

## RELATIONS OF VAGINAL BACTERIAL INFECTION TYPES BASED ON GRAM STAINING WITH PREMATURE RUPTURE OF MEMBRANES

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### Abstract

**Introduction:** Premature rupture of membranes (PROM) is defined as rupture of the membranes before delivery (<37 weeks is called preterm premature rupture of membranes-PPROM, >37 weeks is called premature rupture of membranes-PROM). PPROM complicates 3-8% of pregnancies and is responsible for 20-30% of all preterm births and perinatal morbidity. Bacterial infection in the third trimester of pregnancy is a risk factor for PROM. Vaginal infections originate from pathogenic microorganisms that interfere with the normal vaginal flora. The microorganism causing the infection can usually be found by Gram stain and/or vaginal swab culture. **Objective:** To determine the relationship between vaginal bacterial infection based on Gram stain and the incidence of preterm premature rupture of membranes. **Methods:** This study is observational with a cross-sectional comparative study design. Sampling was conducted from Mei 2020 hingga April 2021. A total of 60 pasien preterm who met the criteria were investigated, with 30 samples experiencing PROM and 30 samples not experiencing PROM. Every sample was performed vaginal swab to examine the existence of bacterial infection based on Gram stain. The chi-square test was used for statistical analysis. **Results:** The most recent education level was SMA (PPROM 57%, non-PPROM 63%). Most were multiparous (PPROM 73%, non-PPROM 67%) and delivered by cesarean section (PPROM 70%, non-PPROM 90%). 63.3% of patients had vaginal infections (PPROM 90%, non-PPROM 63.3%). Most infections are caused by gram negative bacteria. The results of statistical tests showed that there was a significant relationship between bacterial infection and the incidence of preterm premature rupture of membranes ( $p < 0.05$ ) but there was no significant relationship between bacterial species based on vaginal Gram staining and the incidence of preterm premature rupture of membranes ( $p > 0.05$ ). **Conclusion:** Gram stain examination should be carried out routinely as an initial procedure for treating PROM patients so that it can be a guide for selecting the right antibiotic therapy in future studies.

**Keywords:** pprom; infection in pregnancy; bacterial

## Introduction

Premature rupture of membranes (PROM) is defined as the rupture of the membranes before delivery. If the rupture of the membranes occurs before 37 weeks of gestation, it is called preterm premature rupture of membranes (PPROM), whereas if it occurs after 37 weeks of gestation, it is called premature rupture of membranes (PROM). PPROM complicates 3%-8% of pregnancies and is responsible for 20%-30% of all preterm births and perinatal morbidity (Adewumi et al., 2017), (El-Messidi & Cameron, 2010).

One of the currently recognized risk factors for PROM in the literature is the presence of bacterial infection in the third trimester of pregnancy. Contamination of pathogenic microorganisms in the vaginal environment can spread to the cervix and reach the amniotic cavity so that it can trigger local inflammation and/or proteolytic processes resulting in lesions of the membranes.<sup>4</sup> The prevalence of genital infection in women with preterm labor was 16.1% and 21.6% in PPROM.<sup>4</sup> A study in India by Taralekar et al, stated that infections were 2-3 times more common in patients with PPROM compared to PROM (Shivaraju, Purra, Bheemagani, & Lingegowda, 2015).

Vaginal infections are characterized not only by the presence of pathogenic microorganisms, but also by changes in normal vaginal microorganisms resulting in reduced Lactobacillus counts. Several organisms have been associated with PPROM in various parts of the world. Vaginal swab culture performed on women with PROM revealed *Escherichia coli*, *Streptococcus*, *Staphylococcus*, *Bacteroides* and *Klebsiella* bacteria which are the types of bacteria that can be detected quickly by Gram staining. These bacteria can be classified into Gram positive or Gram negative which can be a reference in the administration of specific antibiotics (P A, A.G G, 2018).

Another study conducted by Asrat et al. regarding Gram examination on amniocentesis in PPROM patients found that 77 patients out of 108 patients had Gram negative bacteria and only 31 patients had Gram positive bacteria. Both of these studies showed the same results, namely more Gram-negative bacteria were found on Gram staining (Asrat, Nageotte, Garite, Gocke, & Dorchester, 1990). A study conducted by Rani et al. found that the most bacteria found in vaginal swabs were Gram negative bacteria in the PPROM group. Groups of bacteria *E. coli* I and *Staphylococcus aureus* are the most common types of bacteria found in this group (Rani, Mehra, Gupta, Huria, & Chander, 2014).

