

Isolation and Evaluation of UTI Causing Bacteria Among Pregnant Women Attending Prenatal Clinic

Jitu Kr. Mahanta¹, Manashi Garg²

^{1,2}Dept. of Medical Laboratory Technology & GEA National College (under Srimanta Sankaradeva University of Health Sciences), Assam

ABSTRACT

BACKGROUND: Urinary tract infections (UTIs) are one of the most common bacterial infections affecting pregnant women globally. Pregnancy increases the risk of UTIs due to physiological and anatomical changes in the urinary tract. If left untreated, UTIs can lead to serious maternal and fetal complications including preterm labor, low birth weight, and kidney infections. The rise in antimicrobial resistance further complicates the treatment of UTIs in pregnant women. This study aims to evaluate the prevalence of UTIs and to isolate and identify the causative bacterial agents among pregnant women attending prenatal clinics. This study seeks to highlight the importance of early diagnosis and targeted treatment to reduce maternal morbidity and improve pregnancy outcomes.

MATERIALS AND METHOD: A total of 200 pregnant women attending the prenatal clinic were included in the study. Participants were selected based on the presence of symptoms suggestive of urinary tract infections, such as burning urination, increased frequency, and lower abdominal discomfort. Midstream urine samples (MSU) were collected under sterile conditions. The samples were tested for bacterial growth using standard microbiological techniques. Laboratory investigations were carried out to identify the causative organisms.

RESULT: The study of 200 pregnant women showed that all urine samples demonstrated bacterial growth, confirming a high prevalence of urinary tract infections. *Escherichia coli* was the most common isolate, followed by *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Staphylococcus saprophyticus*. Gram-negative bacteria dominated the results, accounting for 52% of all isolates. Colony morphology and biochemical tests confirmed the identification of each organism, including swarming *Proteus* and mucoid *Klebsiella*. Mixed infections were observed in 10–15% of samples, indicating polymicrobial involvement. Overall, the findings highlight *E. coli* as the leading uropathogen and stress the importance of routine urine culture during pregnancy. These results support early diagnosis and targeted treatment to prevent maternal and fetal complications.

CONCLUSION: The results of the study clearly show that urinary tract infections are widely present among pregnant women attending the clinic. The most common bacteria identified was *Escherichia coli*, along with other organisms such as *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Staphylococcus saprophyticus*. Gram-negative bacteria were found more frequently than Gram-positive ones. These findings indicate that UTIs are a significant health concern during pregnancy and highlight the importance of routine urine culture, early detection, and proper antibiotic treatment to prevent complications for both mother and baby.

Keywords: Urinary Tract Infection, Prenatal Clinic, Gram negative bacteria, uropathogen, pregnancy

1. INTRODUCTION:

Urinary tract infections (UTIs) are a prevalent health concern among pregnant women, representing one of the most common bacterial infections during pregnancy [1]. Pregnancy induces physiological changes such as ureteral dilation, urinary stasis and hormonal influences that favor bacterial colonization and ascent of pathogens. These alterations significantly increase the risk of developing UTIs which may range from asymptomatic bacteriuria to acute pyelonephritis. Physiological and anatomical changes in the urinary tract during gestation increase susceptibility to UTIs, which, if not promptly diagnosed and treated, may result in serious maternal and fetal complications including preterm labor, low birth weight, and pyelonephritis [8]. If not diagnosed and managed promptly, these infections can contribute to adverse maternal and neonatal outcomes including premature delivery, intrauterine growth restriction, hypertensive disorders of pregnancy, and even sepsis [9]. The identification of uropathogens and their antimicrobial resistance profiles is critical in guiding effective treatment, particularly in an era where resistance to conventional antibiotics is on the rise. Empirical therapy, while convenient, may lead to inappropriate treatment if not supported by microbiological evidence, potentially worsening resistance patterns and delaying recovery. The prevalence of UTIs among pregnant women varies significantly across geographic regions and populations depending on factors such as hygiene practices, socioeconomic status, access to healthcare and awareness of UTI symptoms [3]. In many developing countries, routine urine screening is often overlooked due to resource limitations, leading to underdiagnoses and mismanagement. This situation poses a major risk, as untreated infections may ascend to the kidneys, resulting in acute pyelonephritis—a serious condition associated with increased maternal morbidity and even fetal loss^[10]. The burden of antimicrobial resistance (AMR) adds another layer of complexity.

