

Frequency of Microbial Colonization of the Maternal Vagina in Preterm Premature Rupture of Membranes

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Abstract

Objective: To determine the frequency of microbial colonization of the maternal vagina in preterm premature rupture of membranes (PPROM).

Methodology: This cross-sectional study commenced at the Department of Obstetrics and Gynecology, Nishtar Hospital, Multan, Pakistan, from March 2025 to August 2025. A total of 111 pregnant women between 20 to 45 years, who presented with PPRM within 12 hours and had a live fetus with a gestational age between 28-36 weeks were studied. The frequency of commonly isolated pathogens was documented. Culture confirmed vaginal microbial colonization was defined as growth of any predefined organism on high vaginal swab culture. Participants were not selected on the basis of positive culture or suspected vaginal infection. Data analysis was performed using IBM-SPSS 26.0, taking $p < 0.05$ as significant for inferential statistics.

Results: In a total of 111 women, the median age, and gestational age were 29.0 (27.0-32.0) years, and 33.0 (30.0-35.0) weeks, respectively. There were 23 (20.7%) women who were primiparous, while 88 (79.3%) were multiparous. Evaluation of socio-economic status revealed that 45 (30.5%) women belonged to low socio-economic status. Residential affiliation was rural among 69 (62.8%) women. Vaginal swab cultures yielded identifiable microorganisms in all 111 (100%) women. *Escherichia coli* (*E. coli*) was isolated in 32 (28.8%) women, *Enterococcus faecalis* in 22 (19.8%), Group B streptococcus in 18 (16.2%), *Staphylococcus aureus* in 19 (17.1%), and *Staphylococcus haemolyticus* in 20 (8.0%) women.

Gestational age was observed to have significant association with microbial distribution ($p=0.011$).

Conclusion: The frequency of culture confirmed microbial colonization of the maternal vagina was universal among women with PPRM. The culture profile in PPRM in this study supports a mixed aerobic colonisation pattern with substantial contributions from *E. coli*, enterococci, GBS, and staphylococci, and demonstrates gestational age-related differences in organism distribution.

Keywords: Extraembryonic membranes, *Enterococcus faecalis*, Gestational age, *Staphylococcus haemolyticus*.

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Introduction

Preterm deliveries affect around one out of every ten births in developing countries.¹ Preterm premature rupture of membranes (PPROM) is known as a significant health problem and accounts for 40% of all preterm deliveries, and 60-80% of perinatal deaths.²

Many factors contribute to the development of PPRM like genital tract infections, polyhydramnios, antepartum bleeding, traumatic injury, previous occurrence of PPRM during previous pregnancies, and genetic polymorphism.³ From the literature on the clinical predictors, the pathology of the fetal membrane, and the bacterial content in the amniotic fluid, it is evident

that infections play a significant role in the pathophysiology of PROM.⁴ Bacterial enzymes may lead to disruption of the architecture of the fetal membrane resulting in its rupture. The recommended treatment plan involves administration of corticosteroids, tocolytics, and antibiotics.⁵

Because of the absence of rapid diagnosis of organisms, the inappropriate use of antibiotics becomes unavoidable. Empirical broad-spectrum antibiotics are prescribed without confirming the microorganisms.⁶ Inappropriate and extensive use of antibiotics is considered to be one of the factors that have led to resistant patterns in microorganisms.⁷ A

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study that investigated 160 pregnant women with PPROM discovered that 85.1% of the samples had organism growth. Common isolated infections included *Enterococcus faecalis* (17.4%, 85.7% resistance to ceftriaxone), followed by *Staphylococcus aureus* (14.3%, 85% resistance to ampicillin), *Escherichia coli* (11.2%, 66.6% resistance to cefuroxime), and *Staphylococcus haemolyticus* (6.2%, 100% resistance to ampicillin).⁸ In another study, 56 patients with PPROM were matched with 56 pregnant women without PPROM and revealed that *Klebsiella* was the commonest organism isolated, accounting for 32.1%, followed by *Escherichia coli* (19.6%), with the highest sensitivity shown to ciprofloxacin (96.3%), followed by co-amoxiclav (94.4%), ceftriaxone (92.6%), and cefuroxime (90.6%).⁹

