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Original Research Article

A STUDY ON MICROBIOLOGICAL SPECTRA ASSOCIATED WITH PROM BASED ON HIGH-VAGINAL SWAB AND URINE CULTURE AND ITS CLINICAL SIGNIFICANCE IN PREGNANCY AND NEONATAL OUTCOME

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ABSTRACT

Background: Premature rupture of membranes (PROM) is a common obstetric complication with significant maternal and neonatal risks, often aggravated by genitourinary infections. This study aimed to analyse the microbiological spectra associated with PROM using high vaginal swabs (HVS) and urine cultures. **Materials and Methods:** This prospective study was conducted at GVMCH, Villupuram, over a period of 2 years and included 150 antenatal mothers with PROM beyond 32 weeks. HVSs and urine samples were cultured using standard microbiological methods. Maternal outcomes (postpartum fever, wound infection, PPH) and neonatal outcomes (Apgar score, prematurity, sepsis, respiratory distress syndrome, mortality) were assessed. Statistical associations were analysed using the chi-square test, with $p < 0.05$ considered significant. **Result:** Most patients were aged 21–29 years (73.3%), multigravida (63.3%), and from the upper-lower socioeconomic class (52%). Infection was identified as the cause of PROM in 19.3% of cases. E. coli was the most common isolate from HVSs (47.6%) and urine cultures (37.5%). Significant associations were observed between culture-positive PROM and maternal ($p < 0.001$) and perinatal morbidity ($p < 0.001$). Low Apgar scores ≤ 6 occurred in 2% of the neonates ($p < 0.001$). Postnatal mortality was 2.6% ($n = 4$), mainly due to prematurity (50%). First-line sensitivity was observed with gentamicin and ampicillin; resistant isolates required amikacin, imipenem, meropenem, piperacillin–tazobactam, or ceftriaxone. **Conclusion:** PROM complicated by infection is strongly associated with maternal morbidity and adverse neonatal outcomes. E. coli predominates, and culture-guided therapy is essential. An antibiotic policy using gentamicin and ampicillin as first-line agents, with escalation to carbapenems or β-lactam inhibitors, can optimise outcomes.

INTRODUCTION

Prelabour rupture of membranes (PROM) is a condition in which the amniotic sac ruptures, causing amniotic fluid to leak before labour. Urinary tract infections (UTIs) and bacterial vaginosis (BV) are the most common infectious conditions that complicate pregnancy when associated with PROM and play a key role in shaping maternal and neonatal outcomes. UTIs affect up to 26% of antenatal women in Indian tertiary care centres, with Escherichia coli (E. coli) being the most frequently observed pathogen, followed by Klebsiella species, Enterococcus faecalis, and Candida species.[1] The anatomical and hormonal changes during pregnancy cause urinary stasis and ureteral dilatation, which further increase the susceptibility to infections.[2] Asymptomatic bacteriuria, which is reported in 2–10% of pregnancies, if left untreated, up to 40% of these cases progress to symptomatic UTI or pyelonephritis.[3] Pyelonephritis in pregnancy has been associated with maternal complications such as anaemia, pre-eclampsia, and chronic renal problems, as well as fetal complications including prematurity, intrauterine growth restriction, and low birth weight.[1]

In North India, the incidence of BV was reported to be 20.5%, associated with preterm labour, PROM, and adverse neonatal outcomes. BV is characterised by the replacement of normal lactobacilli with anaerobes, Gardnerella vaginalis, and Mycoplasma hominis, organisms that can cause preterm delivery,

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intrauterine infection, and even intrauterine death.[4] The coexistence of BV and UTI along with PROM has been found to aggravate complications, and women with any of those infections are at the highest risk of poor pregnancy outcomes.[4,5] Maternal UTI is significantly associated with neonatal UTI, with pathogens such as E. coli, Klebsiella, and Proteus species being most commonly isolated in both mother and child.[2] The longer the duration from membrane rupture to delivery, the more the risk of neonatal complications. Therefore, PROM cases are associated with an increased risk of prematurity and neonatal infections due to an increased latency period. Indian studies report that babies born to mothers with positive high vaginal swab (HVS) cultures after PROM had higher chances to develop sepsis, with E. coli, Staphylococcus aureus, and UTI-related organisms as frequent pathogens.[6,7]

