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Analysis of risk factor microflora colonization on pregnancy vagina in Makassar

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ABSTRACT

Introduction: Worldwide infection rates based on estimated 20-30 % colonization at 35 weeks gestation. During normal pregnancy, colonization is transient, intermittent, or chronic infection, and the spectrum varies from asymptomatic colonization to sepsis. Intrapartum transmission of bacteria from mother to neonatal will cause the infection to develop into neonatal sepsis during childbirth. This study aimed to determine the proportion of gram-positive and negative bacteria colonization in pregnant women over 31 weeks of age.

Research Methodology: This research uses vaginal swab samples from pregnant women and blood samples from babies who are born, then these samples will be examined in the Unhas Hospital laboratory. Vaginal swab samples via transport medium will be placed in BHIB medium and then incubated at 270 C for 24 hours.

Result: Prevalence of gram-positive bacterium identified Streptococcus species (0.9%), S. Aureus (11.8%), and S. Epidermidis (78.4%). For gram-negative bacteria identified, Enterobacter Sp (32.3%), Providencia Sp (12.7%), Proteus Sp (8.8%), Acinetobacter sp (5.9%), and E. Coli (6.9%). Candida Sp was obtained on SDA culture and gram staining, followed by several 54 samples (55.1%). The symptom was to be Discharge Vagina in over 30% of cases. Of 76.6% were habitually antiseptic. Based on data and statistical analysis, it can be concluded that age has a significance level of 0:01 ($\rho < 0.05$), and contraceptives used to have a significance level of 0.038 ($\rho < 0.05$), had a relationship with a potential fetomaternal infection to the infant.

Conclusion: Maternal age and history of contraceptive use are significantly associated with bacterial colonization in pregnant women. These findings underscore the need to consider demographic and behavioral factors when assessing and managing vaginal health during pregnancy. Effective educational interventions focusing on proper hygiene practices and the risks associated with antiseptic soap use, coupled with targeted monitoring of older pregnant women and those with a history of contraceptive use, are recommended to reduce the risks associated with vaginal microbial colonization.

Keywords*: microflora colonization, risk factors, and pregnancy.

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INTRODUCTION

Sepsis in newborns is a severe medical condition that can affect babies under 28 days old. Sepsis occurs when the body has an extreme response to an infection (Buffington *et al.*, 2023). Newborn babies who experience infections and experience sepsis can experience inflammation throughout their bodies. This inflammation and blood clots cause reduced blood flow to your baby's limbs and vital organs (Gorczyca *et al.*, 2024). This can cause organ failure and even death. Bacterial infection is the most common cause of sepsis in newborn babies. Bacteria such as E. coli, Listeria, and Group B streptococcus are common bacteria that can cause infections that lead to sepsis. Viruses, fungi, and parasites can also cause this condition. For example, herpes simplex virus can cause severe infections in newborns (Jin *et al.*, 2023). There are approximately 1.3 million cases of neonatal sepsis reported worldwide, with deaths being more common in premature and low birth weight babies. Neonatal sepsis is the third leading cause of neonatal death, resulting in 203,000 deaths per year. A global observational study involving more than 3,200 newborns suffering from sepsis in 19 hospitals in 11 countries has shown that many newborns die because antibiotics used to treat sepsis begin to lose their effectiveness. Experiences a diversity of cultural practices and environmental factors that may contribute to differences in the vaginal microbiome among pregnant women. Despite the growing body of research on the vaginal microbiota and its role in pregnancy, there remains limited data on the specific risk factors associated with the colonization of vaginal microflora in this region. Understanding these risk factors is essential for improving prenatal care and developing targeted interventions to prevent microbial imbalances and associated complications (Baker *et al.*, 2023).