PPROM is closely linked with ascending genital tract colonization and infection which may contribute to chorioamnionitis, early onset neonatal sepsis, shortened latency, and preterm birth related morbidity.² Recent microbiome-based evidence indicates that PPROM is associated with disruption of Lactobacillus dominated vaginal flora and increased abundance of potentially pathogenic organisms, including *Streptococcus agalactiae*, *Escherichia coli*, *Staphylococcus aureus*, anaerobes, and other dysbiosis associated bacteria.^{7,8} Microbial patterns are also expected to differ across populations, supporting the need for local microbiological surveillance rather than relying only on data from other regions. Antibiotics are routinely used after PPROM, yet broad spectrum treatment may eradicate some organisms while allowing persistence of others such as *Escherichia coli* and increasing resistant bacterial populations.

Therefore, local data obtained before antibiotic exposure are clinically important to guide empirical treatment, reduce unnecessary antibiotic use, and support maternal and neonatal infection prevention strategies. In Pakistan, recent setting specific data on vaginal microbial colonization among women with PPROM remain limited, particularly from large public sector obstetric units in South Punjab. This gap provided the rationale for the present study which aimed to determine the frequency and organism wise distribution of culture confirmed maternal vaginal colonization among women presenting with PPROM.

Methodology

This cross-sectional study was conducted at the Department of Obstetrics and Gynecology, Nishtar

Hospital, Multan, Pakistan, from June 2025 to February 2026, following approval from the Ethics Review Committee (reference number: 9006/NMU; dated May 12, 2025). A sample size of 111 participants was calculated using the WHO sample size calculator, based on an anticipated frequency of 6.21%⁸ for *Staphylococcus haemolyticus* colonization, a 95% confidence level, and a margin of error of 4.5%.

Pregnant women aged 20–45 years who presented with PPROM within 12 hours were eligible for inclusion. Only women with a live fetus confirmed by antenatal ultrasonography and no documented history of hypertensive disorders, diabetes mellitus, or chronic kidney disease were included. Women with a gestational age of less than 28 weeks or more than 36 weeks, twin pregnancy, intrauterine device (IUD), fetal anomaly, gestational diabetes, polyhydramnios, or placenta previa were excluded. Women who had received antibiotics within 7 days before the onset of PPROM were also excluded.

PPROM was defined as spontaneous rupture of the fetal membranes before 37 completed weeks of gestation and before the onset of labor. The diagnosis was confirmed by sterile Cusco's speculum examination, with visualization of a pool of amniotic fluid in the posterior fornix and/or fluid passing through the cervical os, with or without the Valsalva maneuver.

Participants were recruited using a non-probability consecutive sampling technique. The patients were informed about the objectives and safety of the study, and written informed consent was obtained from all participants before enrollment.

Baseline data of the eligible subjects, which included age (years), gestational age (weeks), parity, and socioeconomic status, were recorded. Socioeconomic status was categorized using World Bank classification, based on monthly household income, as low (≤ 11605 PKR), lower middle (11606–45395 PKR), upper middle (45396–140461 PKR), and high (> 140461 PKR).¹⁰ High vaginal swabs were taken using the commercially available sterile swab sticks, taking all necessary aseptic precautions by the trained obstetrician. Collected samples were sent to the microbiology diagnostic laboratory of the institution for the automated culture. All specimens underwent Gram staining, and microorganisms were identified according to the standard protocol. The frequency of commonly isolated pathogens, which included *Enterococcus faecalis*, Group B streptococcus, *Staphylococcus aureus*,

Escherichia coli, and *Staphylococcus haemolyticus*, was noted. Women were enrolled on the basis of PPRM and eligibility criteria before culture results were available. Culture confirmed vaginal microbial colonization was defined as growth of any predefined organism on high vaginal swab culture. Participants were not selected on the basis of positive culture or suspected vaginal infection. A specifically predesigned proforma was used to record the required information.

The data were analyzed using "IBM-SPSS Statistics" version 26.0. The qualitative data was presented as frequency and percentage. The normality distribution of the quantitative data was checked using the Shapiro-Wilk test. For the representation of quantitative variables, means and standard deviations (SD) or medians and interquartile ranges (IQR) were computed. The data were stratified on age groups, gestational age, parity, and socioeconomic status to determine the effect on distribution of identified microorganisms. A post-stratification chi-square test was applied, considering a p -value <0.05 for significance.