The current study aims to evaluate the prevalence and identify the bacterial pathogens responsible for UTIs among pregnant women attending a prenatal clinic. A descriptive cross-sectional study was conducted in which clean-catch midstream urine samples were collected from pregnant women for the microbiological examination along with standard culture methods and biochemical identification procedures to isolate and characterize the uropathogens. Further, by isolating and identifying the causative organisms and determining their antimicrobial susceptibility profiles, the study aims to offer localized data that can be used to improve clinical management.

2. METHODOLOGY:

This study utilized as a cross-sectional survey conducted in Tolaram Bafna Hospital, Guwahati, Assam to elicit the pregnant women attending in prenatal clinic. A total of 200 pregnant women between 20–38 years of age participated in the study. The mean age of participants was 28.6 ± 5.5 years with the majority belonging to the 25–29-years age group. Participants were well distributed across trimesters: 35.5% in first, 31% in second, and 33.5% in third trimester. A structured consent forms were used while collecting the samples from all the participants’.

Inclusion criteria of the study:

- Patient of the age group 20-38 years.
- Pregnant women of any trimester attending the prenatal clinic.
- Women who present with symptoms suggestive of urinary tract infection (e.g., burning urination, frequent urination, lower abdominal pain).

- Women willing to provide a urine sample and give informed consent.

Exclusion criteria of the study:

- Patient of less than 20 years and more than 38 years.
- Pregnant women currently on antibiotic treatment.
- Women diagnosed with chronic renal diseases or other major urological disorders.
- Women unwilling or unable to provide a urine sample.

2.1 Sample Collection

The patients were instructed to wash their hands and clean the external genital area with sterile water or antiseptic wipe before urination and collect the midstream portion of the urine into the sterile wide-mouthed screw-cap container to avoid contamination. The samples were immediately transported to the microbiology lab within 1–2 hours of collection and stored at 4–8° C but not later than 48 hours.

2.2 Processing of sample prior to study

Samples were let to stand for some times before performing culture for obtaining the room temperature. All the necessary items required for further processing were assembled and gathered together.

2.3 Preparation of culture media for the microbial growth

Selective growth media *viz*; MacConkey's agar, Blood agar and CLED agar were prepared to enable the targeted isolation and cultivation of specific microorganisms while suppressing the growth of other competing flora. The media components were first dissolved in distilled water according to the established laboratory formulations and the pH was adjusted to the required range (typically 7.0 ± 0.2) using sterile 1N HCl or 1N NaOH. The medium was then sterilized by autoclaving at 121°C for 15 minutes. Following sterilization, selective antibiotics were aseptically incorporated into the cooled (45–50°C) medium under laminar airflow conditions. The selective agents were chosen based on the physiological and biochemical characteristics of the target organism to inhibit unwanted microbial populations. The prepared medium was thoroughly mixed and dispensed into sterile Petri plates or culture tubes, and allowed to solidify under aseptic conditions. All batches were subjected to sterility and performance testing using reference strains to ensure selectivity, sensitivity, and reproducibility prior to experimental use.

Following incubation on appropriate solid media under standardized conditions (24–48 h at 35–37 °C), individual colonies were morphological characteristics of the growing bacteria that included colony size (diameter in millimeters), shape (circular, irregular, filamentous), margin (entire, undulate, lobate, curled), elevation (flat, raised, convex, umbonate), surface texture (smooth, rough, wrinkled), opacity (transparent, translucent, opaque), pigmentation, and hemolytic activity were examined visually.

