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Original Research Article

Identification of causative pathogen and its antibiotic sensitivity in cases of preterm premature rupture of membranes

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ABSTRACT

Background: Pre-labor rupture of membranes is defined as amniotic membrane rupture before the onset of labor contractions, and if it happens before 37 weeks, it is called preterm premature rupture of membranes (PPROM). Several organisms commonly present in the vaginal tract are *E.coli*, Group-B *streptococci*, *staphylococcus aureus*, *chlamydia trachomatis*, *Gardnerella vaginalis* and *Enterococcus faecalis* which secrete proteases that degrade collagen thereby weakening the fetal membranes leading to PPROM. Appropriate antibiotic therapy has a significant role in the prevention and treatment of maternal and neonatal complications.

Methods: This was a prospective observational study done in the department of obstetrics and gynaecology, Narayana medical college, Nellore. Selectively 100 patients with complaint of PPROM admitted to labor room were included in the study. Diagnosis of membrane rupture was established by speculum examination, and high vaginal swabs are taken and sent to laboratory for identifying bacteria using gram staining and cultured in aerobic and anaerobic methods. Antimicrobial susceptibility testing of the organisms was performed by disk diffusion method by Kirby and Bauer.

Results: Out of 100, high vaginal swabs had growth in 82 patients, and 18 were sterile. The repeatedly isolated organism in patients with PPROM is *E.coli* amounting 32%, followed by candidal species 20%. Staphylococci are scoring 11% and enterococci 8%. However, organisms like *gardenella vaginalis* and Group B *streptococcus* are least common with a score of 6% and 5% respectively. In this study, *E.coli* is highly sensitive to tigecycline, colistin 100% each and highly resistant to gentamycin and amikacin.

Conclusions: In this study, *E.coli* is related to the maximum number of cases with preterm premature rupture of membranes. Appropriate use of antibiotics significantly lowers maternal morbidity and neonatal mortality.

Keywords: Antibiotics, High vaginal swab, Preterm premature rupture of membranes, Premature rupture of membranes

INTRODUCTION

Pre-labor rupture of membranes previously known as preterm rupture of membranes is described as amniotic membrane rupture before the onset of labor contractions, and if it happens before 37 weeks, it is called preterm premature rupture of membranes (PPROM).¹ Preterm labor is one of the major causes of perinatal mortality and neurological morbidity.² Imbalance in vaginal bacterial colonization in pregnancy makes these women susceptible to the colonization of pathogenic organisms.³ Among women with preterm PROM, clinically evident intraamniotic infection occurs in approximately 15-25% and postpartum infection occurs in approximately 15-20%.⁴ Collagens are the structural component for the tensile strength of membranes. The foremost cause for rupture of membranes is loss of tensile power of amniotic membranes. The pathophysiology of infection-causing PPROM is considered to be due to the production of prostaglandins and matrix-degrading enzymes via microbial endotoxins and pro-inflammatory cytokines (IL-8, IL 1beta, TNF alpha) which are liberated after binding of organisms to pattern- recognition receptors (ex-toll like receptors).⁵ This lead to an increase in metalloproteinase, which is responsible for collagen degradation leading to a decrease in the tensile power of membranes resulting in rupture. The hypothesis is that several organisms that are commonly present in the vaginal flora, including E.coli, B streptococci, staphylococcus aureus and microorganisms that cause bacterial vaginosis, secrete proteases that degrade collagen and weaken the fetal membranes leading to PROM.^{6,7} With regard to the importance of maternal genital tract bacterial colonization as an etiologic factor in PPROM, appropriate antibiotic therapy has a cardinal role in the prevention and treatment of maternal and neonatal complications.

The objective of this study was to diagnose the appropriate organism using a high vaginal swab. To select an appropriate antibiotic therapy to prevent maternal and neonatal complications.

METHODS

This study was prospective observational study from October 2018 to April 2019. Data of 100 patients with singleton pregnancy are admitted to the labor room with complaint of PPROM. Detailed history regarding gestational age, parity, duration of the leak was collected. In detail, the general and obstetrical examination is performed. Diagnosis of membrane rupture was accomplished by proper speculum examination to see amniotic fluid leakage from the cervical os. The amount, color, and smell of the fluid were assessed. Specimens were gram stained and cultured in both aerobic and anaerobic methods for identification of pathogens. In aerobic culture, bacterial colonies are identified by their morphology and biochemical reactions like catalase, coagulase, and esculin hydrolysis for gram-positive organisms. In the case of gram-negative bacteria, testing for motility, catalase, oxidase, sugar fermentation, nitrate reduction, citrate utilization, urea hydrolysis, methyl red, and Voges-Proskauer test is done. In anaerobic culture, bacterial identification was done by colony morphology, pitting of agar, growth character in selected and indicator anaerobic media plates. Presence of candida species in gram stained preparation of vaginal discharge showed gram positive budding yeast cells.