Prolonged anhydramnios after PPROM is associated with a fourfold risk of adverse complications such as fetal death, severe neurologic impairment and severe retinopathy (Tchirikov et al., 2018). The most significant risk to the fetus after delivery with PPROM is the complication of prematurity. Airway obstruction was reported as the most frequent complication of labor. In addition, several complications such as sepsis, intraventricular hemorrhage and necrotizing enterocolitis are also closely associated with prematurity, but they do not occur frequently (Ehsanipoor & Major, 2012).

The diagnosis of PROM must be carried out thoroughly through anamnesis, physical examination and investigations. To determine one of the causes of preterm

premature rupture of membranes, it must go through various examinations such as vaginal inspection, ultrasound to identify the causative bacteria by Gram staining to vaginal swab culture (P A, A.G G, 2018). The researcher wanted to know the relationship between vaginal bacterial infection based on Gram stain and the incidence of preterm premature rupture of membranes.

### Research Methods

This study is a cross-sectional comparative study with an observational study design. The research was carried out at Dr. M. Djamil Padang's Department of Obstetrics and Gynecology. Sampling was conducted from May 2020 to April 2021. Inclusion criteria were 24-34 weeks of gestation, single live fetus as evidenced by ultrasound; the state of the amniotic membrane as evidenced by the litmus test; and willing to be a sample. Exclusion criteria were multiple fetuses, medical abnormalities in pregnant women (diabetes mellitus, hypertension in pregnancy, preeclampsia/eclampsia), and polyhydramnios. A total of 60 preterm patients who met the criteria were investigated, with 30 samples having PPRM and 30 samples non-PPROM. Every sample was performed vaginal swab to examine the existence of bacterial infection based on Gram stain. The chi-square test was used for statistical analysis.

### Results and Discussion

#### A. Results

Characteristics of research respondents consisted of maternal age, education, parity before pregnancy and vaginal bacterial infection (Table 1). The most recent education level was senior high school (PPROM 57%, non-PPROM 63%). Most were multiparous (PPROM 73%, non-PPROM 67%) and delivered by cesarean section (PPROM 70%, non-PPROM 90%). 63.3% of patients had vaginal infections (PPROM 90%, non-PPROM 63.3%). Most infections were caused by gram-negative bacteria (PPROM 46.7%, non-PPROM 36.7%).

**Table 1**  
**Characteristics of Respondents**

Characteristics	PPROM	Non-PPROM
Education levels		
Elementary school	1 (3%)	0 (0%)
Junior high school	8 (27%)	6 (20%)
Senior high school	17 (57%)	19 (63%)
Bachelor degree	4 (13%)	5 (17%)
Parity		
Primipara	8 (27%)	10 (33%)
Multipara	22 (73%)	20 (67%)
Treatment		
Vaginal birth	9 (30%)	3 (10%)
cesarean section	21 (70%)	27 (90%)
Vaginal infection		
Yes	27 (90%)	19 (63,3%)

No	3 (10%)	11 (36,7%)
Gram staining		
Positif	13 (43,3%)	9 (30%)
Negatif	14 (46,7%)	10 (33,3%)
No bacterial	3 (10%)	11 (36,7%)

The relationship of vaginal bacterial infections based on Gram staining with the incidence of preterm premature rupture of membranes is presented in Table 2. Most infections are caused by gram-negative bacteria. The results of statistical tests showed that there was a significant relationship between bacterial infection and the incidence of preterm premature rupture of membranes ( $p < 0,05$ ) but there is no significant relationship between bacterial species based on vaginal Gram staining with the incidence of preterm premature rupture of membranes ( $p > 0,05$ ).