Results

Among the 111 women included in the study, the median age was 29.0 (27.0–32.0) years, with a range of 23–40 years. The median gestational age was 33.0 (30.0–35.0) weeks, ranging from 28 to 36 weeks. Of the total participants, 23 (20.7%) women were primiparous, while 88 (79.3%) were multiparous. Regarding socioeconomic status, 45 (30.5%) women belonged to the low socioeconomic group. Most women

were from rural areas (69, 62.8%). The demographic characteristics of the women with PPRM are presented in Table I.

Table I: Demographic characteristics of women with PPRM. (n=111)

Characteristics		Number (%)
Age (years)	20-30	73 (65.8%)
	31-45	38 (34.2%)
Gestational age (weeks)	28-32	52 (46.8%)
	33-36	59 (53.2%)
Parity	Primiparous	23 (20.7%)
	Multiparous	88 (79.3%)
Socioeconomic status	Low	45 (40.5%)
	Lower middle	38 (34.2%)
	Upper middle	16 (14.4%)
	High	12 (10.8%)
Residence	Urban	42 (37.8%)
	Rural	69 (62.8%)

Vaginal swab cultures yielded identifiable microorganisms in all 111 (100%) women. *Escherichia coli* (*E. coli*) was isolated in 32 (28.8%) women, followed by *Enterococcus faecalis* in 22 (19.8%), *Group B Streptococcus* in 18 (16.2%), *Staphylococcus aureus* in 19 (17.1%), and *Staphylococcus haemolyticus* in 20 (18.0%) women (Figure 1).

Distribution of age groups ($p=0.869$), parity status ($p=0.165$), socio-economic status (0.073), and residential affiliation ($p=0.231$) were not found to have any significant association with the distribution of various micro-organisms (table II). Gestational age was observed to have significant association with microbial distribution as at 28–32 weeks, isolates included *E. coli* in 53.1%, *Enterococcus faecalis* in 27.3%, *Group B*

Table II: Association of characteristics of women with distribution of micro-organisms identified on swab cultures. (n=111)

Characteristics		<i>E. Coli</i> (n=32)	<i>Enterococcus faecalis</i> (n=22)	<i>Group B streptococcus</i> (n=18)	<i>Staphylococcus aureus</i> (n=19)	<i>Staphylococcus haemolyticus</i> (n=20)	P-value
Age (years)	20-30	21 (65.6%)	13 (59.1%)	12 (66.7%)	12 (63.2%)	15 (75.0%)	0.869
	31-45	11 (34.4%)	9 (40.9%)	6 (33.3%)	7 (36.8%)	5 (25.0%)	
Gestational age (weeks)	28-32	17 (53.1%)	6 (27.3%)	14 (77.8%)	9 (47.4%)	6 (30.0%)	0.011
	33-36	15 (46.9%)	16 (72.7%)	4 (22.2%)	10 (52.6%)	14 (70.0%)	
Parity	Primiparous	7 (21.9%)	8 (36.4%)	3 (16.7%)	4 (21.1%)	1 (5.0%)	0.165
	Multiparous	25 (78.1%)	14 (63.6%)	15 (83.3%)	15 (78.9%)	19 (95.0%)	
Socioeconomic status	Low	9 (28.1%)	9 (40.9%)	11 (61.1%)	7 (36.8%)	9 (45.0%)	0.073
	Lower middle	17 (53.1%)	7 (31.8%)	1 (5.6%)	9 (47.4%)	4 (20.0%)	
	Upper middle	5 (15.6%)	4 (18.2%)	3 (16.7%)	-	4 (20.0%)	
	High	1 (3.1%)	2 (9.1%)	3 (16.7%)	3 (15.8%)	3 (15.0%)	
Residence	Urban	17 (53.1%)	8 (36.4%)	4 (22.2%)	7 (36.8%)	6 (30.0%)	0.231
	Rural	15 (46.9%)	14 (63.6%)	14 (77.8%)	12 (63.2%)	14 (70.0%)	

streptococcus in 77.8%, *Staphylococcus aureus* in 47.4%, and *Staphylococcus haemolyticus* in 30.0%, whereas at 33–36 weeks, *E. coli* was identified in 46.9%, *Enterococcus faecalis* 72.7%, *Group B streptococcus* 22.2%, *Staphylococcus aureus* 52.6%, and *Staphylococcus haemolyticus* in 70.0% isolates ($p=0.011$).

