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Prevalence and identification of bacteria causing asymptomatic bacteriuria in pregnant women at Antang Community Health Center, Makassar City

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ABSTRACT. Asymptomatic bacteriuria in pregnancy carries significant risks to maternal and fetal health. Untreated cases can progress to symptomatic urinary tract infections, prematurity, low birth weight, and fetal death. Data on the prevalence and types of bacteria causing asymptomatic bacteriuria in pregnant women, especially in Makassar, are still very limited. This study aims to determine the prevalence of asymptomatic bacteriuria and the types of bacteria causing it in pregnant women at the Antang Community Health Center in Makassar. This study is a quantitative descriptive study, using microbiological examination methods through urine dipstick testing, urine culture, and identification. The subjects were 58 pregnant women without symptoms of urinary tract infections who underwent pregnancy checkups at the Antang Community Health Center in Makassar. The sample used was a mid-stream urine specimen. Based on the demographic characteristics of pregnant women in their first, second, and third trimesters at the Antang Health Center in Makassar City, it shows that the majority of respondents are young pregnant women in the final stages of pregnancy (third trimester). Based on the results of the dipstick examination, it shows that the majority of respondents do not have an increase in leukocytes, which indicates a low possibility of urinary tract infections among pregnant women in their first, second, and third trimesters at the Antang Health Center in Makassar City. The results of urine culture showed that 2 samples with positive dipstick results for leukocyte esterase and nitrite parameters had bacterial colonies of >100,000 CFU/mL. The prevalence of asymptomatic bacteriuria in pregnant women in their first, second, and third trimesters at the Antang Health Center in Makassar City in this study was 3.45%. The types of bacteria that cause it are *Escherishia coli* and *Klebsiella pneumoniae.*

Keywords: Antang Community Health Center; asymptomatic bacteriuria; pregnant women; urine culture; urine dipstick

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INTRODUCTION

The urinary system is a series of organs that function to filter the blood and remove metabolic waste products and excess fluid from the body in the form of urine (Guyton & Hall, 2021). Anatomically and physiologically, this system is highly susceptible to colonisation by microorganisms that can develop into infections (Tortora & Derrickson, 2020). Urinary tract infections (UTIs) remain one of the most common public health problems worldwide, with millions of cases reported annually (WHO, 2020). UTIs can affect patients of all ages, from newborns to the elderly (Mangiri *et al*., 2020). Furthermore, pregnant women are at high risk of developing urinary tract infections (UTIs), whether asymptomatic or symptomatic (Cunningham *et al*., 2018).

Asymptomatic bacteriuria refers to the presence of bacteria in the urine. This is a condition where a urine culture shows significant growth of pathogens, i.e. greater than 100,000 CFU/mL (Fakhrizal, 2017). In a clean midstream urine sample, with no obvious symptoms of a urinary tract infection in the patient (Tille, 2021). This is common in pregnant women, the elderly, and people with diabetes (Nicolle *et al*., 2019). The primary cause of the increased risk of bacteriuria in pregnant women is the anatomical and physiological changes that occur from the first trimester onwards. An increase in progesterone levels (Patterson & Andriole, 2017).

The physiological changes occurring in the urinary tract during pregnancy are influenced by increased levels of the hormone progesterone. Progesterone causes relaxation of smooth muscle throughout the body, including in the ureters, leading to a reduction in urinary peristalsis and the

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occurrence of ureteral dilation or hydroureter (Sherwood, 2018). This condition causes urine flow from the kidneys to the bladder to slow down (urinary stasis). Urine pooled in the urinary tract acts as an ideal growth medium for bacterial colonisation (Black & Hawks, 2021). Furthermore, the physiological increase in plasma volume during pregnancy can reduce urine concentration, meaning that 70% of pregnant women experience glucosuria, which is also a trigger for bacterial growth in urine (Thaslifa *et al*, 2022). This makes them highly susceptible to asymptomatic bacteriuria, which can progress to a urinary tract infection with clinical symptoms (Azami *et al*, 2019).

