Research Article

Germ Patterns and Antibiotic Susceptibility in Pregnancy and Labor with Risk of Infections

Pola Kuman dan Sensitivitas Antibiotik pada Kehamilan dan Persalinan dengan Faktor Risiko Infeksi

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Abstract

Objective: To determine the pattern of germs and antibiotic susceptibility tests in pregnant women related to risk factors for infection in preterm PROM cases, the threat of preterm labor, pathological fluor albus, and prolonged labor in Manado city.

Methods: This study is a cross-sectional descriptive study. A total of 21 samples were obtained, consisting of six preterm PROM cases, five premature contraction cases, five pathological fluor albus cases, and five prolonged labor cases. The study was conducted in Kandou General Hospital and Affiliated Hospitals, Manado. All patients were informed about the study and signed informed consent. Germ pattern and antibiotics susceptibility data were analyzed.

Results: Of 21 samples, the detected microorganisms included *Staphylococcus aureus* (12), *Coagulase-negative staphylococcus* (2), mixed microorganisms (*Staphylococcus aureus + Candida sp, Coagulase-negative staphylococcus + Candida sp*), *Bacillus sp* and *Candida sp*. The susceptible and safe antibiotics for pregnancy were Ampicillin / Sulbactam and Meropenem. The resistant antibiotics were Ceftriaxone, Tetracycline, and Neomycin.

Conclusion: The most common type of bacteria found in pregnant women and women in labor was *Staphylococcus aureus*. Susceptible and safe antibiotics for pregnancy were Ampicillin / Sulbactam and Meropenem.

Keywords: antibiotic sensitivity test, germ pattern, pregnancy and labor infection.

Abstrak

Tujuan: *Mengetahui pola kuman dan uji sensitivitas antibiotik pada perempuan hamil terkait faktor risiko infeksi pada kasus KPD preterm, ancaman partus prematurus, fluor albus patologis dan partus lama di kota Manado.*

Metode: *Studi ini merupakan studi deskriptif potong lintang. Jumlah sampel yang didapatkan sebesar 21 sampel di mana terbagi pada masing-masing kasus KPD preterm 6 sampel, ancaman partus prematurus 5 sampel, fluor albus patologis 5 sampel dan partus lama 5 sampel. Sampel diambil dari RSUP Prof. Dr. R. D. Kandou Manado dan Rumah Sakit Jejaring di kota Manado yang memenuhi kriteria inklusi dan eksklusi. Semua pasien dijelaskan mengenai prosedur penelitian dan penandatanganan inform consent baru dilakukan pengambilan sampel. Data dianalisis dengan cara deskriptif tentang pola kuman dan uji sensitivitas antibiotik pada kehamilan dan persalinan dengan faktor risiko infeksi.*

Hasil: *Dari 21 sampel, sebaran jenis mikroorganisme diantaranya Staphylococcus areus (12), Staphylococcus non koagulase (2), mikroorganisme campuran (Staphylococcus aureus + Candida sp, Staphylococcus non koagulase + Candida sp), Bacilus sp dan Candida sp. Hasil uji sensitivitas antibiotik yang sensitif dan aman untuk kehamilan di antaranya Ampicillin/Sulbactam dan Meropenem. Hasil uji sensitivitas antibiotik yang resisten adalah Ceftriaxone, Tetracycline dan Neomycin.*

Kesimpulan: *Jenis mikroorganisme paling banyak adalah Staphylococcus aureus. Hasil uji sensitivitas antibiotik yang sensitif dan aman untuk kehamilan di antaranya Ampicillin/ Sulbactam dan Meropenem.*

Kata kunci: *infeksi kehamilan dan persalinan, pola kuman, uji Sensitivitas antibiotik.*

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Received: Accepted: Published:

INTRODUCTION

Infectious disease is a global issue that occurs in both developing and developed countries. The infection can be caused by bacteria, viruses, fungi, and parasites, while the transmission may occur intrauterine, during labor, or after birth. Infection is one of the leading causes of death in the world. In Indonesia, the most common causes of maternal death are bleeding (8%), eclampsia (24%), infection (11%), prolonged or obstructed labor (5%), and others (11%). In North Sulawesi, maternal death caused by infection was 8%.[1-3]

