ORIGINAL ARTICLE

Evaluation of Endocervical Microbial Colonization in Premature Rupture of Membranes and Pregnancy Outcomes: A Cross-sectional Observational Study

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Abstract

Objective: Premature rupture of membranes (PROMs) is one of the common causes of preterm birth which has a major contribution to maternal and fetal adverse outcomes. Infection may be contributing to premature rupture of membrane or it may be a consequence. The aim of our study was to assess the common pathogens colonizing the endocervix in women with PROM and pregnancy outcomes.

Materials and methods: In this prospective observational study, seventy antenatal women with PROM after 24 weeks of gestation were studied from January 2020 to July 2021. Two swabs were taken from the endocervix. Swabs were subjected to gram staining and culture. The association of endocervical culture positivity with chorioamnionitis, mode of delivery, and neonatal outcome was evaluated.

Results: In the study, 24.2% of endocervical cultures were positive. The pathogens isolated were *Escherichia coli* in 11.4%, *Staphylococcus hemolyticus* in 2.9%, budding yeast in 2.9%, 1.4% each with *Klebsiella pneumoniae* and Methicillin-resistant *Staphylococcus aureus* (MRSA). The culture was positive in 30.3% of subjects with preterm PROM and 18.9% with PROM ($p = 0.40$). The incidence of chorioamnionitis was higher with endocervical culture-positive cases as compared to culture-negative women ($p = 0.05$). There was no association of endocervical culture positivity with the neonatal intensive care unit (NICU) admission, duration of membrane rupture, and mode of delivery.

Conclusion: In our study, the most common bacteria inhabiting the endocervix in PROM was *E. coli*. There is a definite influence of bacterial endocervical colonization on pregnancy outcomes but further studies are needed to corroborate the findings.

Keywords: Antibiotic sensitivity, Maternal morbidity, Neonatal morbidity, Premature rupture of membranes, Preterm premature rupture of membranes, Treatment protocol, Vaginal microbiota.

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Introduction

The rupture of fetal membranes prior to the beginning of labor contractions is called premature rupture of membranes (PROMs). If rupture of membranes occurs prior to 37 weeks, it is termed as preterm premature rupture of membranes (PPROM).[1] PROM is responsible for around 30–40% of births occurring between 20 and 37 weeks of gestation.[2] In these cases, as the time since membrane rupture increases, the risk of intrauterine infection escalates.[3] Spontaneous labor usually sets in the majority of the term PROM. The likelihood of chorioamnionitis in patients with PPROM can be as high as 20–40%. The objective of the management of cases with PPROM mainly relies on maintaining the equilibrium between the advantages of lengthening the pregnancy duration to decrease the complications due to prematurity and timely delivery to reduce the risk of chorioamnionitis and its adverse outcomes.[4] It has been postulated that cervicovaginal colonization with microorganisms activates the local and innate immune system followed by the release of inflammatory mediators that can lead to remodeling and disruption of membrane architecture and premature rupture.[5]

After a substantial search of the literature, a wide range of studies conducted in various parts of the world have isolated different organisms responsible for PROM and PPROM. In many Western countries, group B streptococcus (GBS) is the predominant microorganism obtained from cervical colonization.[2] However, some studies demonstrated a different range of pathogens involved in cases with PROM. Hence, the antibiotic of choice has not been clear. The studies conducted to correlate the pathogenic colonization of the maternal genital tract with pregnancy outcomes also had mixed results. Thus, the objective of our study was to find out the common bacteria colonizing the endocervix in women with PROM and PPROM and their pregnancy outcomes.