Asymptomatic bacteriuria in pregnancy poses a significant risk to the health of both the mother and the foetus. Untreated cases have a high probability (up to 40%) of developing into acute symptomatic urinary tract infections (pyelonephritis) during pregnancy, and poor obstetric outcomes such as preterm birth, low birth weight, and higher foetal mortality rates (Wahyu *et al*, 2024). As well as increased prenatal mortality and morbidity (Owens *et al*, 2019). Furthermore, it can lead to complications such as anaemia, pre-eclampsia, renal failure, and septicaemia (Ismail & Handayani, 2020).

In pregnancy, asymptomatic bacteriuria is defined as bacteriuria without clinical symptoms, whereas symptomatic UTI is accompanied by symptoms such as dysuria, urgency/frequency, suprapubic pain/lower back pain, or fever (Soewarto, 2021). Gestational age, low socioeconomic status, a history of urinary tract infections, and multiparity are factors contributing to the occurrence of asymptomatic bacteriuria. Furthermore, early marriage, poor sanitation, lack of *personal hygiene* and lack of inadequate antenatal care (ANC) examinations can also cause asymptomatic bacteriuria (Saimin *et al*., 2018).

Globally, the prevalence of BA in pregnant women is estimated to range from 2% to 15% (Smaill & Vazquez, 2019). The prevalence of asymptomatic bacteriuria in Indonesia is 7.3%; in West Jakarta, a relatively high rate of 35.3% was found. A study at M Jamil General Hospital in Padang found a 42% rate of miscarriage among pregnant women with asymptomatic bacteriuria. The bacteria most commonly causing asymptomatic bacteriuria are *Escherichia coli* (70 to 80%), *Klebsiella pneumoniae* (25%) and *Staphylococcus aureus* (25%) (Wabe *et al*, 2020).

The aetiology of bacteria causing asymptomatic bacteriuria in pregnant women is dominated by normal intestinal flora. *Escherichia coli* remains the primary pathogen most frequently isolated, accounting for approximately 70% to 80% of cases (Kalinderi *et al*., 2018). In addition to *E. coli*, other frequently identified microorganisms include *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus* spp., and *Group B Streptococcus* (GBS) (Angsar, 2018).

The highest incidence of asymptomatic bacteriuria in pregnant women occurs between the second and third trimesters of pregnancy, with a peak incidence in the third trimester. The high incidence of asymptomatic bacteriuria during the second and third trimesters is attributed to increased levels of progesterone, which reduces smooth muscle tone; this leads to urinary retention and facilitates bacterial spread during pregnancy, particularly in the second and third trimesters (Riftahani, 2016).

The serious risks associated with undiagnosed asymptomatic bacteriuria in pregnant women have led researchers to recommend routine culture screening for all pregnant women attending antenatal clinics to protect both mother and child from any complications that may arise due to infection. However, in many health centres in Indonesia, healthcare providers' attention is generally focused on the presence of glucose and protein in urine specimens using urine test strips, with little attention paid to the possibility of asymptomatic infection through urine culture testing (Malau & Adipireno, 2019).

Generally, in Community Health Centres (Puskesmas), routine urine testing for pregnant women is carried out using urine test strips rather than urine culture tests, due to the cost implications and the time required for culture results, which tend to be more expensive and take longer (usually 48 hours). The true nature of a urine specimen cannot be fully assessed using urine test strips alone,

as these strips cannot measure the extent of infection or guide antimicrobial therapy, which is typically determined by culture tests (Mano *et al*, 2023).

Based on the above, this study aims to investigate asymptomatic bacteriuria and the causative bacterial species in pregnant women at the Antang Community Health Centre, Makassar. The results of this study are expected to serve as a reference in the development of standard operating procedures (SOPs) for routine urine testing for pregnant women at the Community Health Centre level to prevent more severe pregnancy complications.

MATERIALS AND METHODS

The research method used was descriptive with a *cross-sectional* approach. The population for this study comprised all pregnant women at the Antang Community Health Centre in Makassar during the period July–October 2025. The study sample consisted of urine samples from pregnant women in the first, second and third trimesters at the Antang Community Health Centre in Makassar who met the inclusion criteria and were willing to participate in the study by signing an informed consent form.

