

2026-09

Extended Spectrum Beta Lactamase And Carbapenemase Profile Of Uropathogens Isolated From Pregnant Women In Bahir Dar, Northwest Ethiopia

Hunie, Eyoel

<http://ir.bdu.edu.et/handle/123456789/17478>

Downloaded from DSpace Repository, DSpace Institution's institutional repository



BAHIR DAR UNIVERSITY

COLLEGE OF MEDICINE AND HEALTH SCIENCES

SCHOOL OF MEDICAL LABORATORY SCIENCES

DEPARTMENT OF MEDICAL MICROBIOLOGY

**Extended Spectrum Beta Lactamase And Carbapenemase Profile Of
Uropathogens Isolated From Pregnant Women In Bahir Dar, Northwest
Ethiopia**

BY: Eyoel Hunie (MSc candidate)

A THESIS SUBMITTED TO THE DEPARTMENT OF MEDICAL MICROBIOLOGY,
SCHOOL OF MEDICAL LABORATORY SCIENCES, COLLEGE OF MEDICINE AND
HEALTH SCIENCES, BAHIR DAR UNIVERSITY IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE MASTER OF SCIENCE DEGREE IN MEDICAL
MICROBIOLOGY

BAHIR DAR, ETHIOPIA

SEPTEMBER, 2026

BAHIR DAR UNIVERSITY

COLLEGE OF MEDICINE AND HEALTH SCIENCES

SCHOOL OF MEDICAL LABORATORY SCIENCES

DEPARTMENT OF MEDICAL MICROBIOLOGY

**EXTENDED SPECTRUM BETA LACTAMASE AND CARBAPENEMASE
PROFILE OF UROPATHOGENS ISOLATED FROM PREGNANT
WOMEN IN BAHIR DAR, NORTHWEST ETHIOPIA**

BY: EYOEL HUNIE (MSc candidate)

ADVISORS: 1. DANIEL MEKONNEN (PhD), ASSOCIATE PROFESSOR

2. AWOKE DERBIE (PhD), ASSOCIATE PROFESSOR

**A THESIS SUBMITTED TO THE DEPARTMENT OF MEDICAL MICROBIOLOGY,
SCHOOL OF MEDICAL LABORATORY SCIENCES, COLLEGE OF MEDICINE AND
HEALTH SCIENCES, BAHIR DAR UNIVERSITY IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE MASTER OF SCIENCE DEGREE IN MEDICAL
MICROBIOLOGY**

BAHIR DAR, ETHIOPIA

SEPTEMBER, 2026

DECLARATION

I certify that due acknowledgement has been made, the work is that of the author alone; the work has not been submitted previously, in whole or in part, to qualify for any other academic award. The content of the thesis is the result of work that has been carried out since the official commencement date of the approved research program; any editorial work, paid or unpaid, carried out by a third party is acknowledged; and ethical procedures and guidelines were followed.

Eyoel HunieSignature

Date

Investigator (BSc)

Department of Medical Microbiology

CMHS-SMLS-Bahir Dar University

Bahir Dar, Ethiopia

Email: eyoelhunie9@gmail.com

Tel: +251946467509

ADVISORS' APPROVAL FORM

I hereby certify that I have supervised, read, and evaluated this thesis titled "**Extended Spectrum Beta Lactamase and Carbapenemase Profile of Uropathogens Isolated from Pregnant Women in Bahir Dar, Northwest Ethiopia**" by Eyoei Hunie, prepared under my guidance. Therefore, I recommend that the student has fulfilled the requirements and hence hereby can submit the research to the Department of Medical Microbiology for public defense.

Daniel Mekonnen



14/09/2026

Advisor (*BSc, MSc, PhD*)

Signature

Date

Department of Medical Microbiology

CMHS-SMLS-Bahir Dar University

Bahir Dar, Ethiopia

Email: nigusdanielm@gmail.com

Tel: +251912990288

Awoke Derbie



14/09/2026

Advisor (*BSc, MSc, PhD*)

Signature

Date

Department of Medical Microbiology

CMHS-SMLS-Bahir Dar University

Bahir Dar, Ethiopia

Email: awokederbie1@gmail.com

Tel: +251913059887



Approval of dissertation/thesis for defense result

As members of the board of examiners, we examined this dissertation/thesis entitled “**Extended Spectrum Beta Lactamase and Carbapenemase Profile of Uropathogens Isolated from Pregnant Women in Bahir Dar, Northwest Ethiopia**”. We hereby certify that the thesis/dissertation is accepted for fulfilling the requirements for the award of the degree of “Master of Science in Medical Microbiology.”

Board of Examiners:-

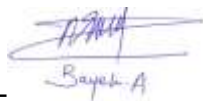
External examiner nameSignatureDate

Professor Baye Gelaw (PhD) -----
-----Department of
Medical Microbiology, School of Biomedical and Laboratory Sciences, College of Medicine and
Health Sciences, University of Gondar

Internal examiner nameSignatureDate

Bayeh Abera (PhD) -----

15/09/2026


Bayeh A

Department of Medical Microbiology, School of Medical Laboratory Sciences, College of
Medicine and Health Sciences, Bahir Dar University

Chairperson's nameSignatureDate

Yesuf Adem (PhD)  15/09/2026

Department of Medical Microbiology, School of Medical Laboratory Sciences, College of
Medicine and Health Sciences, Bahir Dar University

ACKNOWLEDGMENTS

My deepest gratitude goes to my advisors, Dr.Daniel Mekonnen and Dr.Awoke Derbie,for their valuable and unreserved support, comments, and suggestions.

I would also like to express my deepest thanks to Bahir Dar University, College of Medicine and Health Sciences,School of Medical Laboratory Sciences, Department of Medical Microbiology, for giving me this opportunity. My special gratitude also goes to Wollo University for sponsoring my MSc postgraduate study.

I would like to give my acknowledgment toAmhara Public Health Institute, Tibebe Gihon Specialized Teaching Hospital, and Felege Hiwot Comprehensive Specialized Hospital Microbiology Laboratory stafffor their support in providing necessary reagents, culture media, and antibiotic discs.

I would also like to acknowledge the staff members of the antenatal care unit in both hospitals for their collaboration in background data provision and urine sample collections. I also express my sincere gratitude to the technical assistants of the Department of Medical Microbiology, College of Medicine and Health Sciences, School of Medical Laboratory Sciences, Bahir Dar University, for their technical support in microbiological procedures.

Last but not least, my special gratitude goes to all the study participants for their willingness during sample and data collection.

TABLE OF CONTENTS

ACKNOWLEDGMENTS	vi
LIST OF TABLES	ix
LIST OF FIGURES	x
ABBREVIATIONS	xi
ABSTRACT	xii
1. INTRODUCTION	1
1.1Background	1
1.2. Statement of the problem	3
1.3. Significance of the study	5
2. LITERATURE REVIEW	6
2.1 Prevalence and Etiology of UTIs among Pregnant Women	6
2.2. Antimicrobial susceptibility of bacterial isolates andESBL- Carbapenemase Production ..	9
2.3. Factors associated with bacteial UTI among pregnant women.....	11
2.4. Conceptual framework	14
3. OBJECTIVES	16
3.1. General objective.....	16
3.2. Specific objectives.....	16
4. METHODS AND MATERIALS.....	17
4.1. Study setting.....	17
4.2. Study design and period	18
4.3. Population.....	18
4.3.1. Source population	18
4.3.2. Study population.....	18
4.4. Inclusion and exclusion criteria.....	18
4.4.1. Inclusion criteria	18
4.4.2. Exclusion criteria	18
4.5. Variables.....	18
4.5.1. Dependent variable	18
4.5.2. Independent variables	18
4.6. Sample size determination	19

4.7. Sampling technique and procedure	20
4.8. Data collection.....	20
4.8.1. Socio-demographic data	20
4.8.2. Laboratory data.....	20
4.8.2.1. Specimen collection and transportation.....	20
4.8.2.2. Bacterial Isolation and identification.....	20
4.8.2.3. Antimicrobial susceptibility test.....	21
4.8.2.4 Extended spectrum beta-lactamase detection	21
4.8.2.5. Carbapenemase detection	22
4.9. Quality control.....	26
4.10. Data processing and analysis.....	26
4.11. Ethical considerations	27
4.12. Plan for dissemination of results	27
5. RESULTS	28
5.1 Socio-demographic characteristics of the study participants	28
5.2. Pregnancy, behavioral and clinical characteristics of the study participants	29
5.3. Types of the identified bacterial uropathogens.....	30
5.4. Antimicrobial Susceptibility Profile of the isolates	30
5.5. Multidrug resistance (MDR) profile of the isolates	33
5.6. Magnitude of ESBL and carbapenemase production	35
5.7. Factors associated with significant bacteriuria.....	38
6. DISCUSSION	40
7. LIMITATIONS OF THE STUDY	45
8. CONCLUSION.....	46
9. RECOMMENDATIONS	47
10. REFERENCES	48
11. ANNEXES	53

LIST OF TABLES

Table 1: Summary of literature review on Prevalence and etiology of UTIs among Pregnant Women.....	12
Table 2: Summary of literature review on antimicrobial susceptibility of bacterial isolates and ESBL-carbapenemase production among pregnant women	13
Table 3 : Sample size determination using associated factors of UTI among pregnant women .	19
Table 4: Summary of antimicrobial agents, class and role in the study (CLSI, 2025).	23
Table 5: Socio demographic Characteristics of the Study participants (n=260) in FHCSH and TGSTH, Bahir Dar, north-west Ethiopia, 2026.	28
Table 6: Pregnancy, behavioral and clinical characteristics of the study participants (n=260) in FHCSH and TGSTH, Bahir Dar, north-west Ethiopia, 2026.	29
Table 7:- Antimicrobial susceptibility profile of Gram-negative isolates from pregnant women with symptomatic UTI attending ANC at FHCSH and TGSTH, Bahir Dar, north-west Ethiopia, 2026.....	31
Table 8: Antimicrobial susceptibility profile of Gram-positive isolates from pregnant women with symptomatic UTI attending ANC at FHCSH and TGSTH, Bahir Dar, north-west Ethiopia, 2026.....	32
Table 9: Multi-drug resistance profile of bacteria isolated from pregnant women with symptomatic UTI attending ANC at FHCSH and TGSH, Bahir Dar, north-west Ethiopia, 2026	33
Table 9(continued).....	34
Table 10: Magnitude of ESBL-Producing <i>Enterobacteriaceae</i> isolated from pregnant women with symptomatic UTI attending ANC at FHCSH and TGSTH, Bahir Dar, north-west Ethiopia, 2026.....	35
Table 11: Antimicrobial susceptible pattern of ESBL and carbapenemase producing <i>Enterobacteriaceae</i> isolated from pregnant women with symptomatic UTI attending ANC at FHCSH and TGSTH, Bahir Dar, north-west Ethiopia, 2026	36
Table 12: Bivariate and multivariate logistic regression analyses of factors associated with SBU among pregnant women with symptomatic UTI attending ANC at FHCSH and TGSTH, Bahir Dar, north-west Ethiopia, 2026.....	39