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Materials and Methods

In this prospective observational study, seventy antenatal women with PROM after a 24-week period of gestation were studied

from January 2020 to July 2021. PROM was diagnosed by direct visualization of amniotic fluid coming from the cervix or collection of the amniotic fluid in the posterior fornix of the vagina during sterile per speculum examination. A study was conducted on the antenatal women admitted during the study period with the diagnosis of PROM. Pregnant women with singleton pregnancy and those who gave consent for the study were included. Patients refused to participate in the study, and multiple pregnancies and polyhydramnios were excluded. The institutional human ethics committee's approval was taken before starting the study. After written informed consent, two swabs were taken from the endocervix. The cervix was visualized using a speculum without a lubricant. Mucus and secretions from the cervix were removed. Sampling of the endocervical canal was done with two sterile cotton swabs for gram staining and culture. The swab for gram staining was covered with an overlying sterile cover and the swab for culture was placed in a glass tube containing Stuart's transport medium (HiMedia®. Mumbai, India). Swabs were transported to the bacteriology laboratory within half an hour of sampling and were processed within 2 hours of receipt.

Sample Processing

Gram staining—about 150 µL of sample mixture was aspirated by a 2-mL sterile syringe for smear preparation. One drop was put on a cleaned slide and allowed to air dry. After drying, heat fixation was done by passing a slide in the flame. It was subsequently stained by the following steps:

Crystal violet was applied for 1 minute and washed in running tap water. Gram's iodine was applied for 1 minute, washed in running tap water, decolorized by acetone for 2–3 seconds, and counterstained by applying safranin for 30–60 seconds. Then the slide was air-dried and observed under 100× magnification. The smear was observed for the presence of inflammatory cells, epithelial cells, and the presence of microorganisms.

The culture was done on blood agar (BA) and MacConkey agar (MA). Plates were incubated overnight at 35 ± 2°C under ambient air conditions in an aerobic incubator. They were read after 18–24 hours for visible colonies on solid media. If more than three types of colonies grew on culture media, they were considered colonizers and were not processed further for anti-microbial susceptibility testing. If no colonies were observed on the first day, they were further re-incubated and were reported as sterile after 40–48 hours of incubation. Identification and antimicrobial susceptibility testing of the isolates was done by VITEK®-2 Compact (bioMerieux, Marcy-l'Etoile, France) or by conventional tests/Kirby–Bauer Disc diffusion method depending upon the availability and interpreted as per Clinical Laboratory Standards Institute (CLSI) M100-29 document.[6,7]

Detailed examination of all the participants was done for fetal lie, presentation, and fetal heart rate. If the woman was less than 34 weeks period of gestation, she was given an injection of betamethasone 12-mg intramuscular and repeated after 24 hours as a routine protocol. All women with PROM near term were induced with oxytocin infusion. Cases with PPROM with clinical chorioamnionitis or any other contraindication for expectant management were also delivered. After taking a culture course of antibiotics (intravenous cefotaxime 1 gm 12th hourly as a routine protocol in our hospital due to broad coverage) was started in women with expectant management. Patients were observed for signs and symptoms of chorioamnionitis, which were defined as a temperature of more than 38° and the presence of at least two of the following criteria:[3] (A) Maternal heart rate of more than 100/minute; (B) Fetal heart rate more than 160/minute; (C) Uterine tenderness; (D) Malodourous uterine discharge; and (5) maternal leukocytosis, white blood cell count more than 15,000 cells. After delivery, neonatal blood culture was sent in newborns who needed neonatal intensive care unit (NICU) admission and with positive maternal endocervical culture.

Table 1: Demographic and clinical characteristics

Characteristics	*Number*	*%*
Mean age	27.6 ± 3.93 years	
Obstetric score		
Primigravida	43	61.4
Gravida 2	19	27.1
≥Gravida 3	8	11.4
BMI (kg/m^2)		
Underweight (<18.5)	2	2.9
Normal (18.5–22.9)	37	52.9
Overweight (23.0–24.9)	28	40.0
Obese (>25)	3	4.3
Socioeconomic status		
Upper lower and lower	34	48.6
Upper middle + lower middle	34	48.6
Upper class	2	2.9
Gestational age (weeks)		
<32	6	8.57
32–37	27	40
>37	37	51.4
Type of case		
PROM	37	52.9
PPROM	33	47

Statistical Analysis Plan

We used Microsoft Excel for data entry and statistical package for the social sciences (SPSS), version 25.0, for data analysis. The entered data were checked for outliers, duplications, redundancy, and missing values if any. Descriptive statistics measures, that is, mean and standard deviation were used to summarize numerical data, count, and percentage for summarizing nominal data. A comparison of categorical data was done using Chi-square test/Fisher's exact test. Further, $p < 0.05$ was considered significant.