The inclusion criteria for this study were pregnant women (in the first, second and third trimesters) without symptoms of urinary tract infection. The sampling technique used in this study was *the total sampling method*. The equipment and materials used in this study were sampling boxes, urine test strips, urine sample bottles, Erlenmeyer flasks, sterile pipettes (1 ml and 10 ml), straight and curved loops, aluminium foil, a Bunsen burner, Petri dishes, microscope slides and cover glasses, an autoclave, an incubator, a staining rack, wooden clamps, a microscope, MacConkey agar, Gram staining reagents (crystal violet solution, Lugol's solution, alcohol/acetone, carbol fuchsin), biochemical test media (Kliger Iron Agar (KIA), Sulphite Indole Motility (SIM) medium, citrate medium, sugar media, mannitol salt agar), sterile distilled water, 70% alcohol, hand and instrument soap, gloves, laboratory masks, laminar air flow hood, water bath, culture/media cabinet, test tubes and racks, sterile cotton wool, cling film, tissue rolls, label paper, permanent marker.

The research procedure began with the sterilisation of the necessary equipment and materials, followed by the collection of *midstream* urine samples by implementing ethical code procedures that must be adhered to first. This was followed by an examination of the levels of bacteriuria and leukocyturia using the dipstick urinalysis method and urine culture to determine whether the patient had asymptomatic bacteriuria or not. Once it is established that the patient is positive for asymptomatic bacteriuria, bacterial identification is carried out via macroscopic and microscopic examination using Gram staining and viewed under 100× objective magnification, as well as biochemical testing. The stages of urine sample examination, culture and identification will be carried out at the Microbiology Laboratory of the Faculty of Medicine, Hasanuddin University.

Sterilisation of equipment and materials. Sterilisation is carried out using two methods: dry heat (flame sterilisation using a Bunsen burner) for metal instruments, and moist heat for heat-resistant instruments and culture media. Wet heat sterilisation is carried out in an autoclave at 121 °C for 15 minutes. Petri dishes are sterilised in an oven at 160 °C for 2 hours.

Preparation of *MacConkey Agar* (MCA) culture medium. 15.4 grams of MCA was weighed on an analytical balance, then placed in an Erlenmeyer flask together with 400 ml of distilled water. The mixture was heated on *a hotplate* whilst being stirred until it had completely dissolved. The Erlenmeyer flask was then removed and the mouth sealed with a bundle. The flask was subsequently sterilised in an autoclave at 121 °C for 15 minutes. Once the sterilisation process was complete, the Erlenmeyer flask was removed from the autoclave and cooled in an incubator until it reached a temperature of 40–50 °C. The cooled medium was poured into Petri dishes. Once set, the Petri dishes were inverted to prevent water condensation. Finally, the Petri dishes were wrapped in newspaper and labelled with the medium's identification and the date of preparation.

Urine sample collection. The urine sampling technique used is a *non-invasive* method known as the *clean catch technique.* In this *non-invasive* technique, the urine sample is a *midstream* sample,

meaning the initial urine excreted is not collected in the container. Before collecting the sample, the patient is instructed to wash their hands with soap and dry them thoroughly. Use an antiseptic wipe to clean the genital area. Wipe from front to back (from the vagina towards the anus) to prevent contamination from anal bacteria. Afterwards, avoid touching the inside or rim of the sample container to maintain sterility. Then, instruct the patient to urinate a small amount first (10 ml), before collecting the urine in the sterile container provided. The urine collection container used is a plastic container with a wide mouth, which is dry, clean, has a screw cap and seals tightly. The collected urine sample is taken to the laboratory using a *sample box* equipped with *an ice pack* at a temperature of 2–8 °C to maintain sample stability during transport.

Urine sample examination. (a) Urinalysis strip test (Dipstick Test), the urine is placed in a test tube, then *the* urine *dipstick* is dipped into it for approximately 1 minute. Any remaining urine adhering to the strip can be removed by dabbing it on a tissue. Subsequently, *the dipstick* is read using a dipstick reader to determine the test results; (2) Urine culture on *MacConkey* agar, before inoculation, the urine sample is centrifuged (Barnes *et al*, 2021), for 10 minutes at a speed of 2,500–3,000 rpm. Afterwards, the centrifuged urine sample is inoculated onto *MacConkey* differential agar using the 4- quadrant streak plate method. The culture is then incubated for 24 hours at 37 °C; (c) Identification of bacterial isolates, this involves macroscopic and microscopic examination using Gram staining and observation under a 100× objective, as well as biochemical tests comprising the catalase test, mannitol test and novobiocin test for Gram-positive bacteria, and the citrate, sulphite-indole-motility (SIM) and Kliger Iron Agar (KIA) tests for Gram-negative bacteria.

