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ISOLATION OF *ESCHERICHIA COLI* FROM PREGNANT WOMEN'S URINE AT BINANGA HEALTH CENTER MAMUJU

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Abstract

Background: Urinary tract infections (UTIs) and asymptomatic bacteriuria during pregnancy can have detrimental effects on both maternal and fetal health. These conditions are associated with complications such as acute maternal illness, low birth weight, preeclampsia, preterm delivery, and intrauterine growth restriction. The causative agents of UTIs are *Escherichia coli, Klebsiella sp, and Staphylococcus sp.*

Objective: to isolate and identify *E. coli* in the urine samples of pregnant women

Methods: This study employed a descriptive observational design. The research sample included pregnant women attending the Binanga Community Health Center in Mamuju. Urine was cultured in BHIB and incubated at 37°C for 24 hours. Subsequent bacterial isolation was performed by subculturing onto EMBA media and MacConkey agar. Colonies that developed were then analyzed using Gram staining and examined microscopically.

Results: Of 25 sample urine samples from pregnant women, 25 were culture-positive on MacConkey Media and EMBA Media. 14 (56%) isolate colored bacteria metallic green on EMBA media that EMBA media is specific growth for *E. coli* bacteria

Conclusions: 56% of the bacterial isolates from the urine samples of pregnant women were indicated as *Escherichia coli,* based on the presence of metallic green colonies observed on EMBA media.

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INTRODUCTION

During pregnancy, women experience a natural decline in immune function, increasing their vulnerability to bacterial infections. Bacteria in the urine during this period may indicate asymptomatic bacteriuria, an upper urinary tract infection (UTI), or a lower UTI (1). Asymptomatic bacteriuria is the presence of bacteria in the urine without specific clinical symptoms associated with an acute urinary tract infection (3). UTIs are caused by various pathogens, including Gram-positive and Gram-negative bacteria and fungi. About 10% of pregnant women experience urinary tract infections (2), and about 2% to 15% of asymptomatic bacteriuria occurs in all pregnancies (3).

Recurrent urinary tract infections during pregnancy can result in severe complications, adversely affecting the health of both the mother and the fetus (4) If not promptly diagnosed and adequately managed, these conditions may result in complications such as acute pyelonephritis and low birth weight in infants (11), premature birth, preeclampsia, intrauterine growth retardation (7), premature rupture of membranes (7) (13), congenital disabilities and the possibility of miscarriage (14), premature delivery.

Pathogenic bacteria in the urinary tract are generally caused by *Klebsiella sp* (6), *Staphylococcus aureus, Escherichia coli* (11), and *Staphylococcus sp* (4). *Escherichia coli* is the most common causative agent in symptomatic and asymptomatic bacteriuria (7). Rare bacteria result in Infection channel urine is *Enterobacter sp, Pseudomonas aeruginosa* and *Proteus mirabilis*.

Screening for bacterial infections in pregnant women is essential for the early detection of bacteriuria during the initial stages of pregnancy (1). A study on pregnant women reported that asymptomatic bacteriuria was identified in 10.5% of the 715 pregnant women (13) participants among pregnant women who attended eight Community Health Centers in Jakarta, Indonesia, between 2015 and 2017. *Escherichia coli* was identified as the predominant causative agent, accounting for 26.7% of cases, followed by *Klebsiella pneumoniae* at 20% from 10.5% of bacteria causing asymptomatic infections in pregnant women. The presence of these bacteria is feared to affect pregnant women and fetuses.

Urinary tract infections may originate from fecal bacteria and are influenced by hygiene practices and sexual behavior (14). Therefore, routine UTIs screening is crucial for pregnant women to ensure early detection and prevention of complications (17).

Based on the description above, the researcher hypothesizes whether the urine of pregnant women contains pathogenic bacteria. This study aims to isolate and find pathogenic bacteria of *E. coli* in the urine samples of pregnant women.