LIST OF FIGURES

Figure 1: Conceptual framework for bacterial UTIs and ESBL-carbapenemase production of bacterial uropathogens among pregnant women.....	15
Figure 2: Flow chart for urine processing, identification the isolates, AST test and ESBL-Carbapenemase detection, Bahir Dar, 2026.....	24
Figure 3: Some pictures taken in the laboratory during urine culture of pregnant women with symptomatic UTI attending ANC at FHCSH and TGSTH, Bahir Dar, north-west Ethiopia, 2026	25
Figure 4: Isolated bacterial uropathogens among pregnant women with symptomatic UTI attending ANC at FHCSH and TGSTH, Bahir Dar, north-west Ethiopia, 2026	30
Figure 5: Comparison of antibiotic resistance among ESBL and non-ESBL producer isolates from pregnant women attending ANC at FHCSH and TGSTH, Bahir Dar, north-west Ethiopia.	37

ABBREVIATIONS

AMR	Anti-Microbial Resistance
ANC	Antenatal Care
AST	Antimicrobial Susceptibility Testing
CLSI	Clinical Laboratory Standards Institute
CoNS	Coagulase Negative Staphylococci
ESBL	Extended Spectrum Beta Lactamase
ETB	Ethiopian Birr
FHCSHFelege Hiwot Comprehensive Specialized Hospital	
GNRs	Gram-Negative Rods
MDR	Multi Drug Resistant
MHA	Mueller Hinton Agar
SBU	Significant Bacteriuria
SOPs	Standard Operating Procedures
SPSS	Statistical Package for the Social Sciences
TGSTH	Tibebe Gihon Specialized Teaching Hospital
UPEC	Uropathogenic <i>Escherichia coli</i>
UTI	Urinary Tract Infection
WHO	World Health Organization

ABSTRACT

Background: Urinary tract infection (UTI) is a leading cause of morbidity during pregnancy. The rising threat of antimicrobial resistance (AMR) among uropathogens against extended-spectrum beta-lactams and carbapenems poses a serious treatment challenge. However, there is a limited data on ESBL- and carbapenemase-producing uropathogens among pregnant women in Bahir Dar, Northwest Ethiopia.

Objective: To assess the extended-spectrum beta-lactamase and carbapenemase profile of uropathogens isolated from pregnant women in Bahir Dar, Northwest Ethiopia.

Methods and materials: A facility-based cross-sectional study was conducted at Felege Hiwot Comprehensive Specialized Hospital (FHCSH) and Tibebe Gihon Specialized Teaching Hospital (TGSTH) in Bahir Dar, Northwest Ethiopia, from 9 February to 8 May 2026. From the 10 mL of midstream urine sample obtained, 1 μ L of it was inoculated into blood and MacConkey agar for bacterial identification. Antimicrobial sensitivity testing (AST) was performed using the Kirby-Bauer disc diffusion technique. ESBL and carbapenemase production were detected using the combined disc diffusion method and modified carbapenem inactivation methods, respectively. Data were entered into SPSS version 26 for analysis. Logistic regression was done to identify factors associated with SBU and a p-value ≤ 0.05 was considered statistically significant.

Results: From the total 260 pregnant women recruited, the proportion of significant bacteriuria (SBU) was 21.2% (55/260). The majority, 40 (72.7%), of the isolates were Gram-negative rods (GNRs), with uropathogenic *E. coli* (UPEC) as the predominant pathogen, 31 (56.4%). From the total 40 GNRs, 92.5%, 60%, and 57% were found resistant to ampicillin, trimethoprim-sulfamethoxazole, and amoxicillin-clavulanic acid, respectively. On the reverse, 97.5% of GNR isolates were susceptible to meropenem, amikacin, and nitrofurantoin. MDR, ESBL, and carbapenemase production were observed in 58.2%, 16.2%, and 2.5% of isolates, respectively. ESBL and carbapenemase-producing isolates were found 100% MDR. Age (15-24 yrs.), third-trimester gestation, and a history of UTIs showed a statistically significant association with SBU.

Conclusions: A high proportion of SBU was observed, mainly caused by GNRs with high MDR rates. The isolation of ESBL and carbapenemase-producing uropathogens was also a concern. This emphasizes challenges in treating UTIs and highlights the importance of screening for SBU among pregnant women, continuous surveillance of AMR, and rational use of antibiotics.

Key words: Urinary tract infection, ESBL, carbapenemase, pregnant women, Bahir Dar.

1. INTRODUCTION

1.1 Background

Urinary tract infections (UTIs) are among the most common infections that can occur in any region of the urinary system, including the urethra, ureters, bladder, and kidney (Al-Kolaibe and Helmy, 2023). Back pain, lower abdominal pain, fever, vomiting, nausea, chills, dysuria, and urinary urgency are common symptoms of UTIs (Begum *et al.*, 2022).

Each year, around 150 million UTI cases are being identified, and all age groups and populations can be affected (Öztürk and Murt, 2020). UTIs disproportionately affect women because of their shorter urethra and the close proximity of the urethral opening to the anus; moreover, the rate of developing UTIs among pregnant women is higher than non-pregnant women, associated with physiological changes that occur during this period, resulting in urine retention in the bladder, ureteral dilation, and elevated glucose and amino acid levels in urine (Rosana *et al.*, 2016, Biset *et al.*, 2020). Lower socioeconomic status, previous history of UTI, history of catheterization, history of diabetes mellitus, and inappropriate wiping after defecation or urination are factors that increase the risk of UTI during pregnancy (Badran *et al.*, 2015, Getaneh *et al.*, 2021).

UTIs can be caused by a broad range of pathogens, including viruses, fungi, and parasites, although bacteria of the enteric origin are the most common causative agents (THAPA *et al.*, 2015). *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Staphylococcus saprophyticus*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* are the most common identified causes (Akther *et al.*, 2023). UTIs in pregnant women are treated with many classes of antibiotics. First-line therapeutic options include beta-lactams, nitrofurans, and sulfonamides (Habak *et al.*, 2026).

Antibiotic resistance is a rising global problem, and bacterial resistance is spreading as a result of inappropriate antibiotic use and self-medication. These have caused selective pressure on the bacterial population, resulting in the emergence of resistant mutants (Benyagoub *et al.*, 2021). Extended-spectrum beta-lactamase (ESBL) mediated drug resistance is the common mode of resistance in uropathogens (Fares *et al.*, 2023).

ESBLs are enzymes produced by the *Enterobacteriaceae* and other non-lactose fermenters. They hydrolyze and inactivate beta-lactam antibiotics, making them ineffective in treating infections caused by those bacteria that have developed resistance to penicillin, third-generation cephalosporins, and monobactams. The genes that encode ESBL are diverse in nature, with the most dominant gene families worldwide being *bla*_{TEM}, *bla*_{SHV}, and *bla*_{CTX-M}; these genes are commonly found on plasmids that carry antimicrobial resistance (AMR) genes, providing resistance to various antibiotics. Thus, many ESBL-producing bacteria are also resistant to non- β -lactam antibiotics, which are common treatment options; including, aminoglycosides, trimethoprim-sulfamethoxazole, and chloramphenicol (Husna *et al.*, 2023).

Treatment choices for UTIs caused by ESBL-producing pathogens, especially in cases of serious conditions, including pyelonephritis, and considered the last resort antibiotics are carbapenems. Antibiotic resistance for those drugs may arise through carbapenemase production encoded by the common genes such as *bla*_{KPC}, *bla*_{NDM}, *bla*_{OXA}, and *bla*_{IMP}. So, ESBL-carbapenemase-producing bacterial pathogens have become a significant public health concern globally because of their high level of drug resistance, which substantially limits therapeutic options for UTIs (Rodríguez-Baño *et al.*, 2018, Tamma *et al.*, 2021)

Antenatal care (ANC) in developing countries, such as in Ethiopia, does not include routine urine culture screening, and therapy is empirically based. This can lead to the emergence and spread of antibiotic-resistant bacteria, including ESBL and carbapenemase-producing. Periodic surveillance of AMR is important to provide effective empirical treatment, to be aware of emerging pathogens, to develop rational prescribing practices, and to make policy decisions (Belete, 2020). Despite this, there is limited data regarding the extent of ESBL-carbapenemase-producing bacteria in UTIs among pregnant women in Bahir Dar, Northwest Ethiopia. Therefore, this study was aimed at assessing extended-spectrum β -lactamase and carbapenemase profile of uropathogens among pregnant women in Bahir Dar, Northwest Ethiopia.

1.2. Statement of the problem

UTIs are one of the leading causes of maternal and newborn morbidity and mortality during pregnancy and are linked to a number of adverse outcomes for mothers and newborns, including low birth weight, preterm birth, stillbirth, preeclampsia, maternal anemia, sepsis, and amnionitis (Rosana *et al.*, 2016, Yetera *et al.*, 2024). UTIs and other infections were responsible for 10.7% of maternal deaths occurred in 2021 globally (Ribeiro-do-Valle *et al.*, 2024, Cao *et al.*, 2025). UTIs are one of the most common pregnancy-related health concerns, with a global prevalence of 3% to 35%; developing nations, particularly those in Sub-Saharan Africa, have higher prevalence rates (Belete, 2020).

AMR is one of the urgent global health threats, and according to the World Health Organization (WHO) report, bacterial AMR directly led to 1.27 million global deaths in 2019 and contributed to 4.95 million fatalities worldwide (WHO, 2023). A growing concerning aspect of AMR is the emergence of ESBL-carbapenemase-producing uropathogens, and the overall prevalence of ESBL-producing uropathogens in pregnant women is 25% globally, with high estimated prevalence rates found in Africa (45%) (Mansouri *et al.*, 2019, Getie *et al.*, 2023). In developing countries like Ethiopia, AMR and its impact are significant. Around 30.92% and 15.12% of UTIs are caused by ESBL-carbapenemase-producing bacteria in the country, respectively (Tigabie *et al.*, 2025).

Studies showed there is a high burden of AMR and ESBL-carbapenemase-producing bacteria in Bahir Dar. According to studies conducted from various clinical samples, about 85.8% and 16.2% of Gram-negative bacteria were ESBL-carbapenemase-producers, respectively (Moges *et al.*, 2019). In addition, 19.2% and 5.8% of uropathogens isolated from catheterized patients were ESBL and carbapenemase-producers. Based on this study, isolated bacteria showed a high level of resistance to commonly used antibiotics for treating UTIs, and all carbapenemase-producing isolates showed non-susceptibility to many classes of antibiotics tested (Asmare *et al.*, 2024). Poor infection prevention and control practices, inappropriate use of antibiotics, over the counter availability of different antimicrobials, and lack of standard laboratory facilities for antimicrobial susceptibility testing (AST) are factors that might contribute to the occurrence and spread of those superbugs in the area (Ameshe *et al.*, 2022).

UTIs caused by those pathogens during pregnancy raise the risk of maternal and neonatal complications, which might be linked with prolonged hospitalization, increased health costs, high morbidity, and high mortality; therefore, evidence regarding the magnitude of those bacteria among pregnant women is important (Bulabula *et al.*, 2017).

A previous study conducted in Bahir Dar among pregnant women revealed a 9.5% overall prevalence of UTI, while the prevalence among symptomatic was 18.9%. Factors such as history of UTI and low socioeconomic status were associated with increased prevalence in the study area (Demilie *et al.*, 2012, Emiru *et al.*, 2013). Besides, previous studies did not assess the ESBL and carbapenemase-production profile of the isolates, which are the current worldwide threat. Considering this gap, and taking the dynamic nature of AMR into account, periodic data on the AMR profile of uropathogens is important to understand the trend and guide effective treatment of UTI among pregnant women. Therefore, the aim of this study was to assess ESBL and carbapenemase profile of uropathogens among pregnant women in Bahir Dar, Northwest Ethiopia.