**Table 2**  
**The Relationship Between Vaginal Bacterial Infections**  
**And Their Types Based On Gram Staining With**  
**The Incidence Of Preterm Premature Rupture Of Membranes**

	PPROM		Non-PPROM		P value
	N	%	N	%	
Vaginal infection					0,015
Yes	27	90	19	63,3	
No	3	10	11	36,7	
Gram staining					0,051
Positif	13	43,3	9	36,7	
Negatif	14	46,7	10	40	
No bacterial	3	10	11	23,3	

**B. Discussion**

Maternal education plays an important role in the development of PROM. Mothers with higher levels of education tend to be more aware of the health conditions of themselves and their families apart from nutrition and health control during pregnancy. So that mothers with higher levels of education are able to identify changes in their pregnancy quickly (Maryuni & Kurniasih, 2017). This is in line with Nafis' research in 2020 where most of the samples were multipara, as many as 80 percent. Research conducted in Semarang in 2002 showed that there was no relationship between parity and the incidence of premature rupture of membranes (Ayu, Winarsih, & Nooryanto, 2016).

From this study, it was found that the distribution of this study was in accordance with the research of Beevi et al, which showed that the distribution of gram-negative bacteria was the most common bacterial pathogen in cases of preterm premature rupture of membranes. The overall composition of microorganisms before tearing of the membranes and the delivery process in preterm cases from mild to extreme was comparable which gave an increased incidence of chorioamnionitis at preterm gestation. This similarity was caused by the reduction of *Lactobacillus spp.* from the observations after the membrane tear occurred. The latent condition between PROM

and labor tends to be long in early pregnancy, allowing sufficient time for remodeling of the bacterial group in the vagina with pathogenic infiltration associated with ascending infection and chorioamnionitis. The mean latency time after PROM was lower and the rate of chorioamnionitis was higher in women with *Lactobacillus spp.* decreased before PROM. This suggests that vaginal microorganisms with an increase in bacteria before PROM result in early labor as a result of inflammatory activation and stimulation of the preterm labor pathway or as a result of a vaginal pathogenic reaction causing ascending infection and chorioamnionitis when the membrane defenses fail (Brown et al., 2019).

A study conducted by Rani et al. found that the most bacteria found in vaginal swabs were Gram negative bacteria in the PPROM group. Groups of bacteria *E. coli* and *Staphylococcus aureus* are the most common types of bacteria found in this group. While in the control group, the most bacteria found were Gram positive bacteria with the culture results mostly sensitive to gentamicin. This research is in line with the research that has been done by the author by finding the most bacteria are Gram negative bacteria (Saghafi et al., 2018).

A study conducted by Nikolaitchouk found that from 37 research samples, 42 species of bacteria were found from the results of vaginal secretion examinations. In women with bacterial vaginosis, several species were found, including *Atopobium vaginae*, *Peptoniphilus harei*, and *Actinomyces urogenitalis*. The bacterial species found in lesser numbers was *Lactobacillus coleohominis* (Nikolaitchouk, 2009).

A study conducted by Feld et al. who conducted a study using Gram staining in patients with preterm premature rupture of membranes immediately upon admission to the hospital. From a total of 70 samples, 41.7 percent of them were found to be Gram-positive cocci bacteria, 30 percent of which were positive for the results of *Streptococcus haemolyticus* culture. The results of this study indicate that the administration of selective antibiotics according to vaginal Gram swab staining is very useful in administering a suitable antibiotic while waiting for the culture results (Feld & Harrigan, 1987).

In a study by Beevi et al, an analysis of 105 vaginal swabs was performed. The results of the examination showed 31 samples in preterm labor without PROM and 74 samples in those with PROM. The results of further examination showed bacterial culture with 80% negative culture results and 20% positive culture results. In this study, the prevalence of genital infections in women with preterm labor was 16.1% and 21.6% in PPROM (P A, A.G G, 2018). An Indian study by Taralekar et al, stated that there were 2-3 times more frequent infections in patients with PPROM compared to PROM (Shivaraju et al., 2015).

From another study in China, it was stated that broadly speaking, Gram-positive bacteria were cultured in 18.4% of PPROM patients, and the most common was *Group B Streptococcus* and Gram-negative bacteria were also cultured in 12.8% of PPROM patients, and the most frequent was *Group B Streptococcus*. *E. coli*. Both Gram positive and negative had an equally significant relationship between the

occurrence of neonatal sepsis with rapid onset ( $p=0.036$  and  $p=0.001$ , respectively) (Li, Kong, & To, 2019).

Adewumi et al. obtained the results of his study that *Gardnerella vaginalis* presented microscopically and with a positive whiff test in 3 (5.3%) cases of preterm PROM but 1 (1.8%) in the control group. *Trichomonas vaginalis* was seen under the microscope in 3 (5.3%) cases of preterm PROM but 2 (3.6%) in the control group. *Candida albicans* was seen in 2 (3.6%) of the cases but 7 (12.5%) in the control group (Adewumi et al., 2017).