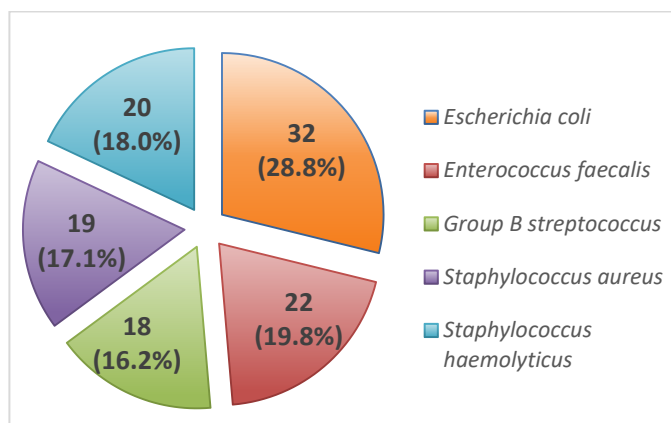


Figure 1. Distribution of micro-organisms identified on swab cultures. (n=111)

Discussion

This study found that vaginal swab cultures yielded growth in 100% women, with *E. coli* isolated in 28.8%, *Enterococcus faecalis* in 19.8%, *Group B streptococcus* 16.2%, *Staphylococcus aureus* in 17.1%, and *Staphylococcus haemolyticus* 18.0%. Abdel-Hakeem et al.,¹¹ reported a similarly high yield among 101 women with PPROM, with only 5.0% showing no growth after 48 hours while *Escherichia coli* was identified in 28.7% which is almost identical to the 28.8% observed in the present study. Ambalpady et al.,⁸ also reported high culture positivity in PPROM with sterile or no growth cultures in only 14.9% samples. A study from Nepal analyzing vaginal microflora in PPROM reported that 74.0% samples were culture positive, and *E. coli* was the commonest organism, isolated in 40.0% samples.¹²

These differences suggest that culture yield in PPROM varies according to timing of sample collection, exclusion of recent antibiotic exposure, specimen site, laboratory methods, and whether colonizing organisms are classified as positive growth. A similar prominence of *E. coli* among women with PPROM has been reported in prospective laboratory-based work from Egypt, where *E. coli* was isolated in 28.7% women, closely aligning with the 28.8% observed here. In endocervical sampling among PROM and PPROM cohorts, *E. coli* has also remained a frequent isolate,

reinforcing that ascending colonisation by enteric gram negative organisms is a recurring microbiological feature across settings.¹³

The frequency of *Group B streptococcus* at 16.2% in PPROM provides clinically relevant context for intrapartum prophylaxis policies in the local setting. Reported GBS prevalence varies widely by geography, sampling site, and study population. A local tertiary care cohort from Lahore that screened pregnant women in the 3rd trimester reported GBS colonisation in 14.0% women, which is directionally consistent with the 16.2% found in this study.¹⁴ In contrast, a retrospective study from Saudi Arabia focusing on PPROM cases described a culture profile dominated by GBS, with GBS reported in 72.9%, and *Candida* in 18.6%, a pattern that differs materially from the mixed aerobic distribution identified here.¹⁵ Differences of this magnitude can plausibly reflect heterogeneity in laboratory workflow, timing of sampling relative to antibiotic exposure, and the case mix in terms of gestational age distribution and referral intensity, since colonisation patterns shift with pregnancy stage and preceding healthcare contact.

The isolation of *Staphylococcus aureus* in 17.1%, and *Staphylococcus haemolyticus* in 18.0% highlights an appreciable burden of staphylococcal colonisation in PPROM. Findings across published PROM and PPROM cohorts show variability in staphylococcal yield, yet *S. aureus* is repeatedly represented among common isolates. In an observational cohort of 100 PPROM cases from India, *S. aureus* was reported in 42.0%, and *E. coli* in 20.0%, with *Candida* in 14.0%, demonstrating a higher proportion of staphylococci than in the present findings while still supporting staphylococci as recurrent organisms in PPROM microbiology.¹⁶ In prospective Egyptian data, *S. aureus* was also frequent in 22.8% women, which approximates the 17.1% observed in this study.¹¹ These cross study similarities support the clinical logic of ensuring empiric regimens cover both gram negative rods and gram positive cocci when antibiotics are indicated, while still aligning with antibiotic stewardship principles.^{17,18}