Risk factors for infections in pregnancy and childbirth include premature rupture of membranes (PROMs), urinary tract infections (UTIs), and vaginal discharge. In India in 2019, the most common bacteria found in pregnancy infections were *Escherichia coli, Candida albicans, Klebsiella,* and *Staphylococcus aureus.* In Manado, the most common infections found in the obstetric case was Staphylococcus epidermis.[4,5]

Genital infections during pregnancy and childbirth are a relatively common cause of morbidity, occurring in one-third of pregnant women. It may lead to restricted intrauterine growth, preterm labor, and premature rupture of membranes, even in asymptomatic women. On the other hand, infections increase the risk of morbidity in infants. Pregnant women are at high risk of infection, especially in the second and third trimesters.[4]

Antibiotics are natural or synthetic compounds that suppress or cease the biochemical processes in organisms, especially in the infection process by microbes. For global purposes, the availability, selection, and use of antibiotics should be conducted appropriately. Therefore, it is necessary to identify pathogenic bacteria and select antibiotics that are effective against these microorganisms. Improper use of antibiotics has resulted in multiple drug resistance against antibiotics. Untreated infections can cause complications in pregnancy and childbirth. In order to prevent these complications, knowledge of the prevalence of various infections in pregnancy is essential for effective treatment and to improve perinatal outcomes.

METHODS

This was a cross-sectional study. The study was conducted at Prof. Dr. R. D. Kandou hospital and affiliated hospitals in Manado from January 2021 to March 2021.

The population of this study was pregnant women with risk factors for infections who visited the obstetric clinic for Antenatal Care (ANC) or emergency room in the Obstetrics and Gynecology Department.

The inclusion criteria were all pregnant women with risk factors for infections in preterm PROM cases, premature contraction, pathological fluor albus, and prolonged labor, and willing to participate in the study. Exclusion criteria were pregnancy with medical complications, such as heart disease, diabetes mellitus, HIV, malignancy, ongoing antibiotics or immunosuppressants treatment, and refusal to participate in the study.

The study subjects were selected through history taking, physical examination, and further examinations. Subjects who met the study criteria and signed the consent form were included in the study. The sample size of this study was 21 samples.

Before subjected to the study procedures, each subject received an explanation of the aims, objectives, and procedures of the study. The samples were taken using a vaginal swab kit. Afterward, a culture with agar media, identification tests, and antibiotic susceptibility tests were conducted. Laboratory examinations were carried out in the Microbiology Laboratory of the Faculty of Medicine, Universitas Sam Ratulangi, Manado

Data were collected and recorded in a study data form that has been prepared and then arranged in a master table. Data were analyzed descriptively.

RESULTS

A total of 21 subjects with risk factors for infections were obtained, including six patients for preterm PROM cases, premature contraction cases, five pathological fluor albus cases, and five prolonged labor cases.

Table 1 showed the characteristics of the study subjects. The most common age group was 20 - 35 years, namely 17 patients (80.95%), with five patients (83.33%) of preterm PROM cases and four patients (80%) with premature contraction, pathological fluor albus, and prolonged labor cases. The majority of the study subjects were senior high school (66.67%) and were housewives. The majority of the study subjects were overweight (66.67%).

Table 2 showed the microorganisms observed

in this study, including three variants of gram-positive microorganisms, one variant of fungal, and two variants of mixed microorganisms (gram-positive bacteria + fungi). The most common type of microorganism found was Staphylococcus aureus with a total of 12 samples (57.14%), followed by coagulase-negative *Staphylococcus* + *Candida sp* (14.28%), *coagulase-negative Staphylococcus* (9.52%), *Staphylococcus aureus* + *Candida sp* (9.52%), *Bacillus sp* and *Candida sp* (4.76%).

Table 3 showed the distribution of microorganisms by the obstetric cases. In the preterm PROM cases, the growth of *Staphylococcus aureus* was observed in four (66.67%) samples, followed by coagulase-negative *Staphylococcus* and *coagulase-negative Staphylococcus* + *Candida sp,* one sample each (16.67%). In premature contraction cases, two samples (40%) showed the growth of *Staphylococcus aureus* and one sample (20%) with *Staphylococcus aureus* + *Candida sp, coagulase-negative Staphylococcus* + *Candida sp*, and *Candida sp*. In pathological fluor albus cases, there were three samples (60%) with *Staphylococcus aureus* growth, one sample (20%) with *coagulase-negative Staphylococcus aureus* growth, and one sample (20%) with *Staphylococcus aureus* + *Candida sp* growth. In prolonged labor cases, three samples (60%) showed the growth of *Staphylococcus aureus,* followed by one sample (20%) with the growth of *coagulase-negative Staphylococcus* + *Candida sp*, and one sample (20%) with *Bacillus sp.*

