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Vaginal flora in preterm premature rupture of membranes and their sensitivity to commonly used antibiotics

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ABSTRACT

Background: Ascending infection is one of the most common etiologies in preterm premature rupture of membranes (PPROM). Antibiotics are used in PPROM to prolong the pregnancy and to prevent infection. But to prevent drug overuse and resistance, microorganism directed antibiotics should be used. So, this study was planned to evaluate vaginal flora in pregnant women with PPROM and their sensitivity to commonly used antibiotics. **Methods:** In this prospective cross-sectional study, 50 pregnant women (cases) preterm premature rupture of membranes and 28 pregnant women (controls) without complication were assessed for the type of vaginal flora and its sensitivity to commonly used antibiotics. **Results:** Among cases 18 (36%) women showed bacteria on Gram's staining of vaginal swabs with Gram-negative bacteria (10/18) being the most common. Among controls, 16 (57%) women showed bacteria on Gram's staining with Gram-positive bacteria being most common. Among cases *Escherichia coli* and *Staphylococcus aureus* were the commonest isolates. Of 8 (16%) positive bacterial cultures in cases, 6 (10%) were sensitive to Gentamicin and 2 (4%) to Ampicillin. **Conclusion:** Lower genital tract flora of pregnant women with PPROM predominantly consists of Gram-negative bacteria, which are sensitive to Gentamicin.

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Key words: Vaginal flora, Preterm premature rupture of membranes, Antibiotics

INTRODUCTION

Preterm premature rupture of membranes (PPROM) complicates about 3% of pregnancies and accounts for 30-40% of preterm deliveries.[1] Ascending choriodecidual infection appears to play an important role in etiology of PPROM.[2] Choriodecidual infection can be diagnosed by processing of amniotic fluid. However, because of invasive nature of procedure, need of expertise and difficulty in cultivating specific organisms, amniocentesis is not routinely done in low-resource settings.[3,4] Antibiotic therapy has been observed to prolong pregnancy and reduce maternal and neonatal morbidity and mortality. CDC also recommends Group B *Streptococcus* prophylaxis in all PPROM.[5]

Limited data is available from India about type of vaginal microflora in PPROM. To circumvent which, broad-spectrum antimicrobial therapy covering Gram positive and Gram-negative aerobic and anaerobic bacteria is used. However, major pathogens of neonatal infections are mostly Gram negative bacteria like *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas* sp, and *Acinetobacter* sp.[6-8] Thus, injudicious use of broad-spectrum antibiotics can lead to emergence of antibiotic-resistant organisms.[9]

This study was planned to evaluate profile of genital tract organisms in Indian women with PPROM and their sensitivity to commonly used antibiotics (ampicillin, cefazolin and gentamicin).

MATERIALS AND METHODS

This cross-sectional study was conducted from April 2009 to August 2010 in Government Medical College & Hospital, Chandigarh. Study protocol was approved by Ethics Committee of the hospital and abiding to Helsinki's declaration. Written informed consent was obtained from enrolled women.

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Patients with live fetus and period of gestation less than 37 weeks were included in the study. Women who presented to the labor and delivery unit with complaints suggesting rupture of chorioamniotic membranes were enrolled as cases. Women who presented to outpatient department at 30-36 weeks of gestation without active labor were enrolled as controls. Patients with maternal complications like multiple pregnancies, antepartum hemorrhage, polyhydramnios, preeclampsia, eclampsia, diabetes mellitus, vaginitis and congenital malformed fetus, already received antibiotics for PPROM were excluded from study. History, general physical and obstetrical examinations were done. Enrolled women were evaluated using a sterile speculum examination. Verification of PPROM was based on pooling of amniotic fluid and alkaline pH with nitrazine test. Swab from the posterior fornix of the vagina was taken and sent for Gram's staining and culture sensitivity.

Microbiological analysis

Vaginal swabs were obtained from cases and controls and sent to the Department of Microbiology. The Gram's staining was done followed by inoculation on Blood and MacConkey agar as per standard protocols. After overnight incubation, plates were checked for growth. Identification of pathogen was done and the significant pathogen was then evaluated for antimicrobial susceptibility testing using commonly used antibiotics for aerobic (Ampicillin, Cefazolin and Gentamicin). Cultures for anaerobic bacteria were not used as growing anaerobes is not cost effective as proven in previous studies and metronidazole is an effective drug for all type of anaerobes.

Statistical analysis

Data was entered in Microsoft Access (Microsoft Corporation, Redmond, WA, USA) and analyzed using Epi-Info (Center for Disease Control, Atlanta, US). Continuous data with normal distribution is represented as mean (standard deviation) and analyzed by student t-test. Continuous data with non-normal distribution is represented as median (interquartile range) and analyzed by Mann-Whitney U test. Categorical data is presented as proportion and analyzed by Chi-square or Fischer exact test.

RESULTS

During the period of study, a total of 50 cases and 28 controls were enrolled. Demographic characteristics and reproductive history of enrolled subjects are summarized in Table 1. Mean age, parity status and gestational age at enrolment were similar among cases and controls.

Cases presented to hospital with median duration of PPROM of 11 h (interquartile range: 8-24). Amniotic fluid index at presentation was 4.3 ± 1.5 cms. Delivery occurred after a median duration of 24 h (IQR: 16.5-55.5) of PPROM. Thirty eight (76%) cases delivered vaginally and 12 (24%) delivered through cesarean section.

