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A comparative study of bacterial colony counts and Enterobacteriaceae isolates in pregnant women across pregnancy trimesters

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ABSTRACT. Urinary tract infections (UTIs) are common during pregnancy and may lead to serious complications for both mother and foetus. Anatomical and hormonal changes throughout gestation, particularly bladder compression and urinary stasis, create favourable conditions for bacterial colonisation and subsequent infection. *Escherichia coli* and other Enterobacteriaceae are the predominant pathogens, and urine culture remains the definitive diagnostic method, with a threshold of $\geq 10^5$ CFU/mL indicating infection. The objective of this study is to investigate the prevalence and distribution of bacterial colony counts and the presence of Enterobacteriaceae in pregnant women across all trimesters, as well as to assess the relationship between gestational age, bacterial load, and the incidence of UTIs. This observational cross-sectional study involved 46 pregnant women at various gestational stages. Midstream random urine samples were collected and subjected to microbiological culture. The number of colony-forming units per millilitre (CFU/mL) and the species of bacteria isolated were recorded and analysed descriptively. UTIs was identified in 9 out of 46 participants (19.6%), characterised by bacterial counts $\geq 10^5$ CFU/mL. The trimester-specific prevalence showed that 11% of cases occurred in the first trimester, 33% in the second trimester, and 56% in the third trimester. The most frequently isolated pathogens were *Escherichia coli* (80%), *Staphylococcus* spp. (75%), *Enterococcus* spp. (25%), and *Klebsiella pneumoniae* (20%). UTIs were more prevalent in the later stages of pregnancy, with the third trimester showing the highest incidence. *E. coli* remained the leading uropathogen. These findings underscore the importance of early screening through urine culture to prevent adverse maternal and neonatal outcomes, particularly during the third trimester.

Keywords: bacterial colony count; Enterobacteriaceae; pregnancy; urinary tract infection; urine culture

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INTRODUCTION

Urinary tract infections (UTIs) during pregnancy is a condition that can have serious consequences for both maternal and fetal health. Pregnant women are more susceptible to UTIs due to anatomical and physiological changes in the urinary tract that accompany pregnancy. Pregnancy causes pressure on the ureters, which can impair complete bladder emptying and thereby increase the risk of bacterial colonization in the bladder (Dewi *et al*., 2018; Kazma *et al*., 2020). This condition is often asymptomatic and frequently goes undetected until it progresses into a more severe infection (Fakhrizal, 2018). The prevalence of UTIs during pregnancy in several Asian countries has been reported as follows: Saudi Arabia: 5% (asymptomatic 4%, symptomatic 1%); Iran: 9.8% (asymptomatic 8.9%, symptomatic 0.6%); Indonesia: 36.5% (asymptomatic 14.8%, symptomatic 21.7%) (Azami *et al*., 2019; Barnawi *et al*., 2024). The prevalence by trimester of pregnancy is as follows: first trimester: 86.9%, second trimester: 70.5%, and third trimester: 24.3%. These figures are influenced by hormonal changes and decreased maternal immunity during the first and second trimesters, while in the third trimester, UTIs are more commonly attributed to anatomical and functional changes (Anozie *et al*., 2016; Laari *et al*., 2022).

Urine culture is the gold standard examination for diagnosing UTIs (Amy, 2016). This examination is used to determine the type of pathogenic bacteria and the number of colonies that grow on culture media, thereby assessing clinical significance. A diagnosis of UTIs is made when the bacterial colony count exceeds 10^5 CFU/mL. The most common causative agents of UTIs in pregnant

women belong to the Enterobacteriaceae family, including *E. coli*, *K. pneumoniae*, and *Enterobacter* sp. (Centers for Disease Control and Prevention (CDC), 2022).

Previous urine culture studies have shown that Gram-negative bacteria are the primary pathogens responsible for UTIs, accounting for approximately 72% of all cases, whereas Gram-positive bacteria are found in about 28% of cases. Among the Gram-negative bacteria, *E. coli* is the most frequently isolated species (51%), followed by *K. pneumoniae* (20%), and other Gram-negative bacteria such as *Proteus* sp and *Acinetobacter* sp, each comprising around 5% of isolates (Grette *et al*., 2020; Rosana *et al*., 2020). Nevertheless, UTIs in pregnant women may also be caused by fungi, particularly *Candida* spp., which are commonly found during the first trimester, with a reported prevalence of up to 27.8%, and are strongly associated with poor genital hygiene (Laari *et al*., 2022; Messina *et al*., 2024). The risk of UTIs during pregnancy may result in serious complications, including an increased incidence of preterm labor (23%), low birth weight (7%), and intrauterine fetal death (6%). Without appropriate and adequate treatment, UTIs can progress to more severe conditions, such as acute pyelonephritis, sepsis, anemia, acute respiratory distress syndrome (ARDS), and even acute renal failure in the mother (Dautt-Leyva *et al*., 2018; Grette *et al*., 2020; UNICEF, 2022).

Most studies on UTIs in pregnant women have been conducted in healthcare facilities, where study participants are typically symptomatic (Dewi *et al*., 2018). This study was conducted to assess the occurrence of UTIs among pregnant women across all trimesters of pregnancy. The identification of asymptomatic UTI cases in this study is expected to serve as a basis for community-level screening efforts and to identify the pathogenic bacteria responsible for the infections.

MATERIALS AND METHODS

Study design and setting. This was an observational analytic study with a cross-sectional design conducted in Pongangan Subdistrict, Gunungpati District, Semarang City, Indonesia. The study aimed to assess the relationship between gestational age and the presence of UTIs as determined by bacterial colony counts and the identification of Enterobacteriaceae isolates in pregnant women.

Participants. A total of 46 pregnant women were recruited as study participants. Inclusion criteria included gestational age of more than 8 weeks and absence of any obstetric or systemic complications during pregnancy. Exclusion criteria were current antibiotic treatment or a known diagnosis of urinary tract anomalies. Participants were selected using a purposive sampling technique from antenatal care attendees in the community.

Ethical considerations. All participants provided written informed consent prior to enrolment. This study received Ethical Clearance approval from the Health Research Ethics Committee of RSI Sultan Agung with reference number 152/KEPK-RSISA/VII/2024.

Urine sample collection and laboratory procedures. Midstream clean-catch urine samples were collected at random times from all participants using sterile urine containers. Samples were transported immediately to the microbiology laboratory for analysis. Each specimen was inoculated on MacConkey agar using a calibrated inoculating loop (0.001 mL) and incubated aerobically at 37°C for 18–24 hours. Colony-forming units were quantified, and a bacterial count $\geq 10^5$ CFU/mL was considered indicative of UTIs.

Data analysis. Data were analysed descriptively and statistically. The primary variables included gestational age (trimester), bacterial colony counts, and the type of bacterial isolates identified. The *Chi-square* test was used to assess the association between gestational age and UTIs prevalence. Statistical significance was set at $p < 0.05$. All analyses were conducted using SPSS.

RESULTS AND DISCUSSION

A total of 46 pregnant women were enrolled as research participants. Based on gestational age, the distribution included 5 individuals in the first trimester, 20 in the second trimester, and 21 in the third trimester. Urine culture is the gold standard diagnostic method for detecting UTIs. In this study, out of 46 cultured samples, 45 showed bacterial colony growth, while 1 sample showed no growth

(sterile). Therefore, only 45 samples were eligible for further analysis to determine the bacterial colony count and identify the types of microorganisms present. Through urine culture examination, bacterial growth and colony count can be quantitatively assessed, as shown in Figure 1. A sample is confirmed as a UTIs if the colony count exceeds the clinical threshold of $\geq 10^5$ CFU/mL, followed by bacterial identification.

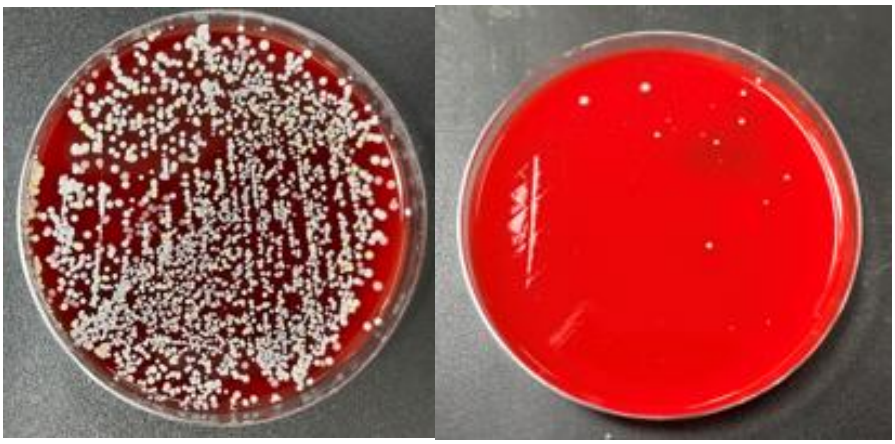

Fig. 1. Bacterial colony count (CFU) in urine culture. (Left) HK >100.000 cfu/ml; (Right) HK <100.000 cfu/ml

The prevalence of urinary tract infections (UTIs) caused by bacteria from the Enterobacteriaceae group was found in 5 samples. Based on Table 1, it was observed that in the first trimester of pregnancy, 2.2% of pregnant women experienced UTIs caused by Gram-negative bacteria. In the second trimester, 6.7% of pregnant women were diagnosed with UTIs, with Gram-negative bacteria identified in 1 out of 3 samples, while the remaining cases were caused by Gram-positive bacteria. In the third trimester, 11% of pregnant women experienced UTIs, with the causative agents distributed as 60% Gram-negative and 40% Gram-positive bacteria.

Table 1. Gestational age categories based on trimesters with urine culture results

∑= 45	Bacterial colony count (cfu/ml)				Type of organism					
	<100.000		>100.000		Gram positive		Gram negative		Fungi	
	n	%	n	%	n	%	n	%	n	%
Trimester 1	4	8,9	1	2,2	3	6,7	2	4,5	0	0
Trimester 2	16	35,6	3	6,7	9	20	10	22,2	1	2,2
Trimester 3	16	35,6	5	11	11	24,4	9	20	0	0

A total of 27 samples (58.7%) showed growth of Enterobacteriaceae, as illustrated in Figure 2. The distribution of Enterobacteriaceae identified as the causative agents of UTIs included *E. coli* (44%) and *K. pneumoniae* (11%). The prevalence of Enterobacteriaceae as UTI pathogens was 100% in the first trimester, 33% in the second trimester, and 60% in the third trimester. Meanwhile, the remaining UTIs were caused by Gram-positive bacteria; however, the specific types of these bacteria were not further identified in this study.

Data analysis using the Chi-square test showed that gestational age ($p = 0.005$), bacterial count ($p = 0.000$), and Enterobacteriaceae presence ($p = 0.238$) were evaluated. Statistically, gestational age and bacterial count demonstrated a significant association ($p < 0.05$) with the incidence of urinary tract infection (UTIs).

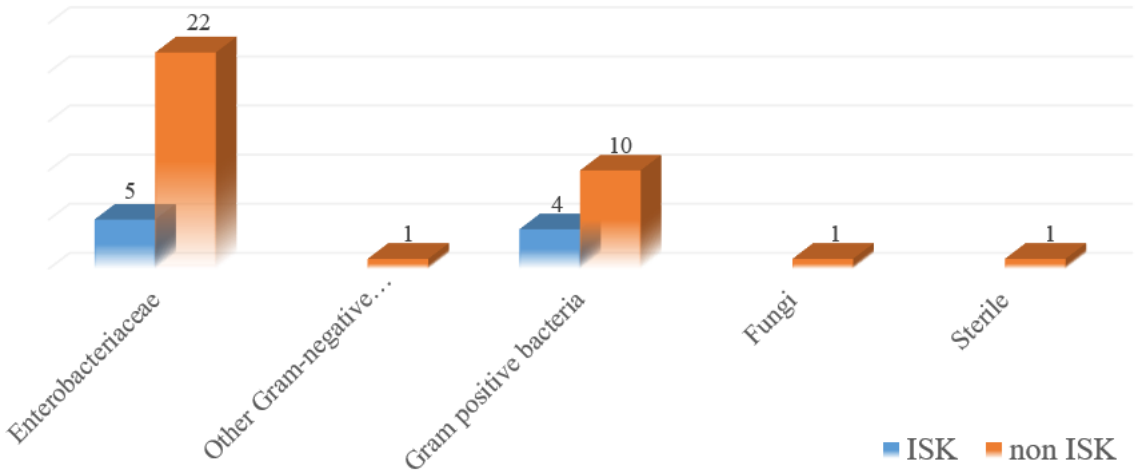

Fig. 2. Bacterial growth from urine culture

The highest prevalence of UTIs occurs during the third trimester, which aligns with findings from a study in Nigeria reporting a significant increase in UTIs incidence among pregnant women in their third trimester. Late-stage pregnancy increases the risk of UTI by up to five times, possibly due to the progressive pressure from the enlarging uterus and the physiological effects of pregnancy hormones on the urinary tract (Ali & Abdallah 2019). Risk factors for UTIs include gestational age, maternal age, frequently holding in urine, improper genital wiping technique, and the water source used for cleaning the genital area. These factors can elevate the risk of UTI, especially in pregnant women (Anozie *et al*., 2016; Hidayah & Siti Fatimah, 2023; Safitri *et al*., 2025).

Proper wiping of the genital area from front (genitals) to back (anus) is a recommended hygienic practice to prevent bacterial contamination from the perianal area to the urinary tract. This is in accordance with previous research findings indicating that improper wiping techniques can increase the risk of UTIs by up to nine times (Sharma *et al*., 2015). In addition, handwashing after urination is essential to prevent the transfer of pathogenic microorganisms that may come into contact with the anal area (Badran *et al*., 2015; Permenkes, 2017a). The quality of water used for personal hygiene also plays a role in UTIs risk. Several studies have shown that well water has a higher rate of *E. coli* contamination compared to clean water from municipal distribution systems, thus increasing the potential exposure to infection-causing bacteria (Cantrell *et al*., 2023; Fatimah *et al*., 2024).

Isolates from the research samples identified as the causative agents of UTIs exhibited round, pink-colored colonies (lactose fermenters), which are characteristic of *E. coli*, accounting for 44% of the cases. *E. coli* was the most frequently identified species responsible for UTIs, followed by *K. pneumoniae* and other bacteria. *K. pneumoniae* displayed mucoid, pink-colored colonies, indicating its lactose-fermenting properties (Amy, 2016). However, in pregnancy-related UTIs cases, *Staphylococcus saprophyticus* is a leading cause of community-acquired UTIs, accounting for about 10–20% of cases, and often leads to recurrent infections despite being treatable (Lawal *et al*., 2021). Although *S. saprophyticus* tends to cause fewer complications compared to *E. coli*, its presence remains clinically relevant and should be considered in the management of UTIs during pregnancy (Mochtar & Noegroho, 2015).

In this study, *E. coli* was responsible for 100% of UTIs cases during the first trimester, a high figure also observed in previous research at around 71.4% (Mahor *et al*., 2021). *E. coli* is a Gram-negative commensal bacterium in the gastrointestinal tract. UTIs may occur due to the spread of bacteria from the anogenital region, often triggered by poor hygiene and improper wiping from the anus toward the genitals. Ultimately, infection occurs due to the bacteria's own virulence; *E. coli* has P fimbriae and S fimbriae, which function as adhesins on epithelial cells of the urinary tract (Biggel *et al*., 2020; Lila *et al*., 2023).

UTIs in the third trimester can lead to complications such as asymptomatic pyelonephritis, which may result in maternal morbidity and increase the risk of miscarriage by up to 72% in the third trimester (Ali & Abdallah, 2019; Grette *et al*., 2020). Therefore, empirical antibiotic therapy is recommended for pregnant women with this condition to prevent complications. Commonly used antibiotics include nitrofurantoin, cephalexin, amoxicillin-clavulanate, and fosfomycin. In contrast, antibiotics such as tetracyclines, fluoroquinolones, and trimethoprim-sulfamethoxazole should be avoided due to risks to pregnancy. Management generally lasts for 7 days, followed by clinical evaluation. If left untreated, asymptomatic bacteriuria can progress to pyelonephritis or other complications in 20–40% of cases, potentially endangering maternal and fetal health. Therefore, routine urine screening during pregnancy is crucial for the early detection of UTIs (Nicolle *et al*., 2019; Permenkes, 2017b; ACOG, 2023)

A key limitation of this study is the use of spot urine samples, which may be susceptible to contamination and variation in bacterial load. Future studies should consider collecting early morning midstream urine samples to improve diagnostic accuracy. Additionally, bacterial identification in this study was limited to culture-based phenotypic methods; advanced biochemical or molecular diagnostic tools could provide more accurate characterisation.

CONCLUSION

This study identified a 20% prevalence of urinary tract infections (UTIs) among pregnant women, with the highest incidence occurring during the third trimester (11%). A total of 58.7% of the bacterial isolates obtained belonged to the Enterobacteriaceae group. This group was identified as the causative agent in 100% of first-trimester cases, 33% of second-trimester cases, and 60% of third-trimester cases. Statistical analysis revealed significant associations between gestational age ($p = 0.005$), bacterial colony count ($p = 0.000$), and the occurrence of UTI. The findings highlight the need to raise awareness among pregnant women regarding the risks associated with UTIs. It is recommended that urine culture testing be incorporated into routine antenatal care, particularly during the second and third trimesters, as an effective strategy for early detection and prevention of complications. Future policies should support community-level education and screening initiatives to reduce the burden of asymptomatic bacteriuria during pregnancy.

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