MATERIALS AND METHODS

Research Design

This study employed a descriptive observational design and was conducted in 2023. Urine samples were collected within the Binanga Community Health Center (PKM) service area in Mamuju Regency. The samples were analyzed at the Microbiology Laboratory of the Mamuju Health Polytechnic. The study sample included pregnant women who agreed to take their urine specimens. Socio-demographic and socio-economic data were taken after

obtaining written consent from each participant. This research has received ethical approval from The Ethics Committee of the Institute of Technology Science and Health Insan Cendikia Medika Jombang Number 084/KEPK/ITSKES-ICME/VII/2023

Research Equipment and Materials

The equipment used in this study is a Stirring Rod, 300 ml Erlenmeyer (Iwaki), 1 L Erlenmeyer (Iwaki), 100 ml Measuring Cup (Iwaki), Spray Bottle, 500 ml Cup (Iwaki Petri disk, Ose, Urine Pot, Sample Box/Container, Reaction Tube (Iwaki), Tube Rack, Electric Heater, Incubator, Oven, Refrigerator, Laminar air flow, Autoclave, Ocular Microscope, Analytical Scale (Sartorius).

The materials used are 70% alcohol, Aquadest, Cotton, Aluminum foil, MacConkey media (Merck), EMBA media (HIMEDIA), BHIB media (HIMEDIA), Gram staining, Inertia oil, 1 ml syringe.

Urine sample collection

The pregnant woman's urine sample is placed in a sterile, wide-mouthed container with a screw-on lid. Before taking the urine sample, the pregnant woman is informed about the correct way to collect urine, such as how to collect mid-stream urine. The urine sample is placed in a refrigerated container/box and brought to the laboratory.

Sample Examination

Urine samples were inoculated into Brain Heart Infusion Broth (BHIB) media for enrichment with comparison 1:1) and then homogenized. Incubated at 37 o C for 24 hours. The BHIB medium was used for 24 hours to isolate bacteria with a colony using a sterile ose round. Sterile ose rounds were scratched on MacConkey agar plate media. This isolation was repeated using EMBA plate media. Both types of media were incubated for 24 hours at 37 o C. After incubation, the shape and color of colony bacteria and the color of MacConkey and EMBA agar plate media were. Growing colonies in MacConkey and EMBA agar plate media were carried out with Gram stain. The preparation that has been colored with Gram staining, observed below the microscope, uses 1000x magnification (using oil inertia).

RESULTS AND DISCUSSIONS

Characteristics of Pregnant Women

Samples in the form of urine taken from the pregnant mother. The characteristics of a Mother's pregnancy are shown in Table 1.

Table 1. Characteristics of Pregnant Women

Description		Frequency (n=25)	Percentage (%)
Body Weight (Kg)	<40	3	12.0
	40-50	2	8.0
	51-60	7	28.0
	>61	13	52.0
Age Pregnancy (Week)	<12	4	16.0
	12-24	3	12.0
	24-36	14	56.0
	>36	4	16.0
Marriage to -	1	22	88.0
	2	3	12.0
Gravida	1	7	28.0
	2-3	13	52.0
	4-5	4	16.0
	>5	1	4.0

Urine samples were obtained from various ages of pregnancies and multiple amounts of pregnancies (gravida). More than half of the Mother's pregnant mothers are overweight 60 Kg and have jobs dominated by the housewife ladder.

Table 2. Colony characteristics and color on Mac Conkey agar plate media

Colony color on MacConkey medium				MacConkey media color changes				Microscopic examination results (Gram Staining)
Pink	White	Purple	Total	Yellowish	Yellow	Do not change	Total	Stem color red
19	5	1	25	8	7	10	25	25

Table 3. Colony Color on EMBA Medium

	Frequency	Percent
Chocolate	1	4.0
Chocolate white	3	12.0
Chocolate purple	2	8.0
Metallic green	14	56.0
Light pink	5	20.0
Total	25	100.0

Based on Table 3, there are isolated colored bacteria that are metallic green. Bacteria that produce color gold on the EMBA media are specific for growth colony bacteria type *E. coli.* The colony color on EMBA media and MacConkey agar plates is seen in Figure 1.