2.4 Preparation of the Biochemical tests

Biochemical tests were performed for the characterization of growing microorganisms following standardized protocols to ensure the reliability of results in research settings. The pure cultures of the bacteria were obtained under optimal growth conditions to ensure metabolic activity. Biochemical assays *viz*; catalase, coagulase, oxidase, Indole, Methyl red and citrate utilization test were designed to evaluate specific metabolic capabilities such as carbohydrate fermentation, enzyme production, substrate utilization or reduction reactions of the growing bacteria. Both positive and negative controls are included to validate test performance. Inoculation is performed aseptically using standardized inoculum densities to maintain consistency across replicates. Incubation conditions, including temperature, atmospheric requirements, and duration, are carefully controlled based on the physiological requirements of the microorganism under study. Following incubation, results were interpreted through observable indicators such as color changes, precipitate formation, gas production, or turbidity, often aided by specific reagents.

3. RESULTS:

3.1 Socio-demographic Characteristics of Study Participants.

Participants were evenly distributed across trimesters—35.5% in the first, 31.0% in the second, and 33.5% in the third trimester—indicating good representation across gestational stages. A total of 200 pregnant women participated in the study. The largest proportion of participants belonged to the 25–29 years age group (29.0%) followed closely by the 20–24 years group (28.5%). Overall, 57.5% of participants were below 30 years of age. The sample was evenly distributed across trimesters providing a representative antenatal population for studying urinary tract infections.

3.2 Distribution of Bacteria Isolated on different selective media

Table 1 depicted the comparative distribution of bacteria growth in all the culture media. Out of 200 urine samples cultured on Blood, Mac Conkey and CLED agar, *Escherichia coli* and *Enterococcus faecalis* showed consistently predominant growth pattern followed by *Proteus mirabilis* with a constant growth percentage of 17%. However, *Staphylococcus saprophyticus* and *Klebsiella pneumonia* also showed a significant growth in all the culture media.

The percentage of bacterial growth is calculated as follows:

% of bacterial growth: Frequency of growth/Total no of participants

Table 1: Comparative Distribution of Bacteria growth on Blood, MacConkey, and CLED Agar .

Organism	Blood (%)	MacConkey (%)	CLED (%)	Mean (%)
<i>Escherichia coli</i>	18.0	17.0	20.0	18.3
<i>Enterococcus faecalis</i>	17.5	20.0	18.5	18.7
<i>Klebsiella pneumoniae</i>	16.5	18.0	16.0	16.8
<i>Proteus mirabilis</i>	17.0	17.0	17.0	17.0
<i>Staphylococcus saprophyticus</i>	17.5	16.5	14.0	16.0

3.3 Colony Morphology

The typical colony patterns observed on blood agar provides important preliminary diagnostic clues based on size, shape, texture, pigmentation, and hemolytic patterns. Colonies typically appears moist, circular, and large with entire margins for *E.coli*. However, the colonies for *Klebsiella pneumoniae* ranges from glossy or mucoid consistency—often associated for thick capsule with convex elevation followed by *Proteus mirabilis* showing swarming pattern as well as *Staphylococcus saprophyticus* with white to cream-colored colony appearance.

On MacConkey agar a typical colony patterns help distinguish lactose-fermenting from non-lactose-fermenting Gram-negative bacteria produce the pink to red colonies due to lactose fermentation and acid production that lowers the pH and causes the neutral red indicator to turn pink indicated the growth of *Escherichia coli* and *Klebsiella pneumonia*. In contrast, non-lactose-fermenting organism that typically showed the colony form as colorless or pale and translucent pointed. However, typical colony morphology on CLED agar varies according to lactose fermentation status. The yellow colonies as a result of acid formation and subsequent color change of the bromothymol blue indicator with smooth, round, and medium-sized, morphology suggested the growth of lactose-fermenting organisms such as *Escherichia coli* along with *Klebsiella* species that characteristically appeared mucoid due to capsule production. In contrast, non-lactose-fermenting organisms such as *Proteus mirabilis* and *Pseudomonas aeruginosa* form

blue to green colonies, reflecting alkaline or non-acidic metabolic byproducts. *Staphylococcus saprophyticus* typically produced smooth, round, convex colonies that are opaque to slightly translucent with a yellow coloration due to lactose fermentation.