The vaginal microflora plays a crucial role in maintaining reproductive health, particularly during pregnancy (Nami *et al.*, 2023). It acts as a natural barrier, preventing the overgrowth of pathogenic microorganisms and protecting both the mother and fetus from infections. The balance of the vaginal microbiota is primarily maintained by lactobacilli, which produce lactic acid to create an acidic environment unfavorable to harmful bacteria and fungi (Weng *et al.*, 2023). However, this delicate balance can be disrupted by various factors, leading to microbial colonization and potential complications. Pregnancy brings about significant hormonal and physiological changes that can alter the composition of the vaginal microflora. Increased levels of estrogen and glycogen create an environment conducive to microbial shifts, which may predispose pregnant women to conditions such as bacterial vaginosis, candidiasis, and urinary tract infections (Mady *et al.*, 2023). These conditions, if left untreated, can lead to adverse outcomes, including preterm birth, low birth weight, and neonatal infections. Several risk factors contribute to these microbial imbalances, including maternal age, socioeconomic status, hygiene practices, dietary habits, and a history of contraceptive use. Behavioral factors, such as using antiseptic soaps and douching, may further disrupt the natural microbiota, exacerbating the risk of colonization by pathogenic organisms. Despite the growing awareness of the importance of vaginal health during pregnancy, there is still limited research addressing the interplay of these risk factors in diverse populations (Obuobi & Škalko-Basnet, 2024).

This study has provided a wealth of high-quality data to improve the care of newborns with sepsis. Sepsis in newborns can be cured. Many newborns who develop sepsis recover completely and have no other problems. However, neonatal sepsis is one of the leading causes of infant death (Huang *et al.*, 2024). The sooner the baby gets treatment, the better the results—the risk of death from sepsis increases by approximately 7.6% for every hour that passes without treatment. The colonization of vaginal microflora during pregnancy plays a critical role in maternal and neonatal health. A balanced vaginal microbiota, dominated by beneficial bacteria such as Lactobacillus species, is essential for maintaining a healthy vaginal environment and preventing infections. However, various risk factors can disrupt this balance, leading to colonization by potentially harmful microorganisms, which may increase the risk of complications such as preterm labor, bacterial vaginosis, and neonatal infections. Vaginal microbiota plays a crucial role in maintaining the health of the reproductive system, particularly during pregnancy (Lan & Chen, 2023). An optimal balance of microorganisms in the vagina is

essential to prevent infections and ensure maternal and fetal well-being. However, disturbances in the vaginal microflora, including the overgrowth of pathogenic microorganisms, can lead to various complications such as bacterial vaginosis, preterm birth, and other adverse pregnancy outcomes. Various factors, including hormonal changes, lifestyle, hygiene practices, diet, and socio-demographic characteristics, influence the composition of vaginal flora during pregnancy. Understanding the risk factors contributing to vaginal microflora colonization is particularly important in regions with unique socio-cultural, environmental, and healthcare conditions. In Makassar, Indonesia, factors such as lifestyle, socioeconomic status, hygiene practices, and access to healthcare may influence the vaginal microbiota of pregnant women. Despite its importance, there is limited research on this topic in the local context. This study aims to analyze the risk factors associated with vaginal microflora colonization during pregnancy in Makassar. By identifying these factors, the research seeks to contribute to developing targeted interventions to improve maternal and neonatal health outcomes in the region.

RESEARCH METHODOLOGY

This research uses vaginal swab samples from pregnant women and blood samples from babies who are born; then, these samples will be examined in the Unhas Hospital laboratory. Vaginal swab samples via transport medium will be placed in BHIB medium and then incubated at 270 C for 24 hours. After that, the sample will be cultured on Blood Agar (BA) medium, Mc. Concey (MC) and Sabarause Dextrose Agar (SDA) were then incubated for 24 hours. The colonies from the culture results will then undergo a confirmation test regarding the type and specifications of the bacteria being examined. A microscopic examination is carried out to see the morphology. The results of each sample examination are then tabulated and statistically analyzed to see and assess the relationship between symptoms that appear in mothers and babies born as a result of fetomaternal transmission of infection.

RESULT

This study aims to identify microflora in the vagina of pregnant women to determine the prevalence and percentage of several microflora that can cause symptoms in pregnant women or those that are asymptomatic using culture techniques with vaginal swab samples. Apart from that, this research also serves as a screening for several cases of sepsis in infants, which have been reported in several cases related to fetomaternal infections caused by Group B Streptococcus (GBS) bacteria. Samples obtained from the hospital in the form of vaginal swabs using Medium Stuart (Medium transport) were placed in BHIB broth and then planted in 3 culture media, namely Blood Agar (BA), Mc. Concey (MC), and Sabarause Dextrose Agar (SDA). Moreover, biochemical tests and gram staining are carried out to differentiate and confirm the microflora to see the morphology and bacterial colonies.