A study reported that approximately 85% of cases with E. coli as the predominant bloodstream pathogen during pregnancy are resistant to commonly used antibiotics, such as ampicillin. These resistant strains not only complicate the maternal management but also increase the likelihood of neonatal infections.[8] While untreated infections risk maternal morbidity, irrational use of antibiotics contributes to resistance and may modify the neonatal microbiota with long-term consequences.[9] Recent Indian data highlight rising rates of extended-spectrum β-lactamase (ESBL) and AmpC β-lactamase production among E. coli and Klebsiella isolates from antenatal women. Similarly, increasing reports of non-albicans Candida resistant to fluconazole also highlight the seriousness of antimicrobial resistance.[1]

Region-specific surveillance of microbiological spectra in pregnant women is important for tailoring empirical therapy. Establishing an antibiotic policy for pregnant women based on local prevalence and susceptibility patterns will improve maternal outcomes and reduce neonatal morbidity. Thus, this study aimed to analyse the prevalence of microbiological spectra among antenatal mothers, assess the impact of UTIs on maternal and neonatal outcomes, and develop an evidence-based antibiotic policy relevant to the local population visiting the Government Viluppuram Medical College Hospital (GVMCH).

MATERIALS AND METHODS

This prospective study was conducted on 150 antenatal mothers attending the antenatal OP department of GVMCH, Villupuram, for 2 years. The Institutional Ethics Committee approved the study, and written consent was obtained from all patients before inclusion.

Inclusion criteria

Pregnant women who provided consent aged 18–40 years with singleton gestations beyond 32 weeks, including both primigravida and multigravida, PROM cases with a confirmed history of leaking PV by speculum examination, cervical dilatation < 3 cm, and absence of uterine contractions.

Exclusion Criteria

Antenatal mothers with a gestational age < 32 weeks, multiple gestations, and maternal complications such as PIH, heart disease, anaemia, or a previous caesarean section. Immunocompromised mothers, including those with HIV infection, tuberculosis, diabetes mellitus, pre-eclampsia, a history of malignancy, or autoimmune disorders. Women with congenital anomalies of the urinary system, chronic renal impairment, haemodynamic instability, or mental illness.

Methods: All eligible patients were admitted to the labour ward and started on intravenous ampicillin 1 g, and then managed individually. Detailed history was obtained, including age, socioeconomic status, obstetric history, time and nature of membrane rupture, amount of liquor drained, prior interventions, history of coitus or genital infections, and any previous cervical surgery. Socioeconomic status was assessed using the Modified Kuppuswamy Socioeconomic Scale (3-29 scores). Clinical examination included nutritional status, anaemia, vital signs, and obstetric findings, including gestational age, liquor volume, uterine activity, and foetal presentation, along with foetal well-being assessment. Speculum examination was performed to confirm leakage, assess cervical dilatation, and determine the status of the membranes. Two HVSs were collected under aseptic precautions from the posterior and lateral fornices, and a midstream clean-catch urine sample (5 ml) was obtained in a sterile container. For catheterised patients, urine was collected through the catheter port.

HVSs were processed for aerobic bacterial and fungal cultures on MacConkey agar, blood agar, and Sabouraud dextrose agar, while urine samples were cultured on CLED agar using the standard loop method (0.01 ml). Plates were incubated at 35°C for 24 h, and growth of ≥ 105 CFU/ml was considered significant. Labour progress was closely monitored, and delivery was conducted either vaginally (spontaneous or instrumental) or by caesarean section, depending on maternal and foetal conditions. For pregnancies below 34 weeks, corticosteroids were administered before the intervention, and no tocolysis was used. Maternal outcomes, including chorioamnionitis (maternal fever with tachycardia or foul-smelling amniotic fluid), mode of delivery, and postpartum complications, were recorded. Neonatal outcomes, including birth weight, Apgar scores (0–10), sepsis, respiratory distress syndrome (RDS) (tachypnoea and cyanosis), morbidity, and mortality, were assessed. Newborns with complications were admitted to the NICU for further evaluation and followed until discharge, and mothers were monitored until discharge.