Bacteria were seen on Gram's staining of high vaginal swab in 18 (36%) cases and 16 (57%) controls. Gram-negative bacteria were most common among cases (10/18), while Gram-positive bacteria were most common among controls (15/16). Culture of high vaginal swab grew normal flora in 11 (22%) cases and 11 (39.3%) control subjects. Pathogenic organism grew in 9 (18%) of cases in which *E. coli* was most common followed by *Staphylococcus aureus* and 1 (2%) grew *Candida* species. In control group 4 (14.2%) of culture grew pathogenic organism in which 2 (7.1%) were *S. aureus* and 2 (7.1%) *Candida* species (Table 2).

Of 8 (16%) positive bacterial culture in cases, 6 (10%) were sensitive to Gentamicin and 2 (4%) to Ampicillin. Out of 4 (14.3%) culture, which had grown organism in control group, 1 (3.5%) each was sensitive to Ampicillin and Gentamicin, 1 (3.5%) to Cefazolin.

None of preterm neonates developed early onset sepsis.

Table 1: Demographic characteristics and reproductive history in cases and control groups

Variable	Cases (n=50)	Controls (n=28)
Age, yr[a]	25.0±3.1	24.7±3.9
Booked in antenatal clinic of hospital[b]	11 (22)	28 (100)
Urban residence[b]	28 (56)	21 (75)
Education[b]		
Nil	2 (4)	0
Less than Class 6	5 (10)	6 (21.4)
Matric	20 (40)	3 (10.7)
Secondary	13 (26)	8 (28.5)
Graduate	4 (08)	4 (14.2)
Postgraduate	6 (12)	7 (25)
Gravida[c]	2 (1-3)	2 (1-3)
Parity[c]	1 (0-1)	1 (0-1)
Previous live birth[c]	0 (0-1)	0 (0-1)
Mean gestational age (wk)[a]	34.2±1.6	33.1±1.6

[a]Values are given as mean±SD, [b] number (percentage) or [c] median (interquartile range)

Table 2: Culture of high vaginal swab in cases and control group[a]

Type of microorganism	Cases n=(50)	Controls (n=28)
Escherichia coli	3 (6)	0
Staphylococcus aureus	2 (4)	2 (7.1)
Pseudomonas aeruginosa	1 (2)	0
Acinetobacter species	1 (2)	0
Klebsiella oxytoca	1 (2)	0
Candida species	1 (2)	2 (7.1)
Contaminants	3 (6)	0
Normal flora	11 (22)	11 (39.3)
Sterile	27 (54)	13 (46.3)

[a]Values are given as number (percentage)

DISCUSSION

In this cross-sectional study, we performed lower genital tract culture in pregnant women with PPROM. Most common isolated bacteria were *E. coli* followed by *S. aureus*. In resource-limited settings where microbiological evaluation of amniotic fluid is not feasible, identification of bacteria in high vaginal swab can guide antibiotic therapy in women with PPROM. Previous studies have shown good correlation between genital tract flora and organism grown in amniotic fluid or blood of neonates with early onset sepsis. Karat et al. reported *S aureus* (28%) and *E coli* (20%) as significant vaginal isolates in-patient with PPROM.[7] Carroll et al. reported that positive genital tract cultures for aerobic and anaerobic organisms predicted 40% of positive fetal blood and 53% of positive amniotic fluid cultures. In more than 75% of cases with positive amniotic fluid cultures, the same organisms were recovered from high vaginal swabs.[10] Out of 97 patients enrolled, 62% high vaginal swabs grew organism. Most common organism was *Mycoplasma* species followed by *Candida albicans*, *S agalactiae* and *E coli*. McDonald et al. too reported that the presence of *Bacteroides*, *K pneumoniae* and *H influenzae* in patients with preterm labor is associated with PPROM.[11] There is lack of data on profile of genital tract bacteria among Indian women with PPROM. To the best of our knowledge, there is only one previous study from India, which reported microbiological correlates of PPROM.[7] Antibiotic therapy in PPROM has been associated with significant reduction in incidence of chorioamnionitis, birth within one week of starting antibiotics and improved neonatal outcomes. Due to difficulty in obtaining amniotic fluid samples and various reports indicating mixed bacterial growth in amniotic fluid cultures, broad-spectrum antibiotics are prescribed during expectant management of PPROM.[4] ACOG also recommends a 7-day course oral or parenteral of Ampicillin or Amoxicillin and Erythromycin in pregnant women with PPROM who are remote from term.[12] This approach although simplistic can lead to inadequate treatment if causative organisms are resistant or not sensitive to these antibiotics. Also wide-spectrum resistance to penicillin group of antibiotics has been reported previously from India and other developing countries.[8,12]

In the present study, none of the culture grew Group B Streptococci and two-third isolates were sensitive to Gentamicin. One major limitations of the present study is small sample size. Due to this reason, we are unable to analyze association between microbiological profile and gestation at presentation or length of pregnancy. A large proportion of mothers did not grow any organism in their vaginal swabs and lack of use of special culture media for *Mycoplasma* may account for decreased rate of isolation.

To summarize, our study provides important data about microbiological correlate of PPROM in Indian pregnant women and most of pathological isolates were sensitive to Gentamicin. Larger studies are needed to formulate antibiotic policy for management of women with PPROM in resource-limited settings.

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Authors Contribution:
SR – designed the study, data collection, analysis and written the manuscript**; RM & AH** – data analysis and revising the manuscript; **VG & JC** – microbiological inputs and revision of manuscript**.**

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