The results of a study conducted by Saghafi et al found that 136 patients with PROM had positive endocervical cultures which consisted mainly of Gram-negative (31%) and 29% Gram-positive and 8% fungal species. The most common pathogens found in endocervical culture were *E. coli* (24.2%) and *epidermal Staphylococci* (14.7%), *Staphylococci saprophitices* (12.5%), *Enterococci* (11.7%) and *Candida* (11.7%).<sup>57</sup> Other studies were conducted. by Asrat et al. regarding Gram examination on amniocentesis in PPRM patients found that 77 patients out of 108 patients had Gram negative bacteria and only 31 patients had Gram negative bacteria. Both of these studies showed the same results, namely more Gram-negative bacteria were found on Gram staining (Asrat et al., 1990).

In a case-control study, Saremi et al examined maternal risk factors which included vaginal culture, history of abortion and cervical length in 121 control subjects (without PROM) and 121 subjects with PROM. The results of this study stated that there was no significant relationship between the number of pregnancies, cerclage procedures, and vaginal cultures with preterm PROM (Dars, Malik, Samreen, & Kazi, 2014).

In another study by Al Riyami et al., 44 women with preterm PROM were surveyed to find out the association of risk factors and their side effects in Omani women. The results of this study indicated that the most important risk factor was a history of infection in 24 study subjects. In addition, there was no significant relationship between gestational age, parity, maternal age and caesarean section. Infection had a significant role both as a risk factor and a cause of PROM in 27% of the study subjects. In conclusion, the researchers stated that infection is still a high risk factor in patients with preterm PROM (Al Riyami, Al-Ruheili, Al-Shezaw, & Al-Khabori, 2013).

In this study, the Odd Ratio value was found to be 5.2. With the Odd Ratio assessment, it can be interpreted that patients with infection have a 5.2-fold risk of developing PROM. Based on the table above, it can also be seen that the overall p value in this study was 0.02, so it can be concluded that there is a significant relationship between the relationship between gram vaginal bacteria infection and the incidence of preterm premature rupture of membranes. The weakness in this study is that researchers are limited to carrying out Gram staining on the results of vaginal swab examinations, further research should be carried out with bacterial culture examination to determine the pattern of bacterial infection in PROM.

### **Conclusion**

There was no significant difference between the sample characteristics in the PROM and Non-PROM groups. Most cases of PROM were found to be infected with the most bacteria being Gram-negative bacterial species. There is a relationship between bacterial infection and the incidence of early PROM. Gram stain examination should be performed routinely as an initial procedure in treating PROM patients in determining appropriate antibiotic therapy while waiting for culture results and preventing resistance to broad-spectrum antibiotics. Further research needs to be done by examining bacteria through culture to find out more about the pattern of bacterial infection in PROM and test resistance to these bacteria.