The association between gestational age and organism distribution ($p=0.011$) suggested that the microbial profile at presentation differs across 28 to 32 weeks and 33 to 36 weeks. Gestational age linked shifts in colonisation have been described in other PPROM cohorts, including work that observed gestational age

related differences in *Candida* colonisation and suggested that microbial ecology and antimicrobial susceptibility can vary across earlier versus later PPROM windows.¹⁹ From a clinical standpoint, gestational age stratified differences matter because empiric antibiotic choices in PPROM are often initiated before culture results, and gestational age at presentation is an immediately available variable that can support risk based prescribing and laboratory prioritisation.^{20,21} The gestational age signal also has relevance for neonatal infectious risk pathways.²² In a large retrospective cohort comparing preterm labour and PPROM, abnormal vaginal colonisation in PPROM was independently associated with early onset neonatal sepsis after adjustment, and concordance between maternal vaginal isolates and neonatal blood cultures was described for *E. coli* and *S. aureus* in PPROM.²³

The culture-based approach used in this study aligns with routine clinical microbiology but should be interpreted in the context of microbiome literature that frames PPROM as a dysbiosis state characterised by reduced *Lactobacillus* dominance and enrichment of diverse taxa, including organisms not reliably captured by standard aerobic culture. A systematic review synthesising microbiome focused studies reported that PPROM is associated with depletion of naturally prevalent *Lactobacillus* species and increased representation of organisms including *Streptococcus agalactiae*, *Escherichia coli*, and *Staphylococcus aureus*, alongside anaerobes and fastidious bacteria.²⁴

Culture based results still provide actionable information for immediate antibiotic selection, yet the microbiome evidence supports expanding future work to include molecular methods that capture Urea plasma, *Mycoplasma*, and anaerobic communities, especially since amniotic cavity colonisation studies identify Urea plasma as a frequent organism in intra amniotic contexts.²⁵

Clinical implications follow directly from the organism mix and the stewardship aim stated in the rationale. Empiric antibiotics in PPROM are often initiated to reduce chorioamnionitis risk and to prolong latency, yet broad spectrum exposure can select for resistance. In a retrospective cohort evaluating vaginal microbial colonisation before and after antibiotic administration following PPROM in 438 pregnancies delivering before 29 weeks, eradication occurred in multiple microbial groups including Group B streptococci, while *E. coli*

showed persistence and resistant bacteria increased after antibiotic treatment.²⁶ In a setting where *E. coli* was present in 28.8% PPROM cases at baseline, this persistence signal supports careful selection of empiric regimens that balance coverage with the risk of resistance amplification, paired with prompt de-escalation once culture results are available. The present data also support the value of maintaining local antibiograms for PPROM isolates, since organism prevalence alone does not determine effective therapy.

The single centre design and non-probability consecutive sampling limit generalisability beyond the institutional catchment, especially where referral patterns and pre admission antibiotic exposure differ. The organism panel was restricted to selected aerobic bacteria cultured on blood agar and MacConkey agar, which does not capture anaerobes, genital mycoplasmas, or *Lactobacillus* dominated states. Antimicrobial susceptibility results were not presented, limiting direct translation into empiric regimen optimisation. Maternal and neonatal clinical outcomes including latency duration, chorioamnionitis, neonatal sepsis, and NICU admission were not analysed. Prospective follow up with predefined outcome adjudication would permit assessment of organism specific risk and inform targeted prevention strategies.

Conclusion

The frequency of culture-confirmed microbial colonization of the maternal vagina was 100% among women with PPROM. The culture profile showed a mixed pattern of aerobic organisms, with substantial contributions from *E. coli*, enterococci, GBS, and staphylococci. The distribution of these organisms also varied according to gestational age. These findings highlight the importance of using local microbiological data to guide antibiotic selection in women with PPROM. Culture results may also help clinicians tailor and de-escalate antibiotic therapy when appropriate, thereby reducing unnecessary antibiotic exposure. Future studies should incorporate broader microbiological methods and multicenter designs that link colonization patterns with maternal and neonatal outcomes to better determine their clinical sign.

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