3.4 Biochemical test

Biochemical characterization was performed to differentiate the different growing organisms on each culture media. *Escherichia coli* and *Enterococcus faecalis* demonstrated positive results for indole production, methyl red (MR), lactose fermentation, and citrate negativity. In contrast, *Klebsiella pneumoniae* was indole negative (except some strains), MR negative, Voges–Proskauer (VP) positive, citrate positive, and produced mucoid lactose-fermenting colonies, *Proteus mirabilis* exhibited distinct biochemical features, including urease positivity. Unlike the other isolates, *Proteus mirabilis* showed characteristic swarming motility. These biochemical profiles provided reliable differentiation of the isolates and supported accurate identification for further microbiological and clinical analysis. *Staphylococcus saprophyticus* however appeared as coagulase-negative coccus.

4. DISCUSSION:

The study demonstrated that UTIs are highly prevalent among pregnant women, consistent with global and national reports. The predominance of *E. coli* aligns with previous literature from Iran, India, Pakistan, Nigeria, and East Africa—all reporting *E. coli* as the leading uropathogen due to its virulence factors enabling ascension and adherence within the urinary tract. The isolation of *Enterococcus faecalis* as the second most common organism is significant, as Enterococci are increasingly recognized in UTIs during pregnancy, particularly in crowded antenatal settings where hygiene may vary. *Klebsiella pneumoniae* and *Proteus mirabilis* also appeared frequently, indicating a shift toward more diverse uropathogenic profiles. Physiological changes during pregnancy—urinary stasis, ureteral dilatation, and altered immunity—likely contributed to the high culture positivity rate. Notably, third trimester samples had more *E. coli* isolates, aligning with studies that show increasing risk of bacteriuria as gestation advances. The consistent colony morphology across all three selective media validates their utility in routine antenatal screening. Biochemical results also confirmed reliable identification, with nearly all isolates showing classical reactions. The near-complete bacterial detection across at least one medium (100%) shows that single-medium cultures may miss significant infections. The findings add to the evidence that culture-based diagnosis is essential, and empirical therapy without laboratory support is risky against the rising evidence of antimicrobial resistance globally.

5. CONCLUSION:

The study concludes that urinary tract infections are highly prevalent among pregnant women, with a consistent bacterial pattern dominated by *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Staphylococcus saprophyticus*. The use of Blood Agar, MacConkey Agar, and CLED Agar proved effective in isolating all significant organisms, supported further by biochemical tests. Given the high rate of bacterial growth and predominance of Gram-negative pathogens, the study strongly recommends routine UTI screening in all antenatal visits regardless of symptoms. Early identification and appropriate management can significantly reduce adverse outcomes such as preterm labor, pyelonephritis, and low birth weight.

ACKNOWLEDGMENT

The authors would like to acknowledge the support from GEA National College for providing the facilities required as well as Tolaram Bafna Hospital Guwahati, Assam, to get the patient sample data.

REFERENCE:

1. Delzell J. E, Lefevre M. L. Urinary tract infections during pregnancy. *American Family Physician*, 2000; 61(3), 713–721.
2. Matuszkiewicz-Rowińska J, Małyszko J, Wieliczko M. Urinary tract infections in pregnancy: Old and new unresolved diagnostic and therapeutic problems. *Archives of Medical Science* 2015, 11(1)67–77.
3. Getachew T, Taddese B. D, Gizachew M. Bacterial profile and antibiotic susceptibility pattern of urinary tract infection among pregnant women attending antenatal care at Dessie Referral Hospital, Northeast Ethiopia. *BMC Research Notes* 2021; 14, 203.
4. Glaser A. P , Schaeffer, A. J. Urinary tract infection and bacteriuria in pregnancy. *Urologic Clinics of North America* 2015; 42(4), 547–560.
5. Alemu A, Moges F, Shiferaw Y, Tafess K, Kassu A, Anagaw B, Agegn, A. Bacterial profile and drug susceptibility pattern of urinary tract infection in pregnant women at University of Gondar Teaching Hospital, Northwest Ethiopia. *BMC Research Notes* 2021; 5(1)197
6. Turpin C. A, Minkah B, Danso K. A, Frimpong E. H. Asymptomatic bacteriuria in pregnant women attending antenatal clinic at Komfo Anokye Teaching Hospital, Kumasi, Ghana. *Ghana Medical Journal* 2007; 41(1) 26–29.
7. Kazemier B. M, Schneeberger C, Miranda E. Costs and effects of screening and treating low-risk women with a singleton pregnancy for asymptomatic bacteriuria, the ASB trial. *BMC Pregnancy and Childbirth* 2015;(15) 264.
8. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing 2020.
9. Olamijulo J. A, Oduyebo O. O. Antibiotic resistance pattern of uropathogens from asymptomatic bacteriuria in pregnancy in Lagos, Nigeria. *Nigerian Postgraduate Medical Journal* 2016;23(1)1–5.
10. Sheffield J.S, Cunningham F.G. Urinary tract infection in women. *Obstetrics & Gynecology* 2005, 106(5)1085–1092.
11. Schnarr J, Smaill, F. Asymptomatic bacteriuria and symptomatic urinary tract infections in pregnancy. *European Journal of Clinical Investigation* 2008; 38(S2)50–57.
12. Azami M, Jaafari Z, Masoumi M, Shohani M, Badfar G, Mahmudi L, Abbasalizadeh, S. Prevalence and risk factors of asymptomatic bacteriuria and urinary tract infections among pregnant women in Iran: a systematic review and meta-analysis. *BMC Pregnancy and Childbirth* 2019;19(1)381.
13. Ghouri F, Hollywood A, Ryan K. A systematic review of non-antibiotic measures for the prevention of urinary tract infections in pregnancy. *BMC Pregnancy and Childbirth* 2018;18(1) 99.
14. Le J, Briggs G. G, McKeown A, Bustillo G. Urinary tract infections during pregnancy. *Annals of Pharmacotherapy* 2004, 38(10)1692–1701.
15. Yang L, Jie, Y. Urinary tract infection during pregnancy and risk of preeclampsia: A systematic review and meta-analysis. *Medicine (Baltimore)* 2018; 97(36).
16. Habak P. J, Griggs R. P. Urinary Tract Infection in Pregnancy. StatPearls Publishing 2022.
17. Belete M. A, Saravanan M. A systematic review on drug-resistant urinary tract infection among pregnant women in developing countries in Africa and Asia; 2005–2016. *Infection and Drug Resistance* 2020; (13)1465–1477.

18. Kebira A. N, Gitau W, Nyerere A. Antimicrobial resistance pattern of Escherichia coli strains from pregnant and non-pregnant women attending antenatal clinics in Nairobi, Kenya. *East African Journal of Public Health* 2009; 6(3)269–273.
19. Okonko I. O, Ijandipe L. A, Ilusanya A.O, Donbraye-Emmanuel O. B, Ejembi J, Udeze A. O, Egun, O. C, Fowotade A, Nkang, A. O. Detection of urinary tract infection (UTI) among pregnant women in Oluyoro Catholic Hospital, Ibadan, South-Western Nigeria. *Malaysian Journal of Microbiology* 2010; 6(1)16–24.
20. Hamdan H. Z, Ziad A. H, Ali, S. K, Adam I. Epidemiology of urinary tract infections and antibiotics sensitivity among pregnant women at Khartoum North Hospital. *Annals of Clinical Microbiology and Antimicrobials* 2011;10(2).
21. Masinde A, Gumodoka, B, Kilonzo A, Mshana S. E. Prevalence of urinary tract infection among pregnant women at Bugando Medical Centre, Mwanza, Tanzania. *Tanzania Journal of Health Research* 2009;11(3)154–159.
22. Mulu W, Abera B, Yimer, M. Prevalence of antimicrobial susceptibility patterns and associated risk factors of asymptomatic bacteriuria among pregnant women attending Felege Hiwot Referral Hospital, Bahir Dar, Northwest Ethiopia. **Ethiopian Journal of Health Sciences** 2012; 22(2)121–128.
23. Tadesse A, Negash M, Ketema L. S. Bacterial uropathogens and their antimicrobial susceptibility profile among pregnant women attending antenatal care at Mizan Tepi University Teaching Hospital, Southwest Ethiopia. **Infection and Drug Resistance** 2020; (13)3967–3975.
24. Haider G, Zehra N, Munir A. A, Haider A. Risk factors of urinary tract infection in pregnancy. **Journal of the Pakistan Medical Association** 2010; 60(3)213–216.