Results

In the study, the mean age of participants was 27.6 ± 3.93 years. Forty-three women (61.4%) were primigravida, and 27 women (38.6%) were multigravida (Table 1). Thirty-seven women (52.9%) had normal body mass index (BMI) and 28 women (40%) were overweight. Thirty-seven women (52.9%) had term PROM and thirty-three (47.1%) had PPROM. On the evaluation of endocervical culture, 53 women (75.7%) were culture negative. Out of 17 women with endocervical culture positive, 8 (11.4%) had *Escherichia coli*, 2 (2.9%) had *Staphylococcus hemolyticus*, two (2.9%) had budding yeast, one each (1.4%) with *Klebsiella pneumoniae* and Methicillin-resistant *Staphylococcus aureus* (MRSA) and three women (4.2%) had more than 3 organisms (Fig. 1). The culture was positive in 30.3% of subjects with PPROM and 18.9% with PROM. The association was not statistically significant ($p = 0.40$). Features of chorioamnionitis were seen in two women with endocervical culture positivity (11.8%)

(Table 2). The organisms detected were *E. coli* and *Klebsiella*. None of the culture-negative patients had features of chorioamnionitis ($p = 0.05$). Out of seventeen culture-positive subjects, nine (52.9%) of their newborns had NICU admission. However, no significant association ($p = 0.16$) was observed between NICU admission and culture positivity (Table 2). The mean duration of rupture of membranes was 5.90 ± 4.77 hours in culture-negative subjects and 6.03 ± 3.62 hours in culture-positive subjects. This association was also not significant, that is, $p = 0.69$ (Table 2). Figure 2 shows antimicrobial susceptibility pattern of the most common organism cultured, that is, *E. coli*, for which a high degree of sensitivity was shown with gentamycin and cotrimoxazole. There was low sensitivity with many other antimicrobials.

Discussion

Premature rupture of membrane is the common cause of preterm labor. Management of preterm birth is a challenge to obstetricians as well as neonatologists. Many risk factors are identified for the occurrence of PPROM, like previous preterm labor, over-distension of the uterus (twins), infections, pregnancy-induced hypertension, black race, known case of diabetes, smoking, age less than 20 and more than 35 years.[8–10]