The following is data on the results of bacterial examinations in pregnant women

Demographic	n	%
Bacteria		
Gram	21	21.4
-positive	77	78.6
Gram-negative		
Mushrooms	54	54
Candida *Sp*	46	46
Non-Candida *Sp*		

The table above shows that 69 samples (78.6%) of rectovaginal pregnant women contained gram-negative bacteria, which was more than the gram-positive bacteria, which were 21 samples (21.4%). Meanwhile, Candida Sp was identified in the culture medium, and gram staining was done in 54 samples (54%). Non-candida was identified in 46 samples (46%).

Table 2 Frequency of bacterial types in vaginal microflora colonization examination of pregnant women

Jenis Bakteri	n	%
Bakteri Gram Positif	21	21.4
GBS	1	1
S. Aureus	12	12.2
S. Epidermidis	8	8.2
Gram negative	77	78.6
Eschericia coli	7	7.1
Klebsiella Pneumonie	4	4.1
Klebsiella Oxytoca	1	1
Enterobacter Agglomerans	18	18.4
Enterobacter Aerogenes	13	13.3
Enterobacter Cloacae	2	2.1
Enterobacter Hapniae	1	1
Providencia Alcalifaciens	13	13.3
Proteus Vulgaris	6	6.1
Proteus Mirabilis	3	3
Acinetobacter Sp	6	6.1
Alkaligenes Faecalis	2	2.1

The table above shows that the most commonly found gram-positive bacteria was S. Aureus in 12 samples (12.2%) compared to other gram-positive bacteria. Meanwhile, the gram-harmful bacteria Enterobacter Aggomerans was the most abundant among the other gram-negative bacteria, namely 18 samples (18.4%). The symptoms that often appear are vaginal discharge in around 30% of pregnant women, and 76.6% of mothers who experience vaginal discharge stated that they often use antiseptic soap.

After conducting statistical analysis of several risk factors for microflora colonization in the rectovaginal of pregnant women, several data were obtained that were not correlated with bacterial colonization, including maternal education with a significance level of 0.824, more excellent than 0.05 ($p>\alpha$), husband's occupation with a significance level (p=0.414), frequency of pregnancy with a level of significance (p=0.816), number of births with a level of significance (p=1.000), history miscarriage with a significance level (p=0.343), vaginal discharge symptoms with a significance level (p=0.652) and pregnant women with a history of surgery with a significance level (p=0.905). Of the several risk factors tested statistically, the following data shows that there is a relationship or correlation between risk factors and bacterial colonization of the vagina, namely age with a significance level of 0.01 ($\rho<0.05$) and history of using contraceptives with a significance level of 0.038 ($\rho<0.05$)

DISCUSSION

The findings of this study reveal a significant prevalence of bacterial and fungal colonization in the vaginal microflora of pregnant women, particularly during the later stages of pregnancy. The predominance of gram-negative bacteria (75.5%) over gram-positive bacteria (20.6%) highlights the potential vulnerability of the vaginal microbiota to shifts in balance during pregnancy. Gram-negative bacteria, such as Enterobacter sp., Providencia sp., and Proteus sp., are known opportunistic pathogens that can pose risks of infections, including urinary tract infections and preterm birth. Similarly, the high prevalence of Candida sp. (55.1%) aligns with existing literature, indicating an increased susceptibility to fungal colonization during pregnancy due to hormonal changes that alter the vaginal pH and immune response. The frequent occurrence of vaginal discharge (30%) and the reported use of antiseptic soap (76.6%) among those experiencing discharge raise essential concerns. Antiseptic soaps, while perceived as hygienic, can disrupt the natural vaginal flora, potentially exacerbating microbial imbalances. This underscores the importance of educating pregnant women on appropriate hygiene practices to preserve the protective functions of the vaginal microbiota (Mondal et al., 2023).