## BIBLIOGRAPHY

- Adewumi, O., Olofinbiyi, B., Oyekale, O., Loto, O., Abu, S., & Sotunsa, O. J. (2017). Microbiological pattern in preterm prelabour rupture of the fetal membranes in South-Western Nigeria. *Obstetrics & Gynecology International Journal*, 6(4), 4–10. [Google Scholar](#)
- Al Riyami, Nihal, Al-Ruheili, Intisar, Al-Shezaw, Fatma, & Al-Khabori, Murtadha. (2013). Extreme preterm premature rupture of membranes: risk factors and fetomaternal outcomes. *Oman Medical Journal*, 28(2), 108. [Google Scholar](#)
- Asrat, Tamerou, Nageotte, Michael P., Garite, Thomas J., Gocke, Steven E., & Dorchester, Wendy. (1990). Gram stain results from amniocentesis in patients with preterm premature rupture of membranes—comparison of maternal and fetal characteristics. *American Journal of Obstetrics and Gynecology*, 163(3), 887–889. [Google Scholar](#)
- Ayu, Revitalia Kusumaning, Winarsih, Sri, & Nooryanto, Mukhamad. (2016). Pola Bakteri dan Uji Kepekaan Antibiotik pada Preterm Premature Rupture of Membranes di RSUD dr. Saiful Anwar Malang Periode 2011-2013. *Majalah Kesehatan FKUB*, 2(1), 51–61. [Google Scholar](#)
- Brown, Richard G., Al-Memar, Maya, Marchesi, Julian R., Lee, Yun S., Smith, Ann, Chan, Denise, Lewis, Holly, Kindinger, Lindsay, Terzidou, Vasso, & Bourne, Tom. (2019). Establishment of vaginal microbiota composition in early pregnancy and its association with subsequent preterm prelabor rupture of the fetal membranes. *Translational Research*, 207, 30–43. [Google Scholar](#)
- Dars, Saira, Malik, Safia, Samreen, Irum, & Kazi, Roshan Ara. (2014). Maternal morbidity and perinatal outcome in preterm premature rupture of membranes before 37 weeks gestation. *Pakistan Journal of Medical Sciences*, 30(3), 626. [Google Scholar](#)
- Ehsanipoor, Robert M., & Major, Carol A. (2012). Premature Rupture of Membranes. *Women's Health Review: A Clinical Update in Obstetrics-Gynecology (Expert Consult-Online and Print)*, 101. [Google Scholar](#)
- El-Messidi, Amira, & Cameron, Alan. (2010). Diagnosis of premature rupture of membranes: inspiration from the past and insights for the future. *Journal of Obstetrics and Gynaecology Canada*, 32(6), 561–569. [Google Scholar](#)
- Feld, Steven M., & Harrigan, John T. (1987). Vaginal gram stain as an immediate detector of group B streptococci in selected obstetric patients. *American Journal of Obstetrics and Gynecology*, 156(2), 446–448. [Google Scholar](#)
- Li, Y. Y., Kong, C. W., & To, William W. K. (2019). Pathogens in preterm prelabour rupture of membranes and erythromycin for antibiotic prophylaxis: a retrospective analysis. *Hong Kong Medical Journal*, 25(4), 287. [Google Scholar](#)



Relations Of Vaginal Bacterial Infection Types Based On Gram Staining With  
Premature Rupture Of Membranes

- Maryuni, Maryuni, & Kurniasih, Dedeh. (2017). Risk factors of premature rupture of membrane. *Kesmas: Jurnal Kesehatan Masyarakat Nasional (National Public Health Journal)*, 11(3), 133–137. [Google Scholar](#)
- Nikolaitchouk, Natalia. (2009). *The female genital tract microbiota: composition, relation to innate immune factors, and effects of contraceptives*. [Google Scholar](#)
- P A, A.G G, C. S. (2018). High vaginal swab study in preterm labour and preterm premature rupture of membranes and its relationship with neonatal sepsis. *Journal of Evidence Based Medicine and Healthcare*, 5(30), 2249–2254. [Google Scholar](#)
- Rani, Shikha, Mehra, Reeti, Gupta, Varsha, Huria, Anju, & Chander, Jagdish. (2014). Vaginal flora in preterm premature rupture of membranes and their sensitivity to commonly used antibiotics. *Asian Journal of Medical Sciences*, 5(4), 58–60. [Google Scholar](#)
- Saghafi, Nafiseh, Pourali, Leila, Ghazvini, Kiarash, Maleki, Asieh, Ghavidel, Mahdis, & Babaki, Mohsen Karbalaeezadeh. (2018). Cervical bacterial colonization in women with preterm premature rupture of membrane and pregnancy outcomes: A cohort study. *International Journal of Reproductive Biomedicine*, 16(5), 341. [Google Scholar](#)
- Shivaraju, Pradeep, Purra, Pallavi, Bheemagani, Navatha, & Lingegowda, Krishna. (2015). Vaginal infections and its relation to preterm labour, PPRM, PROM and its outcome. *International Journal of Reproduction, Contraception, Obstetrics and Gynecology*, 4(5), 1422–1427. [Google Scholar](#)
- Tchirikov, Michael, Schlabritz-Loutsevitch, Natalia, Maher, James, Buchmann, Jörg, Naberezhnev, Yuri, Winarno, Andreas S., & Seliger, Gregor. (2018). Mid-trimester preterm premature rupture of membranes (PPROM): etiology, diagnosis, classification, international recommendations of treatment options and outcome. *Journal of Perinatal Medicine*, 46(5), 465–488. [Google Scholar](#)

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