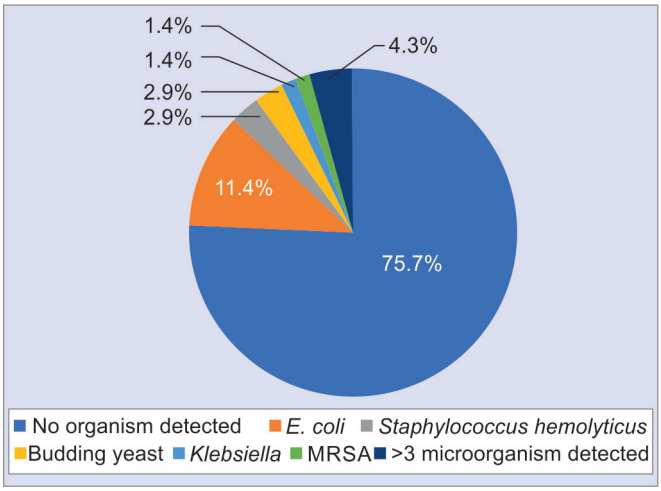

Fig. 1: Organisms found in swab culture

The most common risk factor identified in our study with PROM was urinary tract infection (24.3%), whereas both anemia (18.2%) and urinary tract infection (15.1%) were present in PPROM patients (Table 3). In addition to these, the most common risk factor in culture-positive subjects was having a positive family history of diabetes (41.1%) followed by Urinary tract infection (17.6%) and anemia (17.6%) (Table 4). A study conducted by Byonanuwe et al. also found Urinary tract infection as a risk factor for PROM. They concluded the need for regular screening and management of urinary tract infections in pregnant women especially with a history of recurrent abortions.[8] According to the study conducted by Assefa Natnael Etsay et al., women with abnormal vaginal discharge will experience 3.31 times the likelihood of development of PROM during the index pregnancy.[9] So, there may be a contribution of genitourinary infection in the pathophysiology of PROM.

Gram Staining and Culture Characteristics

In our study, Gram-positive bacilli were detected in 10%, Gram-negative bacilli in 7.1%, and Gram-positive cocci in 2.9% of the cases. The most common pathogens were *E. coli* (11.4%) and *S. hemolyticus* (2.9%). An Indian study by Kerur et al. revealed similar results with *E. coli* as the most common pathogen (38.2%) followed by *Klebsiella* (4.9%).[10] Another study was conducted in Iran by Saghafi N et al.,[1] in which positive endocervical culture was found in 68% of PPROM patients. The most common pathogens were *E. coli* (24.2%) and *Staphylococci epidermis* (14.7%). Group B streptococcus was detected in only three cases (2.2%).[1]

The scenario in Western countries is quite different where the predominant pathogen isolated was GBS. The results obtained by a study conducted by Lajos et al.[11] showed GBS as the most common organism (9.4%) followed by *Candida*, *Streptococcus pneumoniae*, *E. coli*, and *Enterococcus* spp. They demonstrated a high rate of endocervical culture positivity even with the use of a nonselective culture media in cases with PROM and preterm labor.[11] Loeb et al. from the United States conducted a study in PPROM cases from 20–25 weeks and also noted GBS as the major pathogen in placenta specimens.[12]

The use of antibiotics in PPROM has completely been evidence-based medicine. According to studies, taking antibiotics for PPROM

Table 2: Correlation of endocervical culture positivity with chorioamnionitis, NICU admission, mode of delivery, and mean duration of rupture of membranes

	Endocervical culture positive (n = 17)	*Endocervical culture negative (n = 53)*	*Total (n = 70)*	*p-value*
PROM	7	30	37	0.40
PPROM	10	23	33	
Features of chorioamnionitis				
Present	2 (11.8%)	0	2 (2.9%)	0.05
Absent	15 (88.2%)	53 (100%)	68 (67.1%)	
NICU admission				
Yes	9 (52.9%)	18 (34%)	27 (38.6%)	0.16
No	8 (47.1%)	35 (66%)	43 (61.4%)	
Mode of delivery				
Vaginal delivery	7 (41.2%)	25 (47.2%)	32 (45.7%)	0.79
LSCS	10 (58.8%)	28 (52.8%)	38 (54.3%)	
Mean duration of rupture of membranes (hours)	6.03 ± 3.62	5.90 ± 4.77	5.93 ± 4.49	0.69

is linked to longer pregnancy latency and decreased chances of fetal and maternal infection. Thus, most of the guidelines recommend antibiotics for all women with PPROM.[13] An appropriate antibiotic regimen is proposed by many guidelines with slight differences in the suggested regimen. The ORACLE study, a randomized trial recommended erythromycin for cases with PPROM. They found many advantages of using erythromycin such as lengthening of the pregnancy duration along with favorable neonatal outcomes.[14] Ampicillin plus erythromycin is one of the most commonly recommended combinations.[15] However, bacterial resistance has increased over the years. Also, these recommendations are not based on the epidemiological and ethnic differences in the pathogenic bacteria.