Sample size: The sample size was calculated using the formula n = 4PQ/D2, where p = 10% and D = 5%. Thereby providing n = 144, which is rounded off to 150.[9]

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Statistical analysis: Data were analysed using IBM SPSS Statistics (v25), with categorical variables presented as frequencies and percentages. Associations were assessed using the chi-square test and Fisher's exact test; a p-value < 0.05 was considered statistically significant.

RESULTS

Most patients were aged 21–29 years (73.3%) and belonged to the upper-lower socioeconomic group (52%). Most were booked cases (95.3%), multigravida (63.3%), term pregnancies (72.6%), and had cephalic presentations (91.3%). The most common cause of PROM was infection (19.3%), although the majority could not be identified (49.3%). PROM was confirmed in 86.7% of the cases with absent membranes, while 13.3% had their membranes intact [Table 1].

Footnotes: Data are presented as frequencies and percentages.

Most had clear liquor (96%), with latency < 6 h in 48.7% and 6–12 h in 40.7%. Vaginal delivery was more frequent (54%), and preterm deliveries occurred in 19.3% of the casesApgar scores were satisfactory (7 – 9) in the majority, although 2% had low scores (≤ 6). Maternal morbidity included postpartum fever (41.4%), wound infection (48.2%), and postpartum haemorrhage (PPH) (10.4%). Perinatal morbidities included prematurity (26.8%), neonatal sepsis (24.4%), and respiratory distress syndrome (RDS) (24.4%). Postnatal mortality was mostly due to prematurity (50% of cases). E. coli was the most common isolate from both HVS (47.6%) and urine culture (37.5%) [Table 2].

Table 1: Baseline maternal and obstetric characteristics

Categories		Count (%)
Age (years)	< 20	30 (20%)
	21 – 29	110 (73.3%)
	30 – 40	10 (6.7%)
Socioeconomic status	Lower middle	29 (19.3%)
	Upper lower	78 (52%)
	Lower	43 (28.7%)
Booked cases		143 (95.3%)
Parity	Primi	55 (36.6%)
	Multi	95 (63.3%)
Gestational age (weeks)	< 34	12 (8%)
	34 – 37	29 (19.3%)
	> 37	109 (72.6%)
Fetal presentation	Cephalic	137 (91.3%)
	Breech	13 (8.7%)
Aetiology of prom	Infection	29 (19.3%)
	Recent Coitus	27 (18%)
	Mal-presentation	17 (11.3%)
	Cervical surgery	2 (1.3%)
	Not known	74 (49.3%)
Membrane present		20 (13.3%)

Table 2: Maternal, labour, and neonatal outcomes

Categories		Count (%)
Colour of the liquor	Clear	144 (96%)
	Meconium	6 (4%)
	Blood stained	0
Latency period (hours)	< 6	73 (48.7%)
	6 – 12	61 (40.7%)
	> 12	16 (10.6%)
Mode of delivery	Vaginal	81 (54%)
	Caesarean section	67 (44.7%)
	Assisted breech	2 (1.3%)
Maturity of the fetus	Preterm	29 (19.3%)
	Term	121 (80.7%)
Apgar score	2	1 (0.7%)
	6	2 (1.3%)
	7	25 (16.7%)
	8	85 (56.7%)
	9	37 (24.7%)
Maternal morbidity (n = 29)	PPH	3 (10.4%)
	Postpartum fever	12 (41.4%)
	Wound infection	14 (48.2%)
Perinatal morbidity (n = 41)	Prematurity	11 (26.8%)
	Neonatal sepsis	10 (24.4%)
	Respiratory distress	10 (24.4%)
	Birth asphyxia	4 (9.8%)
	SGA	5 (12.2%)
	Meningitis	1 (2.4%)