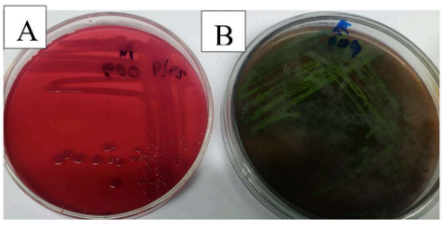

Figure 1. Colony Gram bacteria negative urine Mother pregnant on MacConkey Agar (A) and EMBA (B) media, a metallic green color on media EMBA provides specific characteristics existence growth bacteria *E. Coli*

Pregnancy induces a range of physiological and hormonal changes, particularly evident during the first and second trimesters. The enlargement of the ureter continues until it happens during labor—progesterone and estrogen hormones increase, which can cause a decrease in ureter and bladder tone. The increase in plasma volume during pregnancy leads to a reduction in urine concentration and an expansion of bladder capacity. These changes make it easier for bacteria to travel to the urethra and kidneys, developing bacteriuria (4).

This study's mid-stream urine samples collected from pregnant women were inoculated into BHIB enrichment broth and then cultured on various specific media, including agar plates. Mac Conkey and EMBA media. Mac Conkey Agar Media is a specific medium for growing Gram-negative bacteria, while EMBA Media is specific for growing *E. coli* (gram-negative bacteria) (16). Colonies on EMBA media appear to be a specific metallic green color. The bacteria that give the metallic green colony color indicate *Escherichia coli* bacteria. However, to ensure that it is necessary to test biochemical characteristics, Bacteria present in the urine during pregnancy are often classified as asymptomatic bacteriuria, which occurs in approximately 2% to 15% of cases(17).

Asymptomatic bacteriuria occurs in most pregnancies, and urinary tract infections are predominantly caused by pathogenic bacteria such as *Staphylococcus aureus* (11) and *Staphylococcus sp* (12). *Klebsiella sp*. (6). *Escherichia coli* species are commonly responsible for both symptomatic and asymptomatic bacteriuria (2)(22). Asymptomatic bacteriuria is the presence of bacteria in the urine without the typical symptoms of an acute urinary tract infection(3). Bacterial screening in pregnant women needs to be done to detect the presence of bacteriuria in early pregnancy. Bacteria that infrequently cause urinary tract infections include *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Enterobacter* species. In contrast, Gram-positive microorganisms cause UTIs, such as *Staphylococcus saprophyticus, Streptococcus, Staphylococcus haemolyticus*, and *Staphylococcus aureus*(4).

The discovery of *E. coli* bacteria in the urine of pregnant women is feared to cause urinary tract infections. Uropathogenic *E. coli* (UPEC) is the primary pathogen responsible for urinary tract infections, typically causing uncomplicated cases (23). Uropathogenic *Escherichia coli* (UPEC) initially colonizes the human host before adhering to the bladder epithelium. Following adhesion, the bacteria invade urothelial epithelial cells, replicating and forming dense intracellular communities resembling biofilms (26).

Based on research from Ashriady (20) found that Gram-negative bacteria were detected in the urine samples of pregnant women and research by Rosana (13) that study conducted on pregnant women found that asymptomatic bacteriuria is present among this population, with *Escherichia coli* identified as the primary causative agent followed by *Streptococcus agalactiae, Klebsiella pneumoniae, Enterobacter cloacae, Staphylococcus saprophyticus, Enterococcus faecalis, Acinetobacter baumannii* (21).

The urine of pregnant women that is indicated to contain pathogenic bacteria *E. Coli* can cause infection. This infection can be caused by vesicourethral reflux, urinary tract obstruction, use of new urethral instruments, and septicemia (22). In a survey by Mastuti Widianingsih in 2016, 12 (40%) of pregnant women's urine from 30 pregnant women's urine samples examined at the Bhayangkara Hospital were identified as positive for *E. coli*. Positive E. coli identification is characterized by the appearance of small, round colonies with a semi-mucoid texture and positive lactose fermentation, evidenced by a color change on MacConkey agar (23)

Urinary tract infections may originate from fecal bacteria and are influenced by hygiene practices and sexual behavior (14). Therefore, routine UTIs screening is crucial for pregnant women to ensure early detection and prevention of complications (17).