Our study revealed *E. coli* as the most common pathogen for which gentamicin was sensitive in 100% of samples followed by cotrimoxazole (50%), meropenem (37.5%), amikacin (37.5%) (Fig. 2). In a study conducted in Hong Kong by Li YY et al., mixed outcome

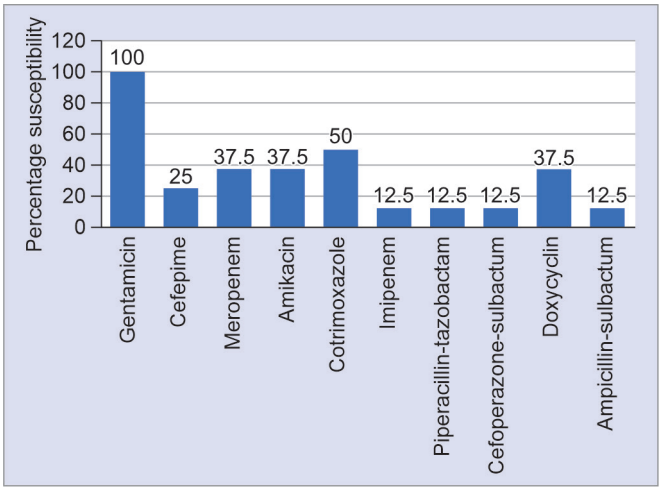

Fig. 2: Antibiotic sensitivity pattern of *E. coli*

Table 3: Distribution of risk factors

Risk factors	*PPROM*	*PROM*
1. Anemia	6 (18.2%)	1 (2.7%)
2. COVID-19 positive	1 (3.0%)	1 (2.7%)
3. Vaginal candidiasis	3 (9.1%)	2 (5.4%)
4. Gestational diabetes	2 (6.1%)	2 (5.4%)
5. Urinary tract infection	5 (15.1%)	9 (24.3%)

was noted with both Gram-positive GBS in 18.2% of specimens and Gram-negative *E. coli* in 12.8% of PPROM patients. In the above study, gram-positive bacteria (GBS) had a high rate of resistance to erythromycin, and *E. coli* had high rates of resistance to ampicillin (70.3%) and gentamycin (33.3%).[16] In a study by Saghafi N et al., *E. coli* being the most common organisms in endocervical culture of PPROM cases, was sensitive to penicillin, but had resistance to cephalosporin, cefotaxime or macrolide in 40% of cases. So, to overcome the increasing rate of antibiotic resistance and varied geographic distribution, frequent studies are needed to formulate the most effective management protocol suitable for a particular population.

Maternal and Neonatal Outcomes

In the present study, lower segment cesarean section (LSCS) was performed in 58.8% of participants where the culture was positive while the LSCS rate was 52.8% where the culture was negative. No significant association ($p = 0.79$) was observed between LSCS rate and culture positivity. There was a significant association ($p = 0.05$) between endocervical culture positivity and features of chorioamnionitis (11.8%) in our study. In a study conducted by Yu et al, clinical chorioamnionitis was found in 17.8% of cases of PROM.[17] In a study by Li YY et al., 4.7% of women showed signs of clinical chorioamnionitis as compared to 19.2% of histological chorioamnionitis in placental specimens. In the above study, prelabour rupture of membranes occurred earlier in gestation in patients with early-onset neonatal sepsis than in those without neonatal sepsis (31.1 vs 34.2 weeks; $p < 0.001$).[16] These findings indicate the significance of the duration of rupture of membranes to delivery or latency period. Maternal and neonatal complications increase as the latency period increases.