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Postnatal mortality (n = 4)	Prematurity	2 (50%)
	Birth asphyxia	1 (25%)
	RDS	1 (25%)
HVS c/s (n = 21)	E. coli	10 (47.6%)
	S. Aureus	3 (14.3%)
	Klebsiella	2 (9.5%)
	Proteus	3 (14.3%)
	P. Aeruginosa	3 (14.3%)
Organism of urine c/s (n = 8)	E. coli	3 (37.5%)
	Proteus	2 (25%)
	Klebsiella	2 (25%)
	P. Aeruginosa	1 (12.5%)

Footnotes: High vaginal swab (HVS), small for gestational age (SGA), postpartum haemorrhage (PPH), respiratory distress syndrome (RDS), culture sensitivity (c/s). Data are presented as frequencies and percentages.

No significant relationship was found between vaginal organisms and latency period, delivery mode, or birth weight ($p > 0.05$). However, significant associations were observed with Apgar scores ($p < 0.001$), maternal morbidity ($p < 0.001$), perinatal morbidity ($p < 0.001$), and postnatal morbidity ($p < 0.001$). First-line sensitivity was observed with gentamicin and ampicillin, whereas second-line agents included imipenem, meropenem, amikacin, amoxicillin + clavulanate, piperacillin + tazobactam, ceftriaxone, cefuroxime, and ciprofloxacin [Table 3].

Table 3: Association of HVS organisms with maternal and neonatal outcomes

		Organism of HVS c/s					p-value
		E. coli	**S. Aureus**	**Klebsiella**	**Proteus**	**P. Aeruginosa**	
Latency	< 6	6	2	1	2	2	0.746
	6 – 12	2	1	1	0	1	
	> 12	2	0	0	1	0	
Delivery mode	Vaginal	6	1	1	1	2	0.998
	Caesarean	4	2	1	2	1	
	Assisted breech	0	0	0	0	0	
Maturity	Preterm	2	1	1	2	1	0.244
	Term	8	2	1	1	2	
Birth weight (Kg)	< 2	1	1	1	1	0	0.773
	2 – 2.5	2	0	0	1	1	
	> 2.5	7	2	1	1	2	
Apgar score	2	1	0	0	0	0	< 0.001
	6	0	1	0	0	0	
	7	1	0	0	2	0	
	8	6	2	2	0	2	
	9	2	0	0	1	1	
Maternal morbidity	PPH	3	0	0	0	0	< 0.001
	Postpartum fever	0	8	2	2	0	
	Wound infection	5	2	1	0	3	
Perinatal morbidity	Prematurity	5	2	1	0	1	< 0.001
	Neonatal sepsis	4	3	0	0	2	
	RDS	8	1	1	0	0	
	Birth Asphyxia	4	0	0	0	0	
	SGA	5	0	0	0	0	
	Meningitis	0	0	0	1	0	
Postnatal morbidity	Prematurity	1	0	0	0	0	< 0.001
	Birth Asphyxia	0	1	0	0	0	
	RDS	0	0	0	0	0	

Footnotes: High vaginal swab (HVS), small for gestational age (SGA), postpartum haemorrhage (PPH), respiratory distress syndrome (RDS), culture sensitivity (c/s). P-values calculated using the Chi-square test; Fisher's exact test applied where cell counts were small or zero. Statistical significance was set at $p < 0.05$.

The organisms isolated from urine were not significantly related to latency, delivery mode, or maturity ($p > 0.05$). However, strong associations were found with Apgar scores ($p < 0.001$), maternal morbidity ($p < 0.001$), perinatal morbidity ($p = 0.048$), and postnatal morbidity ($p < 0.001$). First-line antibiotics were ampicillin, penicillin, and erythromycin, whereas second-line options included linezolid, vancomycin, clindamycin, ciprofloxacin, and tetracycline [Table 4].