1.3. Significance of the study

The findings of the study have the following significance;

- This study provided updated data on the types of bacterial uropathogens and their antimicrobial susceptibility profile among pregnant women.
- This study also showed the magnitude of ESBL-carbapenemase production of the isolates among pregnant women.
- The data from the study would be important for policymakers, and stakeholders to be aware of the burden and implement appropriate interventions for controlling the multidrug-resistant (MDR) and ESBL-carbapenemase-producing bacterial pathogens.
- Moreover, the study findings would serve as an input to develop an updated treatment guideline, which would improve the management of UTIs in pregnant women and would also be used as baseline data for researchers for further studies.

2. LITERATURE REVIEW

Clinically, UTIs can be categorized as uncomplicated and complicated. The former happens in a healthy, non-pregnant woman with a neurologically and structurally normal urinary tract, while the latter occurs in patients with compromised urinary tract structure or function, such as during pregnancy or in neurologic impairments that cause urine retention, or in the presence of a foreign body, such as catheters or calculi. UTIs in pregnancy can range from asymptomatic bacteriuria to cystitis and pyelonephritis (Matulay *et al.*, 2016, Silverman and Turrentine, 2023). During pregnancy, pregnant women experience ureteral dilatation, which persists until childbirth. Decreased bladder tone, in addition to reduced ureteral tone, are thought to contribute to increased urine stasis. Around 70% of pregnant women experience glycosuria, which is believed to support bacterial growth (Nowicki, 2002). Gram-positive and Gram-negative bacteria, usually facultative anaerobes of the intestinal flora, are the main causes of infections in the urinary tract (Fatima and Mussa, 2017).

2.1 Prevalence and Etiology of UTIs among Pregnant Women

The prevalence of UTIs among pregnant women varies significantly across countries and regions. Globally, many of the studies conducted have shown Gram-negative rods (GNRs), including uropathogenic *E. coli* (UPEC), as the leading etiologic agents of UTIs in pregnant women. Studies from the Middle East revealed a moderate to high prevalence of UTI. In a study conducted in Southwest Iran, the prevalence of UTI was 9.8% (221 out of 2245), in which GNRs caused 89.6% of the cases. In this study, *E. coli* (173, 78.3%) was the most frequent pathogen isolated from urinary cultures, followed by *S. aureus* (14, 6.33%) and *Enterobacter spp* (12, 5.4%) (Omidifar *et al.*, 2020). Likewise, a study conducted in Iraq on 5,042 pregnant women reported an overall SBU prevalence of 12.40%, of which 31.68% (198) were symptomatic. *Klebsiella pneumoniae* isolates at 92 (14.31%) were the second most dominant bacteria following *E. coli* (363, 56.45%), while CoNS were the predominant Gram-positive pathogens at 41 (6.38%) (Taha, 2024).

Furthermore, a study carried out in the United Arab Emirates, recruiting 682 pregnant women, found a 17.9% prevalence of UTI, reporting that *Group B Streptococcus* (25%) isolates were the second most commonly isolated bacteria following *E. coli* (27%) (Dube *et al.*, 2022).

The high UTI prevalence was recorded in Yemen at 60% among 350 recruited pregnant women, where the three most often isolated pathogens were *Escherichia coli* accounted for 90/210 (42.9%), *Staphylococcus aureus*, 50/210 (23.8%), and *Klebsiella pneumoniae*, 25/210 (11.9%) (Al-Badaii *et al.*, 2023).

In Africa, UTIs remain a major concern in pregnant women. A study conducted in Johannesburg, South Africa, on 1984 specimens belonging to pregnant women reported a total prevalence of symptomatic UTI of 16.8%, where *E. coli* isolates were the most prevalent (49.9%), followed by *Klebsiella* species (14.4%) and *Enterococcus faecalis* (12.9%) (Orji *et al.*, 2022). A high prevalence was reported in Southwestern Uganda, where SBU was observed in 35% of pregnant women (140/400). Unlike many of the studies, *Klebsiella pneumoniae* was the predominant isolate at 52 (37.41%), followed by *Escherichia coli* at 40 (28.78%) (Johnson *et al.*, 2021).

UTIs continue to be a prevalent maternal health issue, according to research done in Ethiopia. Even though the overall etiologic pattern is similar to that reported globally, the prevalence and the distribution of bacterial isolates vary between locations. A study conducted in Bale Zone, Southeast Ethiopia, by recruiting 169 pregnant women indicated that the overall prevalence of UTI was 26% (44/169), with symptomatic cases accounting for 18/51 (35.3%); based on this study, from the total of 44 bacteria isolated, *E. coli* 12/44 (27.3%), *K. pneumoniae* 9/44 (20.5%), and *S. marcescens* 4/44 (9.1%) were the commonest bacterial pathogens, and no Gram-positive bacteria were isolated (Taye *et al.*, 2018).

In another similar study conducted at Hiwot Fana Specialized University Hospital, Harar, Eastern Ethiopia, 200 study participants were included, and the prevalence of UTI was 15.5% (31/200). In this study, 13/62 (20.9%) had shown SBU among symptomatic. Similar to other studies, *Escherichia coli* (45.2%) was the most frequently isolated uropathogen, followed by *Proteus spp* (22.6%), *Klebsiella pneumoniae* (16.1%), and *Staphylococcus aureus* (9.7%) (Ejerssa *et al.*, 2021).

A study conducted at Dil Chora Referral Hospital in Dire Dawa, Ethiopia, found a prevalence of SBU among symptomatic pregnant women of 17% (15/88); in line with many other studies, GNRs were predominant 73.1%, and *E. coli* was found to be the most frequent isolate (34.6%), followed by *CoNS*(19.2%), *P. aeruginosa* (15.4%), and *Klebsiella* spp(Derese et al., 2016). Similarly, in another study undertaken at Hawassa,Southern Ethiopia, recruiting 296 pregnant women, *E. coli* was the most frequently isolated bacteria (47.8%), followed by *K. pneumoniae* (17.4%) and *S. aureus* (8.7%); in this study,UTI was observed in 7.8% of participants (Tula et al., 2020).

In another study conducted at Nekemte Specialized Hospital in the East Wollega Zone of Ethiopia, which recruited 296 study participants, the prevalence of SBU among symptomatic pregnant women was 21%.In this study, GNRsaccounted for the majority of the isolates, 27 (61.4%), and *E. coli*, 18 (40%), was the most common bacterial isolate, followed by *CoNS*,9 (20.5%)(Abebe et al., 2025).According to a study conducted at Dessie Referral Hospital, Northeastern Ethiopia, the prevalence of UTI was 15.5% (50/323), while the prevalence among symptomatic was 26.6% (21/79), respectively; similar to the above study, *Escherichia coli* at 17 (33.3%), were the predominant isolates, followed by *CoNS*, 15 (30.0%)(Belete, 2020).

2.2. Antimicrobial susceptibility of bacterial isolates and ESBL- Carbapenemase Production

A developing and significant public health problem that raises morbidity and mortality is antibiotic resistance. Resistance to routinely administered antibiotics in urinary tract infections is constantly increasing, and resistance rates vary from region to region (Sher *et al.*, 2024)

A study was conducted over two years at Nagpur, India. In this study, all GNRs were sensitive to carbapenems (imipenem and meropenem). Nitrofurantoin was found to be an effective antibiotic, in which 87.09% of GNRs and 100% of Gram-positive cocci were susceptible to it. Methicillin resistance was observed in 50% of *Staphylococcus* isolates. However, all were sensitive to nitrofurantoin and vancomycin. Regarding ESBL-Carbapenemase production, ESBL production was detected in 19.35% of *Enterobacterales* and none of the isolates produced carbapenemase (Patil *et al.*, 2024).

In contrast, a study conducted in Nigeria reported a high level of resistance to meropenem (48.6%), imipenem (31.4%), ceftriaxone (20%), and ceftazidime (22.9%); in this study, piperacillin-tazobactam was the most active antibiotic, with 82.9% of isolates sensitive to it, followed by ciprofloxacin (26, 74.3%), ceftazidime (25, 71.4%), ceftriaxone (25, 71.4%), and 34.3% of isolates were carbapenemase-producing (Ogban *et al.*, 2024).

A study from South Africa also showed variation in susceptibility patterns among different uropathogens. Antibiotics such as amoxicillin/clavulanic acid (82%), ciprofloxacin (88%), nitrofurantoin (95%), and gentamicin (93%) were effective against *E. coli* isolates, while those bacteria were less sensitive to ampicillin (24%) and trimethoprim/sulfamethoxazole (38%). In contrast, *E. faecalis* isolates were susceptible to ampicillin/amoxicillin (99%) and trimethoprim/sulfamethoxazole (95%). The prevalence of ESBL producing *E. coli* was 7% and 9% in *K. pneumoniae*. The prevalence of MDR uropathogens was low (4%) (Zwane *et al.*, 2021).

In a study in Kenya, high resistance among the *E. coli* isolates was observed against ampicillin (88.9%), nalidixic acid (86.7%), and cephalosporins. In contrast, the isolates remained highly susceptible to amoxicillin-clavulanic acid (86.6%), imipenem (100%), and nitrofurantoin (98.8%) (Onyango *et al.*, 2018).

S. aureus isolates were fully susceptible to amoxicillin-clavulanic acid, nitrofurantoin, and Chloramphenicol; however, imipenem resistance was observed in 20% of *S. aureus*, 14.3% of *K.*

pneumoniae, and all *Enterococcus* isolates. MDR was observed in 96% of the isolates. Around 20% of isolated *S. aureus* were methicillin resistant, and 16% of *K. pneumoniae* and 22% of *E. coli* isolates were found ESBL producers (Onyango *et al.*, 2018).

In a study carried out in Hargeisa, Somaliland, a relatively high level of resistance was observed against ampicillin (87%), amoxicillin (85%), cefotaxime (57%), and cephalexin (52%) by isolated Gram-negative bacteria. On the other hand, most of the isolates were susceptible to nitrofurantoin. Regarding ESBL profile, 25 isolates (32%) were ESBL producers. Particularly, *P. aeruginosa* 3 (42.9%), *E. coli* 14 (38.8%), *K. pneumoniae* 5 (31.3%), *P. mirabilis* 1 (20%), *Citrobacter Spp* 1 (33.3%), and *Enterobacter Spp* 1 (100%) (Mohamoud *et al.*, 2021).

A study was conducted at ALERT Hospital, Addis Ababa, Ethiopia. In this study, 50% (n = 6/12) of GNRs were ESBL producers, and all were resistant to amikacin and gentamicin while, they were susceptible to nitrofurantoin (83.3%) and chloramphenicol (83.3%). In this study, 60-100% of GNRs developed resistance to ampicillin and trimethoprim/sulfamethoxazole. But all of those isolates were sensitive to nitrofurantoin, meropenem, amikacin, and gentamicin. Among the total GNRs, MDR was observed in 80% of the isolates (Getie *et al.*, 2023).

In another similar study conducted at Hiwot Fana Specialized University Hospital, Harar, Eastern Ethiopia. Among the isolated bacteria, amikacin showed the highest efficacy, with 96.4% of the isolates were susceptible, followed by nitrofurantoin (90.3%) and gentamicin (83.9%); conversely, the isolates showed high level resistance to ampicillin (58.1%), amoxicillin-clavulanate (51.6%), and cotrimoxazole (51.6%) (Ejerssa *et al.*, 2021).