Table 4 showed the results of the antibiotics susceptibility test based on the microorganism. For the most common microorganisms, *Staphylococcus aureus,* sensitive antibiotics included Chloramphenicol, Gentamicin, Tobramycin, Levofloxacin, Moxifloxacin, Ampicillin / Sulbactam, and Meropenem antibiotics. Ampicillin / Sulbactam and Meropenem antibiotics are category B in pregnancy and considered safe. Furthermore, resistant antibiotics to *Staphylococcus aureus* were Ceftriaxone, Tetracycline, and Neomycin. *Candida sp*. were sensitive to all anti-fungal groups.

Table 1. Characteristics of Patients

Characteristics	Diagnosis/Cases									
	Preterm PROM	%	Premature Contraction	%	Pathological Fluor Albus	%	Prolonged labor	%	Total	%
Age (year)										
< 20	1	16,67	0	0	0	0	1	20	2	9.52
20-35	5	83.33	4	80	4	80	4	80	17	80.95
> 35	0	0	1	20	1	20	0	0	2	9.52
Education										
Elementary	0	0	0	0	0	0	1	20	1	4.76
Junior high school	0	0	1	20	1	20	1	20	3	14.28
Senior high school	4	66.67	3	60	4	80	3	60	14	66.67
University/ college	2	33.33	1	20	0	0	0	0	3	14.28
Occupation										
Housewives	4	66.67	3	60	5	100	5	100	17	80.95
Business women	2	33.33	0	0	0	0	0	0	2	9.52
Civil servants	0	0	2	40	0	0	0	0	2	9.52
Body mass index										
Underweight (< 18.5)	0	0	1	20	0	0	0	0	1	4.76
Normal (18.5- 24.9)	1	16.67	0	0	2	40	2	40	5	23.8
Overweight (25-29.9)	4	66.67	4	80	3	60	3	60	14	66.67
Obese (≥ 30)	1	16.67	0	0	0	0	0	0	1	4.76

Table 2. Grouping Types of Microorganisms

Microorganisms		Total	%
Gram-Positive Bacteria	*Staphylococcus aureus*	12	57.14
	Coagulase-negative Staphylococcus	2	9.52
	Bacillus sp	1	4.76
Fungi	*Candida sp*	1	4.76
Mixed (Gram-Positive bacteria + fungi	*Staphylococcus aureus + Candida sp*	2	9.52
	Coagulase-negative Staphylococcus + Candida sp	3	14.28

Table 3. Distribution of Microorganism Growth by the Type of Cases

Microorganisms	Diagnosis/Cases							
	Preterm PROM	%	**Premature Contraction**	%	**Pathological Fluor Albus**	%	**Prolonged labor**	%
Staphylococcus aureus	4	66.67	2	40	3	60	3	60
Coagulase-negative Staphylococcus	1	16.67	0	0	1	20	0	0
Staphylococcus aureus + Candida sp	0	0	1	20	1	20	0	0
Coagulase-negative Staphylococcus + Candida sp	1	16.67	1	20	0	0	1	20
Bacilus sp	0	0	0	0	0	0	1	20
Candida sp	0	0	1	20	0	0	0	0