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Table 4: Association of urine culture organisms with maternal and neonatal outcomes

		Organism of urine C/S				p-value
		E. coli	Proteus	Klebsiella	P. Aeruginosa	
Latency	< 6	2	2	1	0	0.814
	6 – 12	1	0	1	1	
	> 12	0	0	0	0	
Delivery mode	Vaginal	1	2	1	1	0.920
	Caesarean	2	0	1	0	
	Assisted breech	0	0	0	0	
Maturity	Preterm	2	1	1	0	0.124
	Term	1	1	1	1	
Birth weight (Kg)	< 2	2	1	1	0	
	2 – 2.5	0	0	0	0	
	> 2.5	1	1	1	1	
Apgar score	2	1	0	0	0	< 0.001
	6	1	0	1	0	
	7	0	0	1	0	
	8	1	2	0	1	
	9	0	0	0	0	
Maternal morbidity	PPH	0	0	0	0	< 0.001
	Postpartum fever	2	0	0	0	
	Wound infection	1	2	1	1	
Perinatal morbidity	Prematurity	2	1	0	0	0.048
	Neonatal sepsis	0	0	0	1	
	RDS	0	0	0	0	
	Birth Asphyxia	0	0	0	0	
	SGA	0	0	0	0	
	Meningitis	0	0	0	0	
Postnatal morbidity	Prematurity	1	2	1	1	< 0.001
	Birth Asphyxia	1	0	0	0	
	RDS	1	0	0	0	

Footnotes: High vaginal swab (HVS), small for gestational age (SGA), postpartum haemorrhage (PPH), respiratory distress syndrome (RDS), culture sensitivity (c/s). P-values calculated using the Chi-square test; Fisher's exact test applied where cell counts were small or zero. Statistical significance was set at $p < 0.05$.

DISCUSSION

PROM associated with infections is a major obstetric condition that can lead to serious maternal and neonatal adverse outcomes. This study evaluated the microbiological spectra associated with PROM and assessed their impact on maternal morbidity, perinatal complications, and neonatal outcomes. Most patients were aged 21–29 years (73.3%) and belonged to the upper-lower socioeconomic group (52%). Similarly, Naseha et al. reported that the mean age was 24.5 years, and most of them belonged to the 21-30 years of age range.10 Wandile et al. reported that the majority of the mortality occurred in SES class V (37.50%), followed by class III (21.21%).[11] This indicates that younger pregnant women and those with lower economic status have higher chances of PROM and related infections.

In our study, the majority of patients were multigravida (63.3%), had term pregnancies (72.6%), and had cephalic presentations (91.3%), while the most common cause of PROM was infection (19.3%). Similarly, Naseha et al. observed that term pregnancies were the most common (72.6%), and antenatal mothers with term gestation presented with PROM. They also reported cephalic presentations in the majority of cases (91.3%) and identified infections as the most common cause of PROM (19.3%).[10] Additionally, Minarey et al. reported that risk factors associated with PROM include intrauterine infection at an early gestational age, lower socioeconomic status, inadequate prenatal care, inadequate nutrition, and sexually transmitted infections. Further risks include vaginal bleeding, smoking, maternal age (< 20 or > 35), blood group, gravidity, maternal fatigue, lack of treatment during pregnancy, history of cervical surgery, genital infections, and maternal diseases such as pulmonary conditions, hypertension, and diabetes.[12]