Nitrofurantoin (100%), gentamicin (100%), and clindamycin (100%) were found effective antimicrobial agents for Gram-positive bacteria in a study conducted at Hawassa, Southern Ethiopia. In this study, Gram-negative bacteria showed a high level of sensitivity to ciprofloxacin (78.3%), gentamicin (91.3%), and nitrofurantoin (100%) and high resistance against cotrimoxazole (68.2%) (Tula *et al.*, 2020).

A study conducted at Dessie Referral Hospital in northeastern Ethiopia reported that 15.8% of GNRs were ESBL producers. In this study, 80% of GNRs were MDR, and the majority of those uropathogens demonstrated a high rate of resistance (65.0%) for ampicillin, and relatively low

level of resistance to nitrofurantoin (25.0%), norfloxacin (25.0%), and ceftazidime 3 (15.0%). A high level of resistance was observed by Gram-positive bacteria to penicillin 29 (93.5%) and trimethoprim-sulfamethoxazole 23 (79.3%). On the reverse, 100.0% of those isolates were sensitive to nitrofurantoin (Belete, 2020).

A similar study carried out at Gondar, Northwest Ethiopia, revealed that 18.2% of *Enterobacteriaceae* were ESBL producers. High resistance rates among Gram-negative isolates were observed for ampicillin (90.9%), cephalothin (84.8%), and amoxicillin-clavulanate (57.6%). Moreover, fifty-seven percent of Gram-negative bacteria were MDR in this study, and 100% of those isolates were susceptible to nitrofurantoin. Regarding Gram-positive bacteria, they showed low susceptibility to penicillin (89.3%) and cotrimoxazole (75%); however, high susceptibility rates for gentamicin (100%), amikacin (100%), and nitrofurantoin (98.36%) were observed in this study (Biset *et al.*, 2020).

2.3. Factors associated with bacterial UTI among pregnant women

Several factors may increase the occurrence of UTI during pregnancy. A cross-sectional study conducted in Yemen found that age, trimester of pregnancy, gestational age, and residence were significantly associated with UTI. The higher prevalence among women aged 20–40 years may

be related to higher sexual activity. The risk may also increase with advancing pregnancy, particularly in later trimesters, due to hormonal and anatomical changes that cause urinary stasis. Differences by residence may reflect variations in sanitation, access to healthcare, and hygiene practices (Al-Badaii *et al.*, 2023). Similarly, history of UTI, educational status, Parity, and occupation of pregnant women were found significantly associated with UTI in a study conducted in Nepal. According to this study, multiparous, third-trimester, and housewife women had a higher risk of UTI. Multiparous women may have increased risk due to repeated pregnancy-related urinary tract changes and pelvic floor alterations (THAPA *et al.*, 2015).

History of chronic diseases, such as diabetes mellitus and, frequency of sexual intercourse were also factors that are associated with UTI among pregnant women as supported by study conducted in Saudi Arabia. Diabetes may increase UTI risk through impaired immune function, glycosuria, and reduced ability to clear urinary pathogens, while frequent sexual intercourse may facilitate the entry of bacteria into the urinary tract and increase the risk of ascending infection (El-Kashif, 2019, Badran *et al.*, 2015). Direction of wiping after genital wash and postcoital urination were associated with SBU according to study conducted in Harar, Eastern Ethiopia. Wiping from back to front may facilitate the transfer of perineal bacteria toward the urethra, increasing the risk of ascending infection, whereas postcoital urination may help flush bacteria introduced into the urinary tract during intercourse and thereby reduce the risk of SBU (Edae *et al.*, 2020).

A cross-sectional study carried out at Dessie Referral Hospital in Northeastern Ethiopia found that prior history of UTI and catheterization were factors showed a statistically significant association with UTI. Previous UTI may increase recurrence through persistent bacterial colonization, while catheterization provides a surface for bacterial adhesion and biofilm development, facilitating persistence and ascending infection (Belete, 2020).

Table 1: Summary of literature review on Prevalence and etiology of UTIs among Pregnant Women

No	Author. Year	Study design and sample size	Country	Prevalence of SBU	Predominant bacterial isolates
----	-----------------	---------------------------------	---------	-------------------	--------------------------------

1	Omidifar <i>et al.</i> , 2020)	cross-sectional, 2245	Iran	9.8% (221/2245)	89.6% gram negative bacteria <i>E. coli</i> 78.3%, <i>S. aureus</i> (6.33%), <i>Enterobacter spp</i> (12, 5.4%), <i>Klebsiella spp</i> (7, 3.2%),
2	Al-Badaii <i>et al.</i> , 2023	cross sectional 350	Yemen	60% (210/350)	<i>E. coli</i> 90/210 (42.9%), <i>S. aureus</i> ,50/210 (23.8%), and <i>Klebsiella pneumoniae</i> , 25/210 (11.9%)
3	(Orji <i>et al.</i> , 2022).	retrospective cross sectional, 1984 specimens	South Africa	333 (16.8%)	<i>E. coli</i> (49.9%). <i>Klebsiella</i> species (14.4%), <i>Enterococcus faecalis</i> (12.9%), and CoNS (8.9%)
4	(Johnson <i>et al.</i> , 2021)	cross sectional, 400	Uganda	140/400 (35%)	Gram negative bacteria (73%): <i>Klebsiella pneumoniae</i> , 52(37.41%), and <i>E.coli</i> , 40(28.78%) , <i>S. aureus</i> 33(23.57%)
5	(Taye <i>etal.</i> , 2018).	cross sectional,169	Bale Zone, Southeast Ethiopia	44/169 (26%), 18/51 (35.3%) in symptomatic	<i>E. coli</i> 12/44 (27.3%), <i>K. pneumonia</i> 9/44 (20.5%) and <i>S. marcescens</i> 4/44 (9.1%)
6	(Ejerssa <i>et al.</i> , 2021)	Cross sectional,200	Harar,Eastern Ethiopia	15.5% and 20.9% (13/62) symptomatic	<i>Escherichia coli</i> (45.2%), <i>Proteus</i> spp. (22.6%), <i>Klebsiella pneumoniae</i> (16.1%)
7	(Abebe <i>et al.</i> , 2025)	Cross sectional,296	Nekemte, Ethiopia	Symptomatic 21.5% (23/107)	Gram-negative 61.4%, <i>E. coli</i> 18 (40%) and CoNS 9 (20.5%)
8	(Belete, 2020)	Cross sectional,323	Dessie,Ethiopia	Symptomatic 26.6%(21/79)	<i>E. coli</i> 17 (33.3%), CoNS15 (30.0%)

Table 2: Summary of literature review on antimicrobial susceptibility of bacterial isolates and ESBL-carbapenemase production among pregnant women

No	Author.	Study	Country	Findings	MDR, ESBl and carbapenemase
----	---------	-------	---------	----------	-----------------------------

	Year	design and size			profile
1	(Patil et al., 2024)	Cross sectional, 400	India	Gram positive cocci 100% resistance to penicillin and 100% sensitive to nitrofurantoin. Gram negativeshowedhigh susceptibility to carbapenems, nitrofurantoin, gentamycin & amikacin (80-100%)	19.3% ESBL (30% <i>E. coli</i> and 40% <i>K. pneumoniae</i> , 0% carbepenmase)
2	(Ogban et al., 2024).	cross-sectional, 349	Nigeria	Isolatesshowed a high rate of resistance to meropenem (48.6%), imipenem (31.4%), ceftriaxone (20%), and ceftazidime (22.9%).	34.3% carbapenemase-producing
3	(Onyang o etal., 2018).	cross-sectional study,210	Kenya	High resistance among the <i>E. coli</i> isolates was observed against ampicillin (88.9%), nalidixic acid (86.7%), and cephalosporins.	96% MDR, ESBL 16% of <i>K.pneumoniae</i> and 22% of <i>E. coli</i>
4	(Tula et al., 2020)	Cross sectional, 296	HawassaSouthern Ethiopia	Nitrofurantoin (100%), gentamicin (100%), and clindamycin (100%) were found effective antimicrobial agents for Gram-positive bacteria. Gram-negative bacteria showed a high level of sensitivity to ciprofloxacin(78.3%), gentamicin (91.3%), and nitrofurantoin (100%) Both showed high resistance to cotrimoxazole (>50%)	
5	(Biset et al., 2020)	cross-sectional study,384	Gondar, Northwest Ethiopia	High resistance rates among Gram-negative isolates were observed for ampicillin (90.9%), cephalothin (84.8%), and amoxicillin-clavulanate (57.6%). nitrofurantoin was effective antibiotic (100%). Gram postive showed high resistance to cotrimoxazole (75%) and penicillin (89.3%).Gentamycin 100% effective	60% MDR and 18.2% ESBL production

2.4. Conceptual framework

In order to show the link between associated factors and bacterial UTI (SBU) a conceptual framework was developed based on findings of the literature review (Figure 1). The framework

broadly explains the associated factors of bacterial UTI and the link among them by classifying them into four groups: namely socio-demographic, clinical, behavioral, and pregnancy related factors.

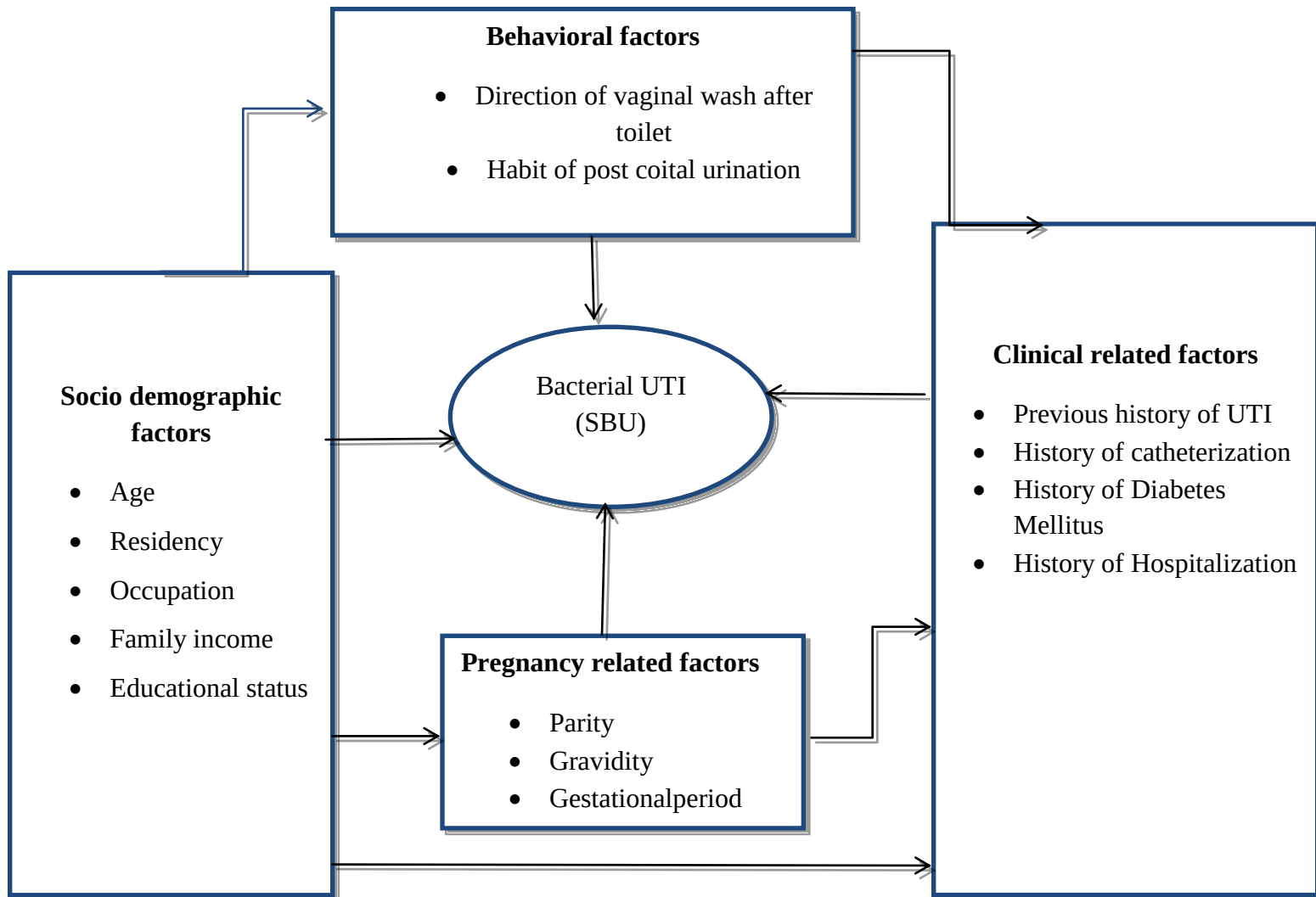


Figure 1: Conceptual framework for bacterial UTIs and ESBL-carbapenemase production of bacterial uropathogens among pregnant women.

3. OBJECTIVES

3.1. General objective

- To assess extended-spectrum beta-lactamase and carbapenemase profile of uropathogens isolated from pregnant women in Bahir Dar, Northwest Ethiopia.

3.2. Specific objectives

- To determine the proportion of significant bacteriuria among pregnant women with presumptive urinary tract infection attending antenatal care.
- To determine the antimicrobial resistance profile of the isolates.
- To determine the magnitude of extended-spectrum beta-lactamases and carbapenemase producing isolates.
- To identify factors associated with significant bacteriuria among pregnant women.

4. METHODS AND MATERIALS

4.1. Study setting

The study was conducted in Bahir Dar, the capital city of Amhara National Regional State, Northwest Ethiopia. It is located 565 km Northwest of Ethiopia's capital city, Addis Ababa. The total population of Bahir Dar city is estimated to be 441,149, with 220,497 males and 220,652 females (Adigeh, 2023). The city currently has three government hospitals and ten health centers. This study was conducted in Felege Hiwot Comprehensive Specialized Referral Hospital (FHCSH) and Tibebe Gihon Specialized Teaching Hospital (TGSTH).

FHCSH was established in 1952 and provides services for people in Bahir Dar city and the surrounding zones. It currently provides comprehensive healthcare services. It serves a population of more than 10 million in the region. The FHCSH has 13 wards with 500 beds and approximately 1420 healthcare staff. ANC is under a branch of the department of gynecology and obstetrics; about 10,967 mothers attend ANC each year, and it has 3 general practitioners, 2 gynecologists, and 17 midwives under ANC.

TGSTH is another hospital located in Bahir Dar. TGSTH was established in 2019 and is a tertiary-level hospital that serves over 7 million populations. At present, the hospital has 12 wards and 503 beds. Additionally, it houses around 1,000 healthcare professionals. It is a modern tertiary-level facility equipped to deliver comprehensive clinical, surgical, and diagnostic services. In ANC ward about 7057 mothers attend each year, and under, ANC there are 3 residents, 4 midwives, and 3 general practitioners.

Urine dipstick, urine microscopy, and culture are the methods used to diagnose UTIs in these hospital laboratories. Studies showed there is a high practice of inappropriate antibiotic usage, including self-medication and non-prescription supply of antibiotics, as well as poor infection prevention and control strategies in the community of Bahir Dar city and healthcare settings (Gebeyehu *et al.*, 2015, Tekle *et al.*, 2025, Yallew *et al.*, 2017). Furthermore, studies showed increasing AMR and the presence of ESBL-carbapenemase-producing pathogens in those study settings (Moges *et al.*, 2019, Asmare *et al.*, 2024).

4.2. Study design and period

An institution-based cross-sectional study was conducted from 9 February to 8 May 2026 in Bahir Dar, Northwest Ethiopia.

4.3. Population

4.3.1. Source population

The source population comprised pregnant women of all age groups attend ANC at selected institutions.

4.3.2. Study population

The study population was pregnant women attending ANC who were clinically suspected of UTI during the study period.

4.4. Inclusion and exclusion criteria

4.4.1. Inclusion criteria

Pregnant women regardless of their gestational period, and attending ANC in the selected facilities with signs and symptoms of UTIs were included in the study.

4.4.2. Exclusion criteria

Pregnant women who took antibiotics two weeks before the time of data collection period were excluded in the study (Mohamoud *et al.*, 2021).

4.5. Variables

4.5.1. Dependent variable

- Significant bacteriuria.

4.5.2. Independent variables

- Socio-demographic status: age, marital status, residence, educational status, occupational status, and family income.
- Pregnancy related factors: Gestational period, gravidity, parity.
- Clinical related variables: Previous history of UTI, history of diabetes mellitus, history of hospitalization last 3 month, history of catheterization last 3 month.
- Behavioral factors: direction of vaginal wash after toilet, habit of post coital urination.

4.6. Sample size determination

The sample size was determined by using single population proportion formula: $n = (Z\alpha/2)^2 P(1 - P)/d^2$ and taking the estimated prevalence of symptomatic UTI among pregnant women, $p = 18.9\%$ (0.189), from a previous study conducted in Bahir Dar (Demilie *et al.*, 2012). Considering a 95% confidence interval ($Z\alpha/2$) and a 5% margin of error (d), the sample size was calculated as $n = (1.96)^2 * 0.189(1 - 0.189) / (0.05)^2 = 236$. Considering a 10% non response rate, the final sample size was 260, and the sample size was determined using the estimated prevalence of ESBL, $P = 18.2\%$ (0.182), which was taken from the study in Gondar (Biset *et al.*, 2020), and the sample size was calculated as $n = (1.96)^2 * 0.182(1 - 0.182) / (0.05)^2 = 229$. By considering a 10% non response rate, the sample size was 252. The Sample size was also calculated using associated factor using Epi Info software, but it was lower. So, the sample size calculated using the prevalence of symptomatic UTI was higher, and the final and minimum sample size was found to be 260. The sample size was proportionally allocated based on yearly ANC flow in the study setting. So, the total flow was 10,967 in FHCSH and 7057 in TGSTH.

The total ANC flow in those study settings was $= 10,967 + 7,057 = 18,024$. So, the sample for each study setting was $\text{FHCSH} = 10,967 / 18,024 * 260 = 158$, and $\text{TGSTH} = 7057 / 18,024 * 260 = 102$.

Table 3 : Sample size determination using associated factors of UTI among pregnant women

Variable	Confidence level (%)	Power(%)	% Outcome in unexposed	AOR	Sample size (n)	After adding 10% non-response rate	Reference
History of catheterization	95	80	11.2	3.270	176	194	(Belete, 2020)
History of UTI	95	80	5.5	8.824	74	81	(Belete, 2020)
Direction of vaginal wash after toilet	95	80	39.3	2.853	134	147	(Edae et al., 2020)
Postcoital urination	95	80	10.7	3.160	194	213	(Edae et al., 2020)
Family monthly income	95	80	4	5.581	168	185	(Emiru et al., 2013)

4.7. Sampling technique and procedure

A convenience sampling technique was used. All pregnant women who fulfilled the inclusion criteria during the study period were consecutively enrolled until the desired sample size was attained.

4.8. Data collection

4.8.1. Socio-demographic data

A structured questionnaire was used to obtain information related to sociodemographic, clinical, behavioral, and pregnancy related data. Those data were collected after explaining the background information, the aim of the study, and obtaining written consent from study participants.

4.8.2. Laboratory data

4.8.2.1. Specimen collection and transportation

From each participant, about 10 ml of clean catch mid-stream urine was collected using a sterile, leak proof, wide-mouth plastic container after giving proper instructions to the women on how to collect midstream urine. The container was labeled with the unique sample number, date, and time of collection. Then the urine specimens were transported to the medical microbiology laboratory of the Department of Medical Microbiology, School of Medical Laboratory Sciences, College of Medicine and Health Sciences, Bahir Dar University, Ethiopia, using an icebox and processed as soon as possible.

4.8.2.2. Bacterial Isolation and identification

A 1 μ L volume of urine specimens were directly streaked in to blood and MacConkey agar. After that, the plates were incubated under the aerobic condition at 37°C for 24 hours, and then colonies were counted to check the presence of significant growth. Colony counts yielding bacterial growth of $\geq 10^5$ CFU/mL of urine were regarded as SBU. Growths of more than two mixed isolates were considered contamination. Identification of bacterial species was done using colony characteristics, gram staining, and a panel of biochemical tests following the standard procedure. The Gram-positive bacteria were identified using catalase, coagulase, and mannitol salt agar. The Gram-negative bacteria were identified by indole production, H₂S production, citrate utilization, urease test, motility test, and carbohydrate utilization tests (Karah *et al.*, 2020, Silverman and Turrentine, 2023, Juszczak *et al.*, 2024).

4.8.2.3. Antimicrobial susceptibility test

AST was carried out by the Kirby-Bauer disc diffusion method. Identical 3-5 pure and fresh colonies from blood agar plate were suspended in 5ml of normal saline and were mixed thoroughly to make the suspension homogenous. The inoculum turbidity was adjusted to 0.5% McFarland standards. Then, the bacterial suspensions were seeded on the surface of the Muller-Hinton Agar (MHA) using a sterile cotton swab. Within less than 15 minutes of inoculum preparation, the antimicrobial impregnated disks were placed on the media using sterile forceps with 15 mm distance from the edge of the plate and not close to 24 mm from each other (center to center), and plates were incubated at 37°C for 24 hours. The zone of inhibitions were measured and interpreted as sensitive, intermediate, and resistant as per the Clinical Laboratory Standards Institute (CLSI) protocol (CLSI, 2025).

The following disks were used for Gram-negative bacteria; Ampicillin (AMP, 10µg), amoxicillin-clavulanic acid (AMC: 20/10 µg), ceftriaxone (CRO: 30 µg), ceftazidime (CAZ: 30 µg), meropenem (MER: 10 µg), gentamicin (GEN: 10 µg), amikacin (AMK, 30µg), Nalidixic acid (NA: 30 µg), ciprofloxacin (CIP: 5 µg), nitrofurantoin (NI: 300 µg) and trimethoprim-sulfamethoxazole (SXT: 23.75/1.25 µg). For Gram-positive bacteria; Penicillin (PEN: 10µg), cefoxitin (30µg), trimethoprim-sulfamethoxazole (SXT: 1.25/23.75µg), tetracycline (TTC: 30µg), gentamicin (GEN: 10µg), nitrofurantoin (NI: 300µg), ciprofloxacin (CIP: 5µg), clindamycin (CL: 10µg), and chloramphenicol (CAF, 30µg) were used. These discs were selected based on the local prescription pattern for UTIs and the recommendations of the CLSI (CLSI, 2025).

4.8.2.4 Extended spectrum beta-lactamase detection

4.8.2.4.1 Screening method

ESBL-production of Gram-negative bacteria was screened by considering the zone of inhibition diameters produced by ceftazidime or ceftriaxone in the disk diffusion technique. Isolates with zone of inhibition diameters of ≤ 22 mm for ceftazidime and ≤ 25 mm for ceftriaxone were considered as likely ESBL producers according to the CLSI 2025 (CLSI, 2025).

4.8.2.4.1 Confirmation of ESBL production

ESBL-production among potential ESBL-producing isolates was confirmed phenotypically using combined disc diffusion method. Comparison of the zone of inhibition was made for the ceftazidime (30µg) versus that of the ceftazidime + clavulanic acid (30 µg/10 µg) which were placed at appropriate distance on MHA plate inoculated with a bacterial suspension of 0.5% McFarland turbidity standards and incubated overnight (18–24 h) at 37 °C. An increase in the inhibition zone diameter of >5 mm for a combination disc versus ceftazidime alone was confirmed as ESBL producer as per CLSI 2025 guideline (CLSI, 2025).

4.8.2.5. Carbapenemase detection

Carbapenemase-production of Gram-negative bacteria was screened by using meropenem disks. The suspension of isolated bacteria was inoculated onto MHA, and then meropenem disk was placed and incubated at 37°C for 18-24 hrs. If the zone of inhibition is ≤ 19 mm, it was considered as a presumptive carbapenemase-producer. Suspected carbapenemase-producing isolate was confirmed by the modified carbapenem inactivation methods, which are recommended by CLSI. After preparation of bacterial suspension of tested isolates in 2 ml trypticase soya broth, meropenem disk (10 µg) was added and incubated at $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 4 hours ± 15 minutes. When the time is completed, meropenem disk (10 µg) was placed on a MHA previously inoculated with meropenem susceptible *E. coli* ATCC 25922 suspension of 0.5 % McFarland turbidity standards and incubated overnight (18–24 hrs.) at 37°C. Bacterial isolates having a zone of inhibition 6–15 mm or presence of pinpoint colonies within a 16–18 mm zone and no inhibition of the meropenem-susceptible *E. coli* ATCC 25922 was confirmed as carbapenemase-producing (CLSI, 2025).

Table 4: Summary of antimicrobial agents, class and role in the study (CLSI, 2025).

Antibiotic class	Selected drugs	Reason for selection	Role in AST	Cross-resistance
Penicillin's	Ampicillin , Penicillin	To asses resistance to locally prescribed drugs for UTI. First line therapy in pregnancy	Routine AST test	amoxicillin and ampicillin are equivalent agents
Penicillins/ β lactamase inhibitor	Amoxicillin-Clavulanic acid	To asses resistance to locally prescribed drugs for UTI First line therapy in pregnancy	Routine AST test	
Cephalosporins	Ceftriaxone, Ceftazidime (3 rd gen),	One of the most commonly used antibiotics for UTIs (sever cases) and used for ESBL tests	Routine AST and screening for ESBL	Cefotaxime and ceftriaxone equivalent agents
	Ceftazidime + Clavulanic acid	For ESBL test	ESBL confirmatory testing	
	Cefoxitin (2 nd -gen)	Surrogate agent for methicillinresistance.		
Carbapenems	Meropenem	Last-resort for MDR Gram negatives and ESBL producing	Routine AST test and carbapenemas e testing	
Aminoglycosides	Gentamicin, Amikacin	Treatment option for sever UTI when first line ineffective	Routine AST test	
Quinolones	Ciprofloxacin,Nalidixic acid	Serve as alternative treatment when first line ineffective Or not avail	Routine AST test	Ciprofloxacin or levofloxacin are equivalent agents
Tetracyclines	Tetracycline	Serve as alternative treatment when first line ineffective Or not avail	Routine AST test	Equivalent tetracyclines (doxycycline, minocycline)
Lincosamides	Clindamycin	Treatment option for UTI in gram positive	Routine AST test	
Sulfonamides	Cotrimoxazole	Broad-spectrum used in UTI	Routine AST test	
Phenicols	Chloramphenicol	Treatment option for sever UTI when first line ineffective	Routine AST test	
Nitrofurans	Nitrofurantoin	First line therapy	Routine AST	

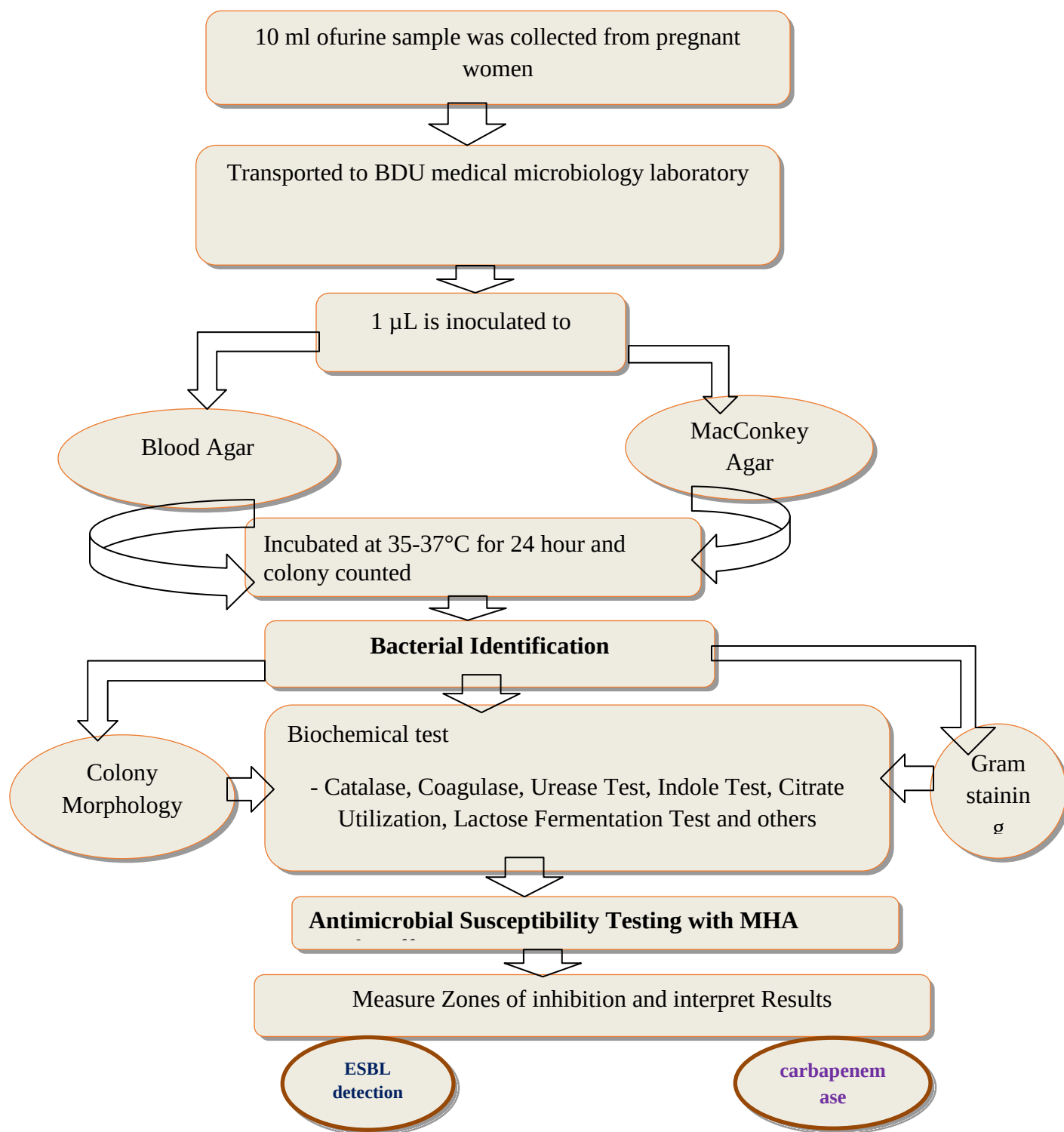


Figure 2: Flow chart for urine processing, identification the isolates, AST test and ESBL-Carbapenemase detection, Bahir Dar, 2026.

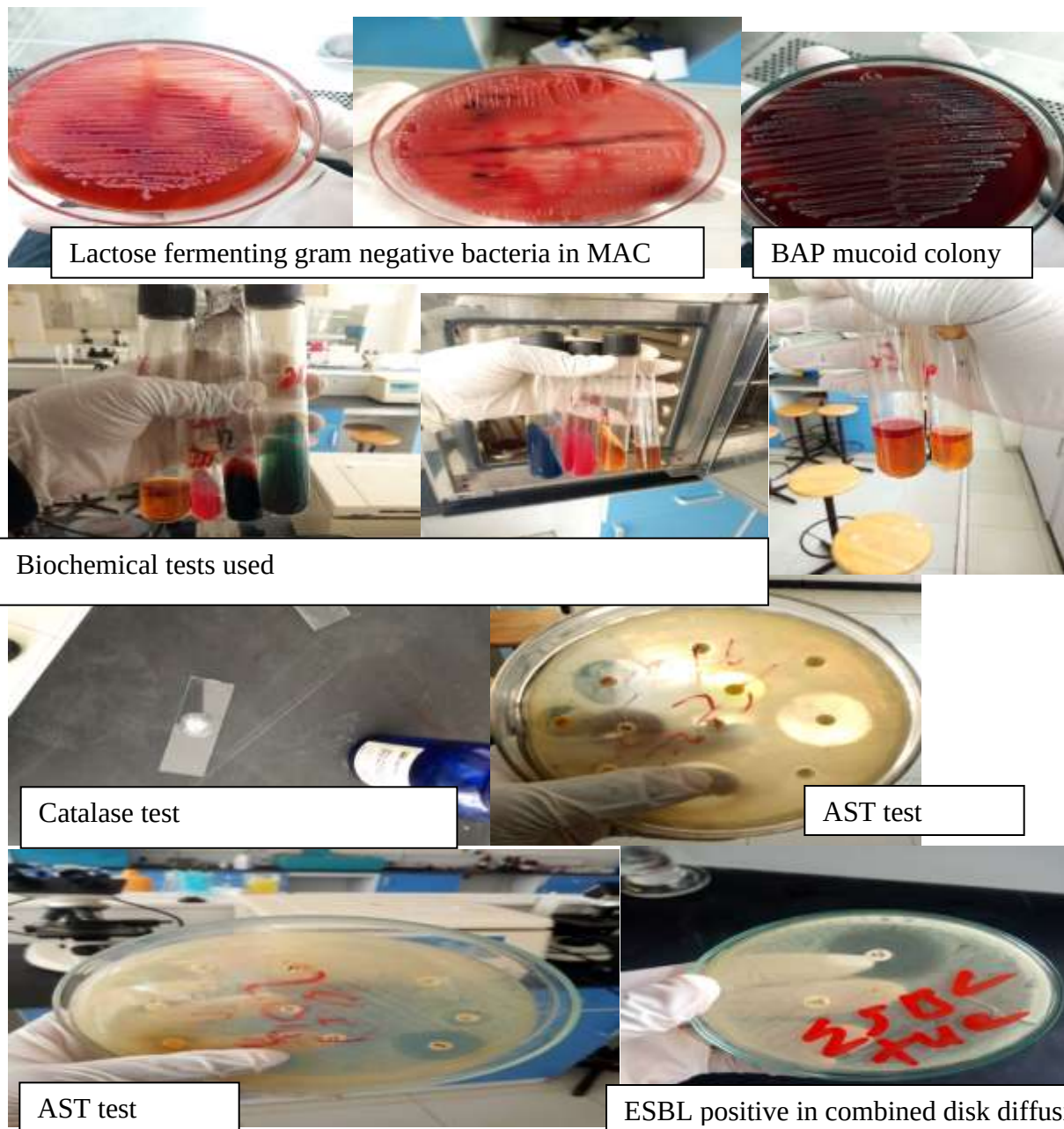


Figure 3: Some pictures taken in the laboratory during urine culture of pregnant women with symptomatic UTI attending ANC at FHCSH and TGSTH, Bahir Dar, north-west Ethiopia, 2026

4.9. Quality control

The data collection tool was adopted from different related pieces of literatures (Biset *et al.*, 2020, Belete, 2020, Mohamoud *et al.*, 2021, Edae *et al.*, 2020), and all the questions in the structured questionnaire were prepared in a clear way and translated in to the local language, i.e. Amharic. To ensure the quality of data, the data collection tool was pretested at Addis Alem Primary Hospital with 5% of the sample size before the data collection period. Training was given for data collectors, and the questionnaires were checked for completeness during and after data collection.

All laboratory tests were done by maintaining Standard Operating Procedures (SOPs). All urine culture specimens were collected by following SOPs and were processed soon after collection. Expiry dates of culture media, antibiotic disks, and reagents were checked. The sterility of culture media was checked by incubating 3–5% of the batch at 35–37°C overnight and observing for bacterial and fungal growth. In addition, the performance of culture media and antibiotic disks was evaluated by using standard quality control strains, such as *E. coli* ATCC 25922 and *S. aureus* ATCC 25923. Standard reference strains such as *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 for ESBL-production, *K. pneumoniae* ATCC BAA-1706 and *K. pneumoniae* ATCC BAA-1705 for carbapenemase-production were used as control, respectively (CLSI, 2025).

4.10. Data processing and analysis

The Data were entered and analyzed using Statistical Package for the Social Sciences (SPSS) version 26. Descriptive statistics such as frequency, percentage, and mean were used to summarize the data. Normality test was used to check the distribution of continuous variables. Multicollinearity of independent variables was assessed by variance inflation factor and tolerance test. Both bivariable and multivariable logistic regression analyses were applied to determine the association between dependent and independent variables. Variable with p value less than 0.2 in bivariable logistic regression were entered into multivariable logistic regression analysis to control the possible effect of confounding variables. A p values ≤ 0.05 with 95% confidence interval were considered statistically significant. Then, the results of the study were finally presented using tables and charts.

4.11. Ethical considerations

The study was approved by the Institutional Review Board of the College of Medicine and Health Sciences, Bahir Dar University, with protocol number 6023/2026. A support letter was obtained from the Department of Medical Microbiology, School of Medical Laboratory Sciences, College of Medicine and Health Sciences, Bahir Dar University. Further support letter and permission were obtained from the administrators of the study health institutions and Amhara Public Health Institute. Then, written informed consent was obtained from the study participants before the collection of clinical data and urine samples. The purpose and importance of the study as well as the rights of participants were clearly explained. The participant's data were coded and put in a secure place, and confidentiality was maintained. Results important for the management of the patients were given to the attending physician.

4.12. Plan for dissemination of results

The result will be presented to the Department of Medical Microbiology, School of Medical Laboratory Sciences, College of Medicine and Health Science, Bahir Dar University. Besides this, the findings of the research will be presented and communicated to the international community through conferences and publication in a peer reviewed journal.

5. RESULTS

5.1 Socio-demographic characteristics of the study participants

In this study, a total of 260 symptomatic pregnant women were included. The mean age of study participants was 28.79 ± 5.1 years. The majority of study participants were urban residents 185 (71.2%), married 234 (90%), housewives 185 (51.9%), and completed secondary school 71 (27.3%) (Table 5).

Table 5: Socio demographic Characteristics of the Study participants (n=260) in FHCSH and TGSTH, Bahir Dar, north-west Ethiopia, 2026.

	Variables	Frequency	Percentage (%)
Age (years)	15-24	49	18.8
	25-34	159	61.2
	35-45	52	20
Residence	Urban	185	71.2
	Rural	75	28.8
Marital status	Single	10	3.8
	Married	234	90
	Divorced	16	6.2
Educational status	Unable to read and write	40	15.4
	Able to read and write	30	11.5
	Primary school	59	22.7
	Secondary school	71	27.3
	College and above	60	23.1
Occupation	House wife	135	51.9
	Private business	64	24.6
	Government employed	46	17.7
	Others	15	5.8
Monthly family income (ETB)	<5,000	48	18.5
	5,000-10,000	159	61.2
	>10,000	53	20

5.2. Pregnancy, behavioral and clinical characteristics of the study participants

Among 260 study participants, 127 (48.8%) and 91 (35%) were in their 3rd and 1st trimesters, respectively. The majority of the participants were multigravidae 178(68.5%)and multiparous 94 (36.2%). Regarding behavioral characteristics, 245 (94.2%) of pregnant women didnot have the habit of urination after sexual intercourse, and 235 (90.4%) of them cleaned their genital area from back to front. Thirty six (13.8%), 11 (4.2%), 19 (7.3%), and 19 (7.3%) of study participants had a history of previous UTI, catheterization, diabetes mellitus, and hospitalization, respectively(Table 6).

Table 6: Pregnancy, behavioral and clinical characteristics of the study participants (n=260) in FHCSH and TGSTH, Bahir Dar, north-west Ethiopia, 2026.

Variables		Frequency	Percentage (%)
Habit of urination after sexual intercourse	Yes	15	5.8
	No	245	94.2
Direction cleaning of genitalia after defecation or urination	Forward (front to back)	25	9.6
	Backward (Back to front)	235	90.4
Gestational period (trimester)	First trimester	91	35
	Second trimester	42	16.2
	Third trimester	127	48.8
Gravidity	Primigravidae	82	31.5
	Multigravidae	178	68.5
Parity	Nulliparous	82	31.5
	Primiparous	84	32.3
	Multiparous	94	36.2
History of UTI	Yes	36	13.8
	No	224	86.2
History of catheterization (in last3 months)	Yes	11	4.2
	No	249	95.8
History of Diabetes Mellitus	Yes	19	7.3
	No	241	92.7
History of hospitalization (in last3 months)	Yes	19	7.3
	No	241	92.7

5.3. Types of the identified bacterial uropathogens

The proportion of significant bacteriuria (SBU) was 21.2% (55/260; 95% CI: 16.0–26.0%). Seven different bacterial species were isolated. The majority of the identified organisms were GNRs at 40 (72.7%), while the remaining 15 (27.3%) were Gram-positive bacteria. No multiple isolates with significant count were identified from a single specimen.

Escherichia coli was the predominant bacterial isolate, accounting for 31 (56.4%), followed by *CoNS* (18.2%), *Klebsiella pneumoniae* 5 (9.1%), *Staphylococcus aureus* 5 (9.1%), *Enterobacter* species 2 (3.6%), *Citrobacter* species 1 (1.8%), and *Proteus mirabilis* 1 (1.8%) (Figure 4).

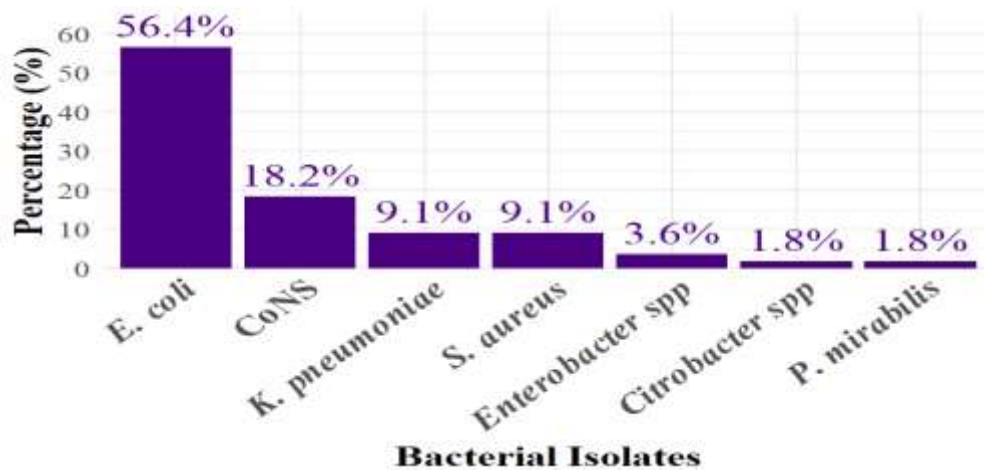


Figure 4: Isolated bacterial uropathogens among pregnant women with symptomatic UTI attending ANC at FHCSH and TGSTH, Bahir Dar, north-west Ethiopia, 2026

5.4. Antimicrobial Susceptibility Profile of the isolates

Summary of the antimicrobial susceptibility profile of the isolates is presented in Table 7 and 8. Among Gram-negative uropathogens (n=40), majority of them showed a relatively higher rate of resistance to ampicillin 37 (92.5%), amoxicillin-clavulanic acid 23 (57.5%), and trimethoprim-sulfamethoxazole 24 (60%). On the other hand, these isolate showed better sensitivity against meropenem, amikacin, and nitrofurantoin, each accounting for 39 (97.5%). Likewise, the Gram-negative isolates showed a relatively low level of resistance against nalidixic acid, ceftriaxone, ceftazidime, ciprofloxacin, and gentamicin, which ranged from 52.5% to 85% sensitivity.

Specifically, *E. coli* isolates were found resistant to ampicillin (90.3%), augmentin (54.8%), and trimethoprim-sulfamethoxazole (58.1%). However, they were found susceptible to nitrofurantoin (100%), amikacin (96.8%), and meropenem (96.8%) (Table 7).

Table 7:- Antimicrobial susceptibility profile of Gram-negative isolates from pregnant women with symptomatic UTI attending ANC at FHCSH and TGSTH, Bahir Dar, north-west Ethiopia, 2026.

Bacterial isolates	Pattern	Antimicrobial agents No (%)										
		AMP	AMC	CRO	CAZ	MEM	AMK	NA	GEN	CIP	NI	SXT
<i>E. coli</i> (n=31)	S	3 (9.7)	14(45.2)	23(74.2)	26(83.9)	30(96.8)	30(96.8)	18(58.1)	27(87.1)	23(74.2)	31(100)	13(41.9)
	I	0 (0)	0(0)	0(0)	0(0)	0(0)	1(3.2)	0(0)	0(0)	0(0)	0(0)	0(0)
	R	28 (90.3)	17(54.8)	8(25.8)	5(16.1)	1(3.2)	0(0)	13(41.9)	4(12.9)	8(25.8)	0(0)	18(58.1)
<i>K. pneumoniae</i> (n=5)	S	0(0)	1(20)	3(60)	3(60)	5(100)	5(100)	1(20)	3(60)	1(20)	5(100)	1(20)
	I	0(0)	1(20)	0(0)	0(0)	0(0)	0(0)	0(0)	1(20)	1(20)	0(0)	0(0)
	R	5(100)	3(60)	2(40)	2(40)	0(0)	0(0)	4(80)	1(20)	3(60)	0(0)	4(80)
<i>Enterobacter species</i> (n=2)	S	0(0)	0(0)	1(50)	1(50)	1(100)	1(100)	1(50)	1(100)	1(50)	1(100)	1(50)
	R	2(100)	2(100)	1(50)	1(50)	0(0)	0(0)	1(50)	0(0)	1(50)	0(0)	1(50)
<i>Citrobacter species</i> (n=1)	S	0(0)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)
	R	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
<i>Proteus mirabilis</i> (n=1)	S	0(0)	0(0)	0(0)	0(0)	1(100)	1(100)	0(0)	1(100)	0(0)	0(0)	0(0)
	R	1(100)	1(100)	1(100)	1(100)	0(0)	0(0)	1(100)	0(0)	1(100)	1(100)	1(100)
Total (n=40)	S	3(7.5)	16(40)	28(70)	31(77.5)	39(97.5)	39(97.5)	21(52.5)	34(85)	26(65)	39(97.5)	16(40)
	I	0(0)	1(2.5)	0(0)	0(0)	0(0)	1(2.5)	0(0)	1(2.5)	1(2.5)	0(0)	0(0)
	R	37(92.5)	23(57.5)	12(30)	9(22.5)	1(2.5)	0(0)	19(47.5)	5(12.5)	13(32.5)	1(2.5)	24(60)

Abbreviations: S=sensitive, I= intermediate, R= resistant, AMP=ampicillin, AMC=amoxicillin-clavulanic acid, CRO=ceftriaxone, CAZ=ceftazidime, MEM=meropenem, AMK= amikacin, NA=nalidixic acid, GEN= gentamicin, CIP=ciprofloxacin, NI=nitrofurantoin, SXT=trimethoprim-sulfamethoxazole.

Similarly, the antimicrobial susceptibility profile of Gram-positive isolates(n=15) is presented in Table 8, and the recorded resistance level ranged from 0% to 100%. A high level of resistance was observed against penicillin 15 (100%), trimethoprim-sulfamethoxazole 12 (80%), and tetracycline 11 (73.3%). However, all of these isolates showed a high rate of sensitivity at 15 (100%) to both nitrofurantoin and gentamicin. *CoNS*, which were the dominant Gram-positive isolate, were resistant to penicillin (100%), trimethoprim-sulfamethoxazole(90%), and tetracycline(70%), while they were found susceptible to gentamicin (100%), nitrofurantoin (100%), and clindamycin (90%)(Table 8).

Table 8: Antimicrobial susceptibility profile of Gram-positive isolates from pregnant women with symptomatic UTI attending ANC at FHCSH and TGSTH, Bahir Dar, north-west Ethiopia, 2026.

Bacterial isolates	Pattern	Antimicrobial agents No (%)								
		PEN	FOX	SXT	TTC	GEN	NI	CIP	CL	CAL
<i>S. aureus</i> (n=5)	S	0(0)	3(60)	2(40)	1(20)	5(100)	5(100)	3(60)	4(80)	3(60)
	I	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(20)
	R	5(100)	2(40)	3(60)	4(80)	0(0)	0(0)	2(40)	1(20)	1(20)
<i>CoNS</i> (n=10)	S	0(0)	6(60)	1(10)	3(30)	10(100)	10(100)	6(60)	9(90)	6(60)
	R	10(100)	4(40)	9(90)	7(70)	0(0)	0(0)	4(40)	1(10)	4(40)
Total (n=15)	S	0(0)	9(60)	3(20)	4(26.7)	15(100)	15(100)	9(60)	13(86.7)	9(60)
	I	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(6.7)
	R	15(100)	6(40)	12(80)	11(73.3)	0(0)	0(0)	6(40)	2(13.3)	5(33.3)

Abbreviations: S= sensitive, I= intermediate
R=resistantPEN=penicillin,FOX=cefoxitin,SXT=trimethoprim-sulfamethoxazole, TTC, tetracycline, GEN- gentamicin,NI=nitrofurantoin, CIP= ciprofloxacin, CL= clindamycin, CAF= chloramphenicol.

5.5. Multidrug resistance (MDR) profile of the isolates

In this study, only 3 (5.5%) of the 55 isolates were found sensitive to all antibiotics tested. The rest, 52 (94.5%), were found resistant to at least one antibiotic used in the study. While 45 (81.8%) of isolates were resistant to two or more antimicrobial agents. In this study, MDR was described as a bacterium developing resistance to at least one agent in three or more classes of antibiotics. The level of MDR was 32 (58.2%).

Among Gram-negative bacteria, 22/40 (55%) and among Gram-positive bacteria, 10/15 (66.7%) were MDR. Specifically, 16 (51.6%) of *E. coli*, 4 (80%) of *K. pneumoniae*, and 1 (50%) of *Enterobacter* isolates were MDR. Among the five *S. aureus* and ten CoNS, 3 (60%) and 7 (70%) of these isolates were MDR, respectively (Table 9).

Table 9: Multi-drug resistance profile of bacteria isolated from pregnant women with symptomatic UTI attending ANC at FHCSH and TGSH, Bahir Dar, north-west Ethiopia, 2026.

Antimicrobial agents	Bacterial Isolates (n=55)							Total N (%)
	<i>E. coli</i> (n=31)	<i>K. pneumoniae</i> (n=5)	<i>Enterobacter</i> (n=2)	<i>Citrobacter</i> (n=1)	<i>P. mirabilis</i> (n=1)	<i>S. aureus</i> (n=5)	<i>CoNS</i> (n=10)	
R0	3	0	0	0	0	0	0	3(5.5)
R1								
AMP	3	1	0	1	0	-	-	5
PEN	-	-	-	-	-	1	1	2
Total	3	1	0	1	0	1	1	7(12.7)
R2								
AMP+SXT	2	0	0	0	0	-	-	2
AMP+AMC	7	0	1	0	0	-	-	8
PEN+SXT	-	-	-	-	-	0	2	2
PEN+TTC	-	-	-	-	-	1	0	1
Total	9	0	1	0	0	1	2	13(23.6)
R3								
AMP+NA+SXT	2	0	0	0	0	-	-	2
AMP+SXT+CRO	1	0	0	0	0	-	-	1
PEN+SXT+TTC	-	-	-	-	-	1	3	4
Total	3	0	0	0	0	1	3	7(12.7)

Table 10(continued).

Antimicrobial agents	Bacterial Isolates (n=55)							Total N (%)
	<i>E. coli</i> (n=31)	<i>K.pneumoniae</i> (n=5)	<i>Enterobacter</i> (n=2)	<i>Citrobacter</i> (n=1)	<i>P. mirabilis</i> (n=1)	<i>S. aureus</i> (n=5)	<i>CoNS</i> (n=10)	
R4								
AMP+NA+SXT+CIP	2	1	0	0	0	-	-	3
AMP+AMC+NA+SXT	1	1	0	0	0	-	-	2
AMP+AMC+CRO+SXT	1	0	0	0	0	-	-	1
AMP+AMC+GEN+SXT	1	0	0	0	0	-	-	1
Total	5	2	0	0	0	0	0	7(12.7)
≥R5								
AMP+AMC+NA+SXT+CIP	1	0	0	0	0	-	-	1
AMP+NA+CRO+SXT+CIP	1	0	0	0	0	-	-	1
AMP+AMC+NA+CRO+CAZ+SXT+CIP	3	1	1	0	0	-	-	5
AMP+NA+CRO+CAZ+SXT+CIP+GEN	1	0	0	0	0	-	-	1
AMP+AMC+NA+CRO+CAZ+SXT+CIP+MEM	1	0	0	0	0	-	-	1
AMP+AMC+GEN+SXT+NA	1	0	0	0	0	-	-	1
AMP+AMC+NA+CRO+CAZ+SXT+CIP+GEN	0	1	0	0	0	-	-	1
AMP+AMC+NA+CRO+CAZ+SXT+CIP+NI	0	0	0	0	1	-	-	1
CIP+SXT+PEN+FOX+TTC	-	-	-	-	-	1	0	1
CIP+SXT+PEN+FOX+TTC+CAF	-	-	-	-	-	0	3	3
CIP+SXT+PEN+FOX+TTC+CAF +CL	-	-	-	-	-	1	1	2
Total	8	2	1	0	1	2	4	18(32.7)
MDR	16	4	1	0	1	3	7	32(58.2)

Abbreviations: R0 - No antibiotic resistance, R1- Resistance to only one drug tested, R2- Resistance to only two drugs tested, R3- Resistance to only three drugs tested, R4- Resistance to only four drugs tested, ≥R5- Resistance to five or more drugs tested, MDR- multi drug resistant (bacteria resistant to at least one agent in three or more classes of antibiotics). –Not applicable/not tested, AMP=ampicillin, AMC=amoxicillin-clavulanic acid, CRO=ceftriaxone, CAZ=ceftazidime, MEM=meropenem, AMK= amikacin, NA=nalidixic acid, GEN- gentamicin, CIP=ciprofloxacin, NI=nitrofurantoin, SXT=trimethoprim-sulfamethoxazole. PEN=penicillin, FOX=cefoxitin, TTC, tetracycline, CL= clindamycin, CAF= chloramphenicol.

5.6. Magnitude of ESBL and carbapenemase production

Forty of the identified isolates were members of Enterobacteriaceae, which include *E.coli* 31 (77.5%), *K.pneumoniae* 5 (12.5%), *Enterobacter species* 2 (5%), *Citrobacter species* 1 (2.5%), and *Proteus mirabilis* 1 (2.5%). Since the method for screening of ESBL productions were not validated for *Enterobacter* and *Citrobacter species*, those isolates were excluded from screening. The proportion of ESBL production among these Enterobacteriaceae was 16.2% (n=6/37). *E. coli* 4 (12.9%) and *K.pneumoniae* 2 (40%) were found ESBL-producing isolates. Only one isolate of *E. coli* was found carbapenemase-producing, and it was also ESBL-producer; thus, the level of carbapenemase-producing *E. coli* was 2.5% (1/40) (Table 10).

Table 11: Magnitude of ESBL-Producing *Enterobacteriaceae* isolated from pregnant women with symptomatic UTI attending ANC at FHCSH and TGSTH, Bahir Dar, north-west Ethiopia, 2026.

Bacterial isolates	ESBL production	
	Positive	Negative
<i>E. coli</i> (n=31)	4 (12.9%)	27 (87.1%)
<i>K. pneumoniae</i> (n=5)	2 (40%)	3 (60%)
<i>Proteus mirabilis</i> (n=1)	0 (0%)	1 (100%)
<i>Enterobacter species</i> (n=2)	NA	NA
<i>Citrobacter species</i> (n=1)	NA	NA
Total (n=40)	6/37 (16.2%)	31/37 (83.7%)

- NA: Not applicable

Regarding the antimicrobial susceptibility profile of ESBL-producing isolates, all (100%) were resistant to ampicillin, 3rd generation cephalosporin, nalidixic acid, ciprofloxacin, and trimethoprim-sulfamethoxazole. In contrast, they were found sensitive to meropenem (83.3%), nitrofurantoin (100%), and amikacin (100%) (Table 11).

