient or attendant. Before collecting specimen, each patient was interviewed and relevant information was recorded systematically in a pre-designed standard data sheet and data was analyzed by appropriate method. The study was approved by protocol approval committee & Ethical review committee of Sir Salimullah Medical College, Dhaka. This cross sectional analytical study was conducted from July 2018 to June 2019.

**Collection of samples**

Two samples were collected from each patient with strict aseptic precaution. One vaginal swab was obtained from the lower third of the vagina by one sterile swab stick.

coccus agalactiae, GBS), *Staphylococcus aureus*, coagulase-negative *Staphylococci* (CONS) and *Enterococcus* spp., are the most common rectovaginal colonizer and are known to be associated with neonatal sepsis. According to data from the American Neonatology Network, gram-positive microorganisms are the etiological agents in 62% of early neonatal sepsis cases and in 79% of late neonatal sepsis. Group B *Streptococcus* (GBS) has been the most frequent etiological agent of neonatal sepsis in developed countries, being responsible for high morbidity and mortality. Upto 30% adults, are commonly colonized with GBS in the gastrointestinal and genital tract but remain asymptomatic. The gastrointestinal tract serves as the primary reservoir for GBS and is the likely source of vaginal or rectal colonization.

Women of childbearing age carry GBS at variable frequencies of 4.6%-31.3% in both developing and developed countries. Pregnant women who are carriers of GBS have 40%-73% potential capacity for vertical transmission of the microorganism and 1%-2% of their newborns develop invasive GBS infection, with significant mortality (5%-20%) and morbidity, especially among premature neonates.

The rates of colonization of *S. aureus* in the women of reproductive age group range from 5% to 26%. Maternal colonization increases the risk of newborn staphylococcal colonization, through vertical transmission and breastfeeding. *S. aureus* is a rare cause of early-onset sepsis (EOS) (<1%) and responsible for 8% to 15% of LOS. Overall, *S. aureus* mortality ranges from 5% to 18%, but it is as high as 25% in very low birth weight infants (VLBW; <1500 g BW).

CONS is a common vaginal colonizer. It is most important bacterial cause of sepsis and meningitis in newborns. 2% to 22.5% of EOS are caused by CONS and 50% of LOS are caused by CONS. Infants born prematurely are at increased risk of CONS sepsis. Neonatal sepsis and meningitis with coagulase-negative *Staphylococci* are associated with significant morbidity and have a mortality rate as high as 10%.

*Enterococci* are among the first bacteria that colonize the neonatal gastrointestinal tract either through oral ingestion of breast milk or from the vaginal and gastrointestinal tract infection or vaginal infection in the current pregnancy and patients who did not give consent for this study. The study was approved by protocol approval committee & Ethical review committee of Sir Salimullah Medical College, Dhaka. This cross sectional analytical study was conducted from July 2018 to June 2019.

**Collection of samples**

Two samples were collected from each patient with strict aseptic precaution. One vaginal swab was obtained from the lower third of the vagina by one sterile swab stick.
Colonization pattern of Gram positive organisms causing neonatal sepsis ...

Subsequently, the swab was carefully withdrawn to prevent contamination with microflora from the vulva and introitus. Then one vaginorectal swabs was obtained from the lower third of the vagina by one sterile swab stick. Subsequently, the swab was carefully withdrawn to prevent contamination with microflora from the vulva and introitus and the swab was inserted 1.5 to 2 cm beyond the anal sphincter and gently rotated to touch the anal crypts. Vaginal and vaginorectal swab were immediately inoculated into 5 ml of Todd-Hewitt broth with 10 µg colistin/ml at the bed side. Then all swabs were transported to the laboratory within 4 hours.

**Culture**

The swabs were inoculated into Todd-Hewitt broth media. The Inoculated Todd-Hewitt broth was incubated for 18-24 hours at 37°C. Then subculture was done into Blood Agar media, MacConkey agar media, HiChrome UTI agar and Chocolate agar media. Blood Agar media, and Chocolate agar media was incubated for 18-24 hours at 37°C with 5% CO₂ (by using a candle jar, 5% CO₂ was generated). MacConkey agar media and HiChrome UTI agar was incubated for 18-24 hours at 37°C. At the end of incubation the culture plates were examined and appropriate colonies were subcultured for pure isolation. If no growth was found, the plates were incubated for an additional 24 hours and re-examined for growth of the organism. If no growth was found on the second examination, the plates were declared as negative. Identification of organisms was performed based on colony morphology, Gram stain, catalase test, CAMP test and bacitracin sensitivity test.

**Statistical analysis**

All necessary information and clinical data were systematically recorded in a pre-designed data collection sheet. Data analysis was done by SPSS version 17.

**Results**

Out of 107 vaginal swab samples 93 (86.92%) yielded bacterial growth and no growth occurred in 14 (13.08%) cases by culture and among 107 vaginorectal swab samples, all (100%) yielded bacterial growth by culture. (Fig: I).