Table 4. Susceptibility Test Results by Microorganisms

Antibiotics	Microorganisms														
	S.a			**CoNS**			**S.a + Candida**			**CoNS + Candida**			**B.sp**		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Chloramphenicol	7	3	2	2	0	0	1	0	1	1	1	1	1	0	0
Erythromycin	1	5	6	0	1	1	0	0	2	0	0	3	0	0	1
Gentamicin	7	4	1	2	0	0	1	0	1	1	1	1	1	0	0
Neomycin	1	2	9	2	0	0	0	1	1	1	0	2	1	0	0
Sulphamethoxazole/ Trimethoprim	3	1	8	0	0	2	2	0	0	1	0	2	1	0	0
Tetracycline	0	3	9	0	0	2	0	0		0	0	3	0	0	1
Tobramycin	6	2	4	1	1	0	1	0	1	1	1	1	1	0	0
Vancomycin	1	7	4	0	2	0	0	1	1	1	0	2	1	0	0
Amikacin	4	3	5	0	1	1	0	0	2	0	1	2	1	0	0
Ciprofloxacin	4	4	4	2	0	0	0	2	0	1	1	1	1	0	0
Cefadroxil	3	1	8	1	0	1	0	0	2	0	0	3	0	1	0
Ampicillin/ Sulbactam	6	1	5	0	0	2	1	0	1	0	0	3	1	0	0
Levofloxacin	6	3	3	1	0	1	1	0	1	1	0	2	1	0	0
Piperacillin/ Tazobactam	4	2	6	0	0	2	1	0	1	0	1	2	1	0	0
Moxifloxacin	6	2	4	0	0	2	0	0	2	1	1	1	1	0	0
Tigecycline	1	4	7	0	1	1	0	1	1	1	1	1	1	0	0
Ceftriaxone	1	2	9	0	0	2	0	0	2	0	1	2	0	1	0
Meropenem	7	4	1	0	0	2	2	0	0	2	0	1	1	0	0

* S = Susceptible, I = Intermediate, R = Resistant, S.a: *Staphylococcus aureus,*
CoNS: *Coagulase-negative Staphylococcus,* B.sp: *Bacillus sp*

DISCUSSION

In this study, 21 pregnant patients met the inclusion and exclusion criteria and had signed the informed consent to participate. The subjects consisted of six preterm PROM cases, five premature contraction cases, five pathological fluor albus cases, and five prolonged labor cases.

The characteristics of the research subjects were assessed in terms of maternal age, education, occupation, and Body Mass Index (BMI). In Table 1, it can be seen that the most common age group was 20 - 35 years (80.95%), with the most common educational background of senior high school (66.67%), housewives as the most common occupation (80.95%). Most of the subjects were overweight (66.67%). Obese pregnant women have a 2.5 to 4.5 times higher risk of infection morbidity than normal-weight patients. Hormonal imbalance may occur in obesity, leading to changes in the balance of normal vaginal flora to pathogenic flora.[6]

In this study, microorganism growth was observed in all 21 samples. These microorganisms included bacteria, fungi, and mixed (bacteria + fungi) (Table 2). Gram-positive bacteria were the dominant microorganisms in this study. A study in Uganda found the highest number of gram-positive bacteria at 63%.[7] The most common microorganism observed in this study was *Staphylococcus aureus* (57.14%). *Staphylococcus aureus* is a gram-positive spherical bacterium with a diameter of 0.8 - 1.0 µm. These bacteria grow at an optimum temperature of 37^0C but form the pigments best at room temperature ($20\text{-}25^0$C). They are mostly observed on the

surface of the skin and mucous membranes of humans. *Staphylococcus aureus* is coagulase-positive and the primary pathogen in humans. *Staphylococcus aureus* is a commensal bacteria that is often found on the skin and urogenital tract. The habit of wiping from front to back is thought to cause translocation of commensal germs on the skin of the vulva.[8,9] There were two variants of microorganisms found in this study, including mixed microorganisms (gram-positive bacteria and fungi), namely *Staphylococcus aureus* + *Candida sp* (9.52%) and c*oagulase-negative Staphylococcus* + *Candida sp*. (14.28%). Coinfection of *Staphylococcus sp*. and *Candida sp*. can increase their pathogenic properties. *Staphylococcus aureus,* in particular, binds to the invasive hyphal form of Candida albicans and increases the mortality and systemic spread in the tissue mucosa.[10]

As seen in Table 3, the most common type of microorganisms in preterm PROM cases was Staphylococcus aureus (66.67%). A study in Uganda (2017) found that the most common microorganisms in preterm PROM were *Staphylococcus aureus* (19.4%), followed by *Escherichia coli* (17.9%), *coagulase-negative Staphylococcus* (13.4%), and *Streptococcus pyogenes* (11, 9%).[7]

In cases of premature contraction, the most common germ was *Staphylococcus aureus* (40%). *Staphylococcus sp*. and *Pseudomonas sp*. were most commonly found in pregnant women with preterm labor without premature rupture of membranes. One of the causes of preterm labor is chorioamnionitis. *Staphylococcus aureus* is also a cause of chorioamnionitis associated with preterm labor. *Staphylococcus aureus* infects soft tissue and forms a strong biofilm on the surface, providing an advantage for bacteria and aiding resistance to antimicrobials. Infection of *Staphylococcus aureus* on the pregnancy membrane will induce the release of proinflammatory cytokines, including IL-1β, IL-2, IL-6, TNF-α, and IFN-, this increase can promote the release of MMP neutrophils which directly contribute to preterm labor.[11]