There was no correlation of culture positivity with the duration of membrane rupture to delivery and NICU admission in our study. The cause of this disassociation may be a small sample size and most of the patients in our study were delivered within 12 hours. On the contrary, a study by Shaghafi N et al. revealed that newborns of women with positive endocervical culture had higher NICU admission rates ($p = 0.004$). Also, there was a significant relation between NICU admission and duration of membrane rupture till sampling ($p < 0.001$).[1]

In our study, neonatal blood culture was sent for newborn babies of women with endocervical culture positivity who needed NICU admission (Table 5). Out of nine cases, four blood cultures were sterile; *E. coli* was detected in two of the neonatal blood cultures, one each with *S. aureus*, *K. pneumoniae,* and *Aerococcus viridans*. Our study did not show the correlation of maternal endocervical

Table 4: Correlation of maternal endocervical culture with neonatal outcome

Neonatal blood culture findings (n = 9)	*Maternal endocervical culture*	*Features of early neonatal sepsis (present/absent)*	*Neonatal outcome*
Sterile	*E. coli*: 3	Present: 1	All alive
4 (44%)	MRSA: 1	Absent: 3	
E. coli	*S. hemolyticus*: 1	Present: 1	Died: 1
2 (22.2%)	Yeast: 1	Absent: 1	Alive: 1
S. aureus	*E. coli*	Present	Alive: 1
1 (11.1%)			
K. pneumoniae	*K. pneumoniae*	Present	Died: 1
1 (11.1%)			
A. viridans	*E. coli*	Absent	Alive: 1
1 (11.1%)			

Table 5: Distribution of risk factors between culture-positive and culture-negative patients

Risk factors	*Endocervical culture positive*	*Endocervical culture negative*
1. Previous PPROM	1 (5.9%)	0
2. Vaginitis	1 (5.9%)	3 (5.6%)
3. Urinary tract infection	3 (17.6%)	10 (18.9%)
4. Anemia	3 (17.6%)	5 (9.4%)
5. Gestational diabetes mellitus	1 (5.9%)	3 (5.6%)
6. Spinal tuberculosis (TB) (treated)	1 (5.9%)	0
7. Family history of diabetes	7 (41.1%)	12 (22.6%)

culture with neonatal blood culture, Similarly, in a study by Głowacka et al., the culture of cervix uteri of PROM did not correlate with bacterial flora of the blood culture of the newborn.[18] Two cases of neonatal death were reported in our study, both had positive maternal endocervical and positive neonatal blood cultures. The cause of the first neonatal mortality in our study was due to sepsis, second newborn died due to respiratory distress syndrome (RDS) and prematurity. Similarly, in a study by Lajos et al., higher occurrences of early-onset neonatal infection were found associated with positive endocervical colonization (25.0 vs 7.3%; $p < 0.01$) and neonatal mortality (two cases in colonized women; $p < 0.02$) when compared with a negative culture of endocervical mucus.[11]

Limitations

The study sample size was small and selective culture media to detect various microorganisms may yield better results.

Conclusion

Our study has shown *E. coli* as the most common organism colonizing the endocervix in cases with PROM. As there are geographic variations in the pathogenic organisms and rising antibiotic resistance, periodic testing and monitoring of antimicrobial susceptibility should be done. In our study, endocervical culture positivity was associated with an increased risk of Chorioamnionitis and poor neonatal outcome. We suggest further large-scale studies to find out the most common pathogen colonizing the endocervix in different regions and also to find out the correlation of isolated microorganisms with the pregnancy outcome.

Clinical Significance

As PROMs can cause significant maternal and fetal compromise, it is important to individualize the management by culture and identify the sensitive antibiotics for safe outcomes. Broad-spectrum antibiotic use might increase Antibiotic resistance and hence region specific and culture-specific antibiotic is preferred.

Author's Contributions

All authors contributed to the study execution and analysis. Poojashree KS and Singh B were involved in the protocol development and data collection. Data management and manuscript writing were performed by Poojashree KS, Singh B, Pushpalatha K, and Gupta A. The initial version of the manuscript was written by Poojashree KS and all authors helped in the revision of the manuscript. All authors have gone through and approved the final manuscript.

Ethical Approval

This study was performed in line with the principles of the Declaration of Helsinki. Institutional Human Ethics Committee approval was taken before starting the study.

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