**Fig I:** Percentage of bacterial growth from vaginal and vaginorectal swab among study population by culture.

Among 107 vaginal swab samples, *Staphylococcus aureus* was the most commonly isolated organism 55 (51.40%), followed by *Enterococci* 22 (20.56%) and Coagulase negative *Staphylococcus* 16 (14.95%). While Group B *Streptococcus* were isolated from 6 (5.61%) vaginal swab samples (Table I).

| Organisms                  | Number | Percentage (%) |
|----------------------------|--------|----------------|
| *Staphylococcus aureus*    | 60     | 56.1           |
| *Enterococci*              | 56     | 52.34          |
| Coagulase negative         | 12     | 11.21          |
| *Staphylococcus*           |        |                |
| Group B *Streptococcus*    | 7      | 6.54           |

Almost similar pattern was found in vaginorectal swab samples. Among 107 vaginorectal swab samples, *Staphylococcus aureus* was the commonest organism 60 (56.1%), followed by *Enterococci* 56 (52.34%) and Coagulase negative *Staphylococcus* 12 (11.21%). Group B *Streptococcus* were isolated from 7 (6.54%) samples (Table II).

| Organisms                  | Number | Percentage (%) |
|----------------------------|--------|----------------|
| *Staphylococcus aureus*    | 55     | 51.40          |
| *Enterococci*              | 22     | 20.56          |
| Coagulase negative         | 16     | 14.95          |
| *Staphylococcus*           |        |                |
| Group B *Streptococcus*    | 6      | 5.61           |
Discussion
Neonates are more vulnerable to infection in the first week of life. It is estimated that infection causes 42% of deaths in the first week of life\(^\text{38}\). Early onset neonatal sepsis is generally caused by microorganisms obtained from the mother generally or during the delivery\(^\text{29}\). Rectovaginal colonization of pregnant women is suspected to be a risk factor for neonatal sepsis\(^{30}\).

The microorganisms that cause early-onset sepsis may vary depending on time and region across the world. Some studies found that gram positive bacteria were the commonest cause of neonatal sepsis\(^{31}\) while one study showed that the frequency of isolation of both gram positive and gram negatives was equal\(^{32}\).

In the present study, 107 vaginal and 107 vaginorectal swab were collected from pregnant women at 35-37 weeks and different microorganisms were isolated and identified from vaginal and vaginorectal swab. All (100%) vaginorectal swab yielded bacterial growth, while in case of vaginal swab 93 (86.92%) showed bacterial growth and 14 (13.08%) were culture negative. Vaginorectal swab was found to be better sampling technique for detection of colonization in pregnant women. This observation is consistent with other studies, which reported that vaginorectal sample is more accurate than vaginal sample\(^{33,34}\).

In this study, isolated organisms from vaginal swab that can cause neonatal sepsis, were *Staphylococcus aureus* (51.40%), *Enterococcus* (20.56%), Coagulase negative *Staphylococcus* (14.95%), Group B *Streptococcus* (5.61%). These findings correlate with the findings of Ekanem et al\(^{35}\), where the commonly found organisms were, Coagulase negative *staphylococcus* (58.6%), *Staphylococcus aureus* (53.1%), *Enterococcus* (40.0%), Group B *Streptococcus* (12.2%). Studies carried out by Tarana and Shamsuzzaman\(^{36}\), reported that *Staphylococcus aureus* (19.64%), Group B *Streptococcus* (12.50%), Coagulase negative *Staphylococcus* (3.57%) were the most frequent organisms isolated from vaginal swab. Shirazi et al\(^{37}\), in their study found a similar pattern of organisms. So it was evident that, although the types of colonizing organisms were similar but the isolation rate of organisms were different in various studies, this may be due to, different laboratory methods used in those studies. Moreover the composition of the vaginal flora is not static but changes over time in response to several endogenous and exogenous influences\(^{38}\).

In this study, isolated organisms from vaginorectal swab were *Staphylococcus aureus* (56.1%), *Enterococcus* (52.34%), Coagulase negative *Staphylococcus* (11.21%), Group B *Streptococcus* (6.54%). These findings correlate with the findings of Hassanzadeh et al\(^{39}\), where the commonly found organisms were, Coagulase negative *Staphylococcus* (26.4%), *Staphylococcus aureus* (7%), *Enterococcus* (25.1%), Group B *Streptococcus* (13.8%). Another study carried out by Javanian et al\(^{40}\), reported that Coagulase negative *Staphylococcus* (55.8%), Group B *Streptococcus* (11.7%) were the most frequent organisms isolated from vaginorectal swab. In this study there is no significant difference between the isolated gram positive organisms from vaginal and vaginorectal swabs. These organisms colonize in the gastrointestinal tract and due to close proximity of anus these organisms may migrate to genital tract and consequently were isolated from vaginal and vaginorectal swabs\(^{41,42,43}\).

According to the present study, *Staphylococcus aureus* and *Enterococcus* spp. were the most common rectovaginal colonizer among the women of 35-37 weeks of pregnancy that can be the potential source of neonatal sepsis.

Conclusion
The findings of the present study revealed that *Staphylococcus aureus* and *Enterococcus* spp. were the most common isolated organisms among the women of 35-37 weeks of pregnancy. Early-onset infections that are caused by organisms prevalent in the maternal genital tract can be prevented through screening program for maternal vaginorectal colonization. Available programs include detection of GBS colonization in the last gestational weeks. These programs allow the reduction of GBS infections in newborns but are not directed to the detection of other pathogens involved in EONS. So the extension of the screening program to other pathogens could identify them and helps to implement the administration of specific antibiotic prophylaxis.

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Hasnat et al

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