Antimicrobial susceptibility testing

It is done using a disc diffusion method of KIRBY and BAUER.⁸ The test organism is inoculated into protease peptone water and incubated for 4-6 hours. The turbidity is matched with McFarland opacity standard 0.5. A sterile cotton swab is immersed into the suspension is used to streak the surface of the Mueller-Hinton agar. Different

antimicrobial discs, 6.0 mm in diameter, charged with appropriate concentrations of antibiotic are placed at a distance of 20 mm apart; inverted plates are incubated for 16-24 hours at 37°C. The degree of antibiotic sensitivity is obtained by measuring the zones of inhibition of growth around the disks.

- Susceptible: zone of inhibition of >10 mm
- Resistant: zone of inhibition <10 mm.

Inclusion criteria

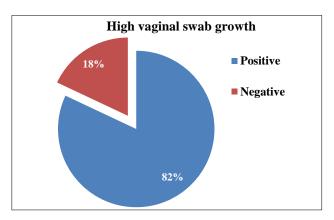
- Singleton pregnancy
- PPROM
- Gestational age between 24-37 weeks
- Chorioamnionitis
- No prior antibiotic use.

Exclusion criteria

- Multiple gestations
- Patients consuming alcohol and smoking tobacco
- Patients refusal for being in the study.

RESULTS

A total of 100 cases with PPROM are taken a high vaginal swab. Out of 100 cases of vaginal swab culture study, 82 cases were culture positive, and 18 cases were sterile (Figure 1).



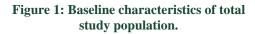


Table 1: Organisms associated with PPROM.

Organism	Total percentage
Escherichia coli	32%
Candida	20%
Staphylococci	11%
Enterococci	8%
Gardnerella vaginalis	6%
Group B streptococcus	5%
No growth	18%

The most common isolated organism is *E.coli* among all cases amounting 32%, followed by *candida species* 20%, *staphylococci* scoring 11% and *enterococci* 8%.

However, pathogens like *Gardnerella vaginalis* and Group B *streptococcus* are least common with a score of 6% and 5% respectively (Table 1).

Sr. no.	Antibiotics	E.coli	Staphylococci	Enterococci	G. vaginalis	Group B streptococci
1	Tigecycline	100%	80%	80%	N/A	N/A
2	Colistin	100%	N/A	N/A	N/A	N/A
3	Linezolid	60%	100%	100%	N/A	N/A
4	Gentamycin	20%	90%	80%	80%	80%
5	Amikacin	20%	N/A	20%	N/A	60%
6	Erythromycin	N/A	20%	N/A	N/A	30%
7	Vancomycin	N/A	60%	30%	N/A	N/A
8	Ciprofloxacin	N/A	N/A	80%	60%	N/A
9	Cefotaxime	80%	N/A	N/A	N/A	100%
10	Amoxycillin+ clavulanic acid	N/A	N/A	60%	N/A	N/A
11	Nitrofurantoin	60%	80%	N/A	N/A	40%
12	Metronidazole	N/A	N/A	N/A	100%	N/A

Table 2: Antibiotic susceptibility of following organisms.

E.coli is highly sensitive to Tigecycline, colistin 100% each and highly resistant to Gentamycin and Amikacin. *Staphylococci* were more sensitive to Linezolid 100%, Gentamycin 90%, Tigecycline, and Nitrofurantoin each 80%. It is mostly resistant to Vancomycin 60% and Erythromycin 20%. *Enterococcus* species are highly sensitive to Linezolid 100%, Tigecycline 80%, Gentamycin, Ciprofloxacin each 80% and it is resistant to Amikacin 20% and Vancomycin 30% (Table 2).

DISCUSSION

In this prospective observational study, antibiotic sensitivity is determined by taking high vaginal swabs in women with PPROM. E.coli is cited as one of the most common organisms amounting 32%, followed by candida 20%. In the antibiogram study, it is highly sensitive to Tigecycline 100%, Colistin 100%, and Cefotaxime 80%. It is mostly resistant to Gentamycin 20% and Amikacin 20%. This study found that staphylococci were more sensitive to Linezolid 100%, Gentamycin 90%, Tigecycline, and Nitrofurantoin each 80%. It is mostly resistant to Vancomycin 60% and Erythromycin 20 %. Enterococcus species are highly sensitive to Linezolid 100%, Tigecycline 80%, Gentamycin, Ciprofloxacin each 80% and it is resistant to Amikacin 20% and Vancomycin 30%. Then comes G. vaginalis 6% and eventually Group B streptococcus 5%.

Administration of broad-spectrum antibiotics prolongs pregnancy, reduces maternal and neonatal infections, and reduces gestational age-dependent morbidity.⁹ Based on available information, in order to reduce maternal and neonatal infections and gestational-age-dependent morbidity, a 7-day course of therapy with a combination of intravenous ampicillin and erythromycin followed by oral amoxicillin and erythromycin is recommended during expectant management of women with PROM.¹⁰

CONCLUSION

Proper detection of causative pathogens by high vaginal swab and administration of appropriate antibiotic therapy improves maternal and neonatal outcome. Most of the pathological isolates were sensitive to Tigecycline, Linezolid, and Gentamycin in our study.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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