In pathological fluor albus and prolonged labor, the most common germ was *Staphylococcus aureus* (60%). The hygiene of the mother also influences the change in acidity and growth of this microorganism during pregnancy. Rapid pH changes lead to the disturbance of vaginal acid-base and the rapid growth of normal and anaerobic microorganisms, resulting in an increased phagocytosis process and the metabolic results of microorganisms that change the vaginal fluorine albus to alkaline and trigger normal vaginal flora to become parasites in the cervical mucosa and vagina. This situation will change the quality of fluor albus and can be categorized as pathological fluor albus. Infections can be a threat to the mother and the fetus during prolonged labor. Frequent examinations of the cervix using fingers lead to vaginal contamination and entry of bacteria into the vagina and uterus.[12,13]

The antibiotic susceptibility test results showed that *Staphylococcus aureus* was susceptible to Chloramphenicol, Gentamicin, Tobramycin, Levofloxacin, Moxifloxacin, Ampicillin / Sulbactam, and Meropenem antibiotics. Aminoglycoside antibiotics (Gentamicin, Tobramycin) and quinolones (Levofloxacin, Moxifloxacin) are classified into category C or D by the FDA and are not recommended for pregnant women. Ampicillin / Sulbactam and Meropenem antibiotics are category B in pregnancy and are considered safe. Ampicillin / Sulbactam is a combined antibiotic class of the penicillin group with β-lactamase inhibitors. The addition of Sulbactam to Ampicillin will increase the effectiveness of Ampicillin. The predominant microorganisms were *Staphylococcus aureus, Streptococcus sp*., and *Escherichia coli*.

Furthermore, the study stated that the sensitive antibiotics considered safe to use during pregnancy included Ampicillin / Sulbactam, Cefixime, Cefuroxim, and Erythromycin. According to the American College of Obstetricians and Gynecologists (ACOG), the administration of broad-spectrum antibiotics reduces infections in both mothers and newborns and may reduce morbidity in pregnancy. Antibiotic treatment for 7 days with parenteral combination administration of Ampicillin and Erythromycin followed by oral Amoxicillin, and oral Erythromycin is recommended in preterm PROM with gestational age <34 weeks.[14] Meropenem is categorized into the Carbapenem antibiotic class. Carbapenem is a broad spectrum β-lactam drug. The Carbapenem group is the last weapon against bacteria that are resistant to other antibiotics.[8]

The antibiotic susceptibility test results showed that resistant antibiotics to Staphylococcus aureus were Neomycin, Tetracycline, and Ceftriaxone antibiotics. A meta-analysis showed that there were 626 samples from 2032 samples of the *Staphylococcus aureus* that were resistant to Ceftriaxone, and there were 1982 samples

from 3019 samples that were resistant to Tetracycline.[15] To minimize the possibility of resistant microbes, proper selection of antibiotics based on their indication, dose, timing, and type must be conducted.

CONCLUSION

The microorganisms observed in vaginal swab cultures of pregnant women with risk for infections in cases of preterm PROM, premature contraction, pathological fluor albus, and prolonged labor were *Staphylococcus aureus*, coagulase-negative *Staphylococcus*, mixed (*Staphylococcus aureus* + *Candida sp*. and *coagulase-negative Staphylococcus* + *Candida sp*.), *Candida sp*., and *Bacillus sp*. The most common microorganism was *Staphylococcus aureus*. Susceptible and safe antibiotics to *Staphylococcus aureus* were Ampicillin / Sulbactam and Meropenem. Resistant antibiotics to Staphylococcus aureus were Ceftriaxone, Tetracycline, and Neomycin antibiotics. *Candida sp*. were susceptible to all anti-fungal groups.

SUGGESTION

Further studies with a larger sample size are necessary to provide more accurate results in determining the pattern of germs in vaginal swab cultures of pregnant women related to risk factors for infection in preterm PROM cases, premature contraction, pathological fluor albus, and prolonged labor in Manado. Furthermore, antibiotics susceptibility studies, including more antibiotics that are safe for pregnancy, are warranted.

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