Maternal morbidity in our study included postpartum fever (41.4%), wound infection (48.2%), and PPH (10.4%). Perinatal morbidities included prematurity (26.8%), neonatal sepsis (24.4%), and RDS (24.4%). E. coli was the most common isolate from both HVS (47.6%) and urine culture (37.5%). Similar to our findings, Kerure et al. reported that maternal complications include anaemia (26%), postpartum fever (23%), abortions (12%), pregnancy-induced hypertension (12%), chorioamnionitis (9%), and PROM (6%).[13] Sethuraman et al. observed that UTI-positive women had higher rates of preterm labour (35.7%), low birth weight (28.6%), NICU admission (20%), and neonatal sepsis (8.6%).[14] Grill et al. evaluated infants born after PROM and before 23 weeks, and reported that early-onset neonatal sepsis occurred in 18.3% of cases.[15] Further supporting our findings, Verma et al. reported that the most commonly isolated organisms were E. coli (14.29%) and Candida species (17.86%) among the pregnant women with positive HVS culture.[16] Hence,

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pregnant women with PROM are prone to severe maternal, perinatal, and postnatal morbidity along with various genital infections.

In our study, infectious organisms were isolated from both urine and the vagina, but none had a significant relationship with latency period, delivery mode, or birth weight ($p > 0.05$). However, both showed similar significant associations with Apgar scores, maternal morbidity, perinatal morbidity, and postnatal morbidity ($p < 0.001$). Strengthening our results, Szubert et al. conducted a retrospective study and reported that patients with abnormal vaginal flora were significantly associated with premature birth (9.09%, $p = 0.038$), poor Apgar score (< 4, $p = 0.024$), longer hospitalisation (6.30 ± 9.87 days, $p = 0.025$), perinatal infections (23.97%, $p = 0.004$), and fetal mortality (4 deaths, $p = 0.045$).[17] Similarly, Michael et al. concluded that UTI in pregnancy can lead to adverse maternal and fetal effects, and this is due to the anatomical changes occurring during pregnancy.[18] Therefore, PROM associated with genital infections can lead to severe fetal adverse events.

In our study, gentamicin and ampicillin were used as first-line agents for HVS isolates, whereas imipenem, meropenem, amikacin, amoxicillin + clavulanate, piperacillin + tazobactam, ceftriaxone, cefuroxime, and ciprofloxacin were used as second-line agents. For urine culture isolates, the first-line effective antibiotics were ampicillin, penicillin, and erythromycin, while the second-line antibiotics were linezolid, vancomycin, clindamycin, ciprofloxacin, and tetracycline. Similarly, Patil et al. observed that E. coli and Klebsiella are highly susceptible to aminoglycosides like gentamicin/amikacin, but are resistant to older β-lactams like ampicillin and co-amoxiclav.[1] Bhavana et al. found high efficacy of piperacillin + tazobactam, imipenem and amikacin.[19] As for the urine cultures, Matalka et al. reported high susceptibility to nitrofurantoin and aminoglycosides but resistance to ampicillin and ciprofloxacin.[20] Thus, empirical therapy should be started with commonly effective agents such as piperacillin + tazobactam, imipenem and amikacin, but escalation should be done based on culture/sensitivity results.

Patients with PROM and positive cultures had higher rates of maternal morbidity, perinatal complications, lower Apgar scores, and more adverse neonatal outcomes. However, the latency period, delivery mode, and birth weight were not influenced by the infection. Larger multicentre studies are required to evaluate these findings and develop region-specific antibiotic protocols to optimise maternal and neonatal outcomes.

Limitations: This study was limited by its single-centre design, small sample size, and short duration of follow-up, which may affect the generalisability of the results. The exclusion of high-risk pregnancies and reliance on culture-based methods may have led to an underestimation of the role of anaerobic organisms in PROM-related morbidity.

CONCLUSION

Escherichia coli was the most common pathogen isolated from both HVSs and urine cultures. Positive cultures were significantly associated with adverse maternal outcomes, such as postpartum fever and wound infection, and neonatal complications, including prematurity, sepsis, respiratory distress, and low Apgar scores. Based on the microbiological spectra, first-line empirical therapy should include gentamicin with ampicillin for HVS isolates and ampicillin with erythromycin for urine isolates. Establishing region-specific antibiotic protocols based on local sensitivity patterns is essential for improving maternal and neonatal outcomes.

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