The statistical associations identified in this study provide further insights into the risk factors for vaginal microbial colonization. Maternal age significantly correlated with colonization, suggesting that physiological changes in older pregnant women may contribute to altered microbial dynamics (Wei *et al.*, 2024). Additionally, the history of contraceptive use was significantly linked to colonization, likely due to the lingering effects of hormonal contraceptives on vaginal ecology. These findings emphasize the need for personalized prenatal care that considers individual demographic and medical histories. From a clinical perspective, the high prevalence of pathogenic bacteria and fungi highlights the need for routine screening of vaginal microflora during pregnancy, particularly in women with identified risk factors (Molina *et al.*, 2024). Early detection and management of microbial imbalances can help prevent adverse outcomes such as preterm labor, infections, and neonatal complications (Roy et al., 2024). Future research should explore the longitudinal dynamics of vaginal microflora throughout pregnancy, including the impact of dietary, environmental, and behavioral factors. Interventions, such as probiotic supplementation, should also be investigated as potential strategies to support healthy vaginal microbiota (Chen *et al.*, 2024).

Bacterial vaginosis occurs when specific bacteria that are naturally present in the vagina grow excessively. This condition disrupts the balance of the number of bacteria in the vagina. There are two types of bacteria in the vagina, namely "good" bacteria and "bad" bacteria (Abavisani *et al.*, 2024). Lactobacillus bacteria are good bacteria that function to limit the growth of harmful bacteria by maintaining the pH or average acidity level in the vagina. Meanwhile, the harmful bacteria that naturally exist in the vagina are anaerobic (Turnip & Kamso, 2024). When the number of good bacteria decreases, the growth of anaerobic bacteria will increase, resulting in bacterial vaginosis (Mirzaei *et al.*, 2023). It is not yet known precisely what causes the balance of bacterial growth in the vagina to be disrupted. However, some factors are thought to increase a woman's risk of experiencing bacterial vaginosis, including Experiencing hormonal changes due to menstruation, pregnancy, or menopause, so the number of Lactobacillus bacteria decreases naturally, Smoke, Having a history of sexually transmitted diseases, Frequently changing sexual partners and not using condoms. It is using antibiotics long term, Using contraception in the form of an intrauterine device (IUD), Using detergents with harsh chemical content to wash underwear, Cleaning the vagina with a vaginal douche, or using soap that can irritate the vagina, such as soap containing perfume and antiseptic soap (Li *et al.*, 2024).

Statistical analysis indicated that age and a history of contraceptive use were significantly associated with bacterial colonization. Older maternal age is often linked with hormonal changes that may alter the vaginal environment, potentially favoring the growth of harmful bacteria (Arnianti et al., 2022). Additionally, contraceptive use, particularly hormonal methods, can affect the vaginal flora by altering the pH and hormonal milieu, which may predispose women to bacterial overgrowth and infections (Wu *et al.*, 2024). The findings suggest that specific attention should be given to older pregnant women and those with a history of contraceptive use during prenatal care, as they may be at higher risk for developing microbial imbalances. Statistical analysis identified maternal age and a history of contraceptive use as significant risk factors for bacterial colonization. These results highlight the multifactorial nature of vaginal microflora disturbances during pregnancy, influenced by demographic, behavioral, and hormonal factors (Çömlekcioğlu *et al.*, 2024). Efforts to promote maternal health should include education on appropriate hygiene practices, discouraging the overuse of antiseptic soaps, and providing targeted care for at-risk groups, such as older pregnant women and those with a history of contraceptive use. Addressing these factors can help prevent microbial imbalances, improving pregnancy outcomes and maternal-fetal health (Bhatia et al., 2024).

CONCLUSION

It can be concluded that it is essential to understand the complex factors that influence vaginal microflora during pregnancy. It is essential to identify and address modifiable risk factors, such as hygiene practices and contraceptive use, to prevent microbial imbalances that may lead to complications during pregnancy. Furthermore, healthcare providers should offer targeted interventions to pregnant women at higher risk, such as those with advanced maternal age or a history of contraceptive use, and recommend appropriate care strategies to manage vaginal discharge and microbial infections. Future studies should explore further the causal relationships between specific risk factors and microbial colonization, as well as the long-term effects on maternal and neonatal health.

Conflict of Interest

This research is interesting because it links bacterial colonization in pregnant women, which can cause sepsis in newborn babies. This research also provides an overview of the types of bacterial colonization of the vagina that cause vaginal discharge, which is associated with lifestyle patterns that can aggravate bacterial colonization of the vagina.

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