Data analysis. The data from this study are presented in narrative form and in tables.

RESULTS AND DISCUSSION

A total of 58 urine samples were collected for this study (August–October 2025) from pregnant women in their first, second and third trimesters at the Antang Community Health Centre in Makassar.

Table 1. Distribution of respondents based on demographic characteristics of pregnant women at the Antang Community Health Centre, Makassar

Characteristics	Category	Frequency (n)	Percentage (%)
	17–30	48	82.75
Gestational age	31–34	10	17.25
	Total	58	100
	Primigravida	26	44.83
Pregnant	Multigravida	32	55.17
	Total	58	100
	First trimester	17	29.31
Gestational age	Second trimester	21	36.21%
	Third Trimester	20	34.48%
	Total	58	100

Demographic data on the age of pregnant women from 58 respondents showed that 48 respondents (82.75%) were aged between 18 and 30 years, whilst 10 respondents (17.25%) were aged between 31 and 34 years. These results indicate that the majority of pregnant women are within the healthy reproductive age range, namely 20–30 years, during which the physiological condition of the reproductive organs and the body's immune system are relatively optimal. Based on the gravidity data from 58 respondents, there were 26 primigravida (44.83%) and 32 multigravida (55.17%). This finding is consistent with the study by Wabe *et al.* (2020), which reported that multigravida have a higher tendency to experience bacteriuria compared to primigravida; this is thought to be due to increasingly pronounced anatomical and physiological changes in the urinary tract during repeated pregnancies. Multigravida are also more susceptible to bacterial colonisation due to the accumulation of risk factors, such as a history of previous urinary tract infections, hygiene practices, or the use of contraceptive devices.

Based on data on gestational age from 58 respondents, there were 17 respondents (29.31%) in the first trimester, 21 respondents (36.21%) in the second trimester, and 20 respondents (34.48%) in the third trimester.

Table 2. Frequency distribution of urine dipstick test results for pregnant women at the Antang Community Health Centre, Makassar

Result	Frequency (n)	Percentage (%)
Positive Leukocytes and/or Nitrite	2	3.45
Positive Leukocytes Negative Nitrite	11	19
Negative Leukocytes and Nitrite	45	77.55

The results of the urine dipstick tests showed that out of 58 urine samples from pregnant women, 11 respondents (19%) tested positive for leukocyte esterase, and 2 respondents (3.45%) tested positive for both leukocyte esterase and nitrite, whilst 45 respondents (77.55%) tested negative for both parameters. This test is a rapid screening method to detect the possible presence of a urinary tract infection or asymptomatic bacteriuria. A positive leukocyte esterase result indicates the presence of white blood cells (leukocytes) in the urine as part of the body's immune response to infection, whilst a positive nitrite result indicates the presence of bacteria capable of reducing nitrate to nitrite, typically Gram- negative bacteria such as *Escherichia coli*.

According to Parwati & Cahyani (2023), a positive leukocyte esterase result but a negative nitrite result may be caused by several factors, such as urine being retained in the bladder for too short a time (less than four hours) so that the bacteria have not had time to convert nitrate into nitrite, low nitrate levels in the urine due to dietary patterns, or a type of bacteria that does not possess the nitrate reductase enzyme. Furthermore, very dilute urine or a high frequency of urination can also reduce the sensitivity of nitrite detection on a dipstick.

Therefore, a positive leukocyte esterase result should still be considered indicative of a urinary tract infection even if the nitrite result is negative. Although the dipstick result indicates a negative nitrite result, the examination should proceed to the urine culture stage, as the dipstick method serves only as an initial screening test with limited sensitivity and specificity, so that 13 samples consisting of 11 respondents who showed positive results on the leukocyte esterase parameter, and 2 respondents who showed positive results for leukocyte esterase and nitrite continued to the culture stage using MCA media.

Table 3. Table of urine culture results on MCA medium for pregnant women at the Antang Community Health Centre, Makassar

Sample Code	Gestational Age (Trimester)	Bacteria Total	Notes
08	1	< 100.00 CFU/mL	Negative
11	**3**	> **100.00 CFU/mL**	**Asymptomatic bacteriuria**
12	1	< 100.00 CFU/mL	Negative
13	1	< 100.00 CFU/mL	Negative
14	3	< 100.00 CFU/mL	Negative
15	2	< 100.00 CFU/mL	Negative
16	1	< 100.00 CFU/mL	Negative
25	1	< 100.00 CFU/mL	Negative
26	**1**	> **100.00 CFU/mL**	**Asymptomatic bacteriuria**
30	2	< 100.00 CFU/mL	Negative
31	2	< 100.00 CFU/mL	Negative
33	3	< 100.00 CFU/mL	Negative
38	3	< 100.00 CFU/mL	Negative

The urine culture results in this study showed that 2 out of 58 samples (3.45%) had a bacterial colony count of ≥100,000 CFU/ml, indicating asymptomatic bacteriuria, whilst 55 samples (96.55%) were negative. This figure is relatively lower compared to the study by Yeni & Welni (2023) in Banda Aceh, which reported a rate of 10–20% among pregnant women in the first trimester.

Table 4. Frequency distribution of asymptomatic bacteriuria (AB) test results in the urine of pregnant women at the Antang Community Health Centre, Makassar

Examination Result BA	Frequency (n)	Percentage (%)
Positive	2	3.45
Negative	55	96.55

Of the 2 pregnant women (3.45%) whose urine samples indicated asymptomatic bacteriuria, these included: Sample code 11, urine from a pregnant woman in the third trimester, multipara (second pregnancy), aged 27 years and Sample code 26, urine from a pregnant woman in the first trimester of pregnancy, a multipara (second pregnancy), aged 24 years. These results indicate that two of the 58 pregnant women who experienced bacterial colonisation of the urinary tract without symptoms of infection (asymptomatic bacteriuria) were multigravidas aged between 18 and 30 years. This finding is consistent with the study by Wabe *et al.* (2020), which reported that multiparous women have a higher tendency to experience bacteriuria compared to primiparous women; this is thought to be due to anatomical and physiological changes in the urinary tract that become more pronounced in subsequent pregnancies. Multigravida are also more susceptible to bacterial colonisation due to the accumulation of risk factors, such as a history of previous urinary tract infections, hygiene practices, or the use of contraceptive devices.

Werter *et al*., (2024), state that dipstick tests have a sensitivity of 80% for detecting UTIs, particularly regarding the leukocyte esterase parameter, although the highest accuracy is achieved when combined with a culture test. Parwati & Cahyani (2023) state that urine culture is *the gold standard* for determining the presence of significant bacteriuria as it can confirm the bacterial colony count and identify the causative microorganism. The culture results on MCA media showed that two types of colonies grew in sample 11, while only one colony grew in sample 26. The colonies that grew on MCA media were then subjected to biochemical tests to identify the bacterial species. The bacterial colony identification results showed *Klebsiella pneumoniae* for sample code 11, *Escherichia coli* for sample code 25, and *Escherichia coli* for sample code 26.

Table 5. Results of identification of bacterial colonies causing asymptomatic bacteriuria in the urine of pregnant women at the Antang Community Health Center using Gram staining and biochemical tests

Identification Results	Code Sample 11		Code Sample 26
	Isolate 1	Isolate 2	Isolate 1
Biochemical Tests:			
Indole	-	+	+
MR	-	+	+
VP	+	-	-
Citrate	+	-	-
Glucose	+	+	+
Sucrose	+	-	-
Lactose	+	+	+
Gram Staining	**Negative, Bacil**	**Negative, Bacil**	**Negative, Bacil**
Type of Bacteria	***Klebsiella pneumonia***	***Escherichia coli***	***Escherichia coli***

CONCLUSION

Based on the research results, the prevalence of asymptomatic bacteriuria among pregnant women at the Antang Community Health Centre in Makassar City in this study was recorded at 3.45%. The results of the identification of the causative bacteria were *Escherichia coli* and *Klebsiella*

pneumoniae. To improve the accuracy of the study, it is recommended that future researchers use a larger sample size so that the results are more representative. Furthermore, antibiotic resistance testing against the bacterial species causing asymptomatic bacteriuria should be conducted to gain a deeper understanding of potential antibiotic resistance.

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