Urinary tract infections (UTIs) can contribute to various complications, including low birth weight, premature rupture of membranes, preterm delivery, and intrauterine growth restriction (15). Urinary tract infections (UTIs) and How to avoid them (4). If not promptly diagnosed and adequately treated, these conditions may lead to serious complications such as acute illness, pyelonephritis, low birth weight in newborns (11), preeclampsia, premature birth, obstruction intrauterine growth (7), amniotic fluid broken early (13), congenital disabilities and possible miscarriage (14). Urinary tract infections can also lead to serious maternal complications such as postpartum endometritis, sepsis, and, in severe cases, maternal shock (25)

Socioeconomic factors, a history of abortion, and abnormal vaginal discharge can cause urinary tract infection (UTI) among pregnant women. In addition to the average level of knowledge, health education is critical to increase awareness and encourage timely intervention. To overcome UTI in pregnancy, a public health strategy is needed. This strategy is efficacious in improving maternal health outcomes and quality of care. Public health care impacts the quality of life and causes work productivity problems (26).

The presence of UTI in pregnant women causes persistent infection, which can eventually lead to urinary tract obstruction or kidney abscess. However, the presence of pathogenic bacteria, especially *E. coli*, in pregnant women's urine can potentially cause transmission to

neonates and infants. Infection by *E. coli* will contribute to the emergence of several infectious diseases, such as diarrhea and sepsis in neonates and infants.

CLINICAL IMPLICATION

E. coli present in the urine of pregnant women has significant potential to cause urinary tract infections (UTIs). A UTI is typically diagnosed when bacterial counts reach $\geq 10^6$ colony-forming units per milliliter of urine. If left untreated, such infections can become chronic and progress to more severe complications, including urinary tract obstruction or renal abscess formation. Furthermore, the presence of pathogenic bacteria, particularly *E. coli,* in the urine of pregnant women poses a risk of vertical transmission to neonates and infants, contributing to conditions such as neonatal sepsis and pediatric diarrhea.

Pregnant women with UTIs may also serve as bacterial dissemination reservoirs within the broader community. If delivery occurs in a healthcare facility, there is an added risk of nosocomial spread, potentially increasing hospitalization duration and associated healthcare costs.

Therefore, implementing rapid urine screening and quantitative urine cultures during pregnancy is essential, as these represent the diagnostic gold standard. Early identification and management of bacteriuria, especially asymptomatic bacteriuria, are critical to preventing complications. Moreover, given the growing concern of antimicrobial resistance, heightened vigilance is necessary to mitigate the risk of resistant bacterial strains propagating within community and healthcare settings.

LIMITATIONS

This study offers valuable insights, particularly regarding the detection of bacteria in the urine of pregnant women. This focus is relevant given the persistently high incidence of diarrhea in children and neonatal sepsis. However, the authors acknowledge certain limitations, particularly in identifying *E. coli* strains. The study relied solely on selective media for *E. coli* growth without performing confirmatory biochemical tests to characterize the bacterial isolates. Additionally, bacterial counts per milliliter of urine were not conducted, which limits the ability to confirm bacteriuria in the pregnant participants. These limitations were primarily due to constraints in research funding and laboratory resources, which affected the ability to perform comprehensive biochemical testing and quantitative analysis of bacterial loads.

CONCLUSIONS

56% of bacteria indicated as *Escherichia coli* were identified in the urine of pregnant women by the characteristic metallic green sheen of its colonies on Eosin Methylene Blue Agar (EMBA) media.

CONFLICT OF INTEREST

The authors affirm that this research was carried out without commercial or financial affiliations that could be interpreted as a potential conflict of interest.

AUTOR CONTRIBUTIONS

AM and RDH designed the study, performed the experiments, and analyzed the data. AM, RDH wrote and edited the manuscript. AA contributed to the discussion on the interpretation of the results. All authors read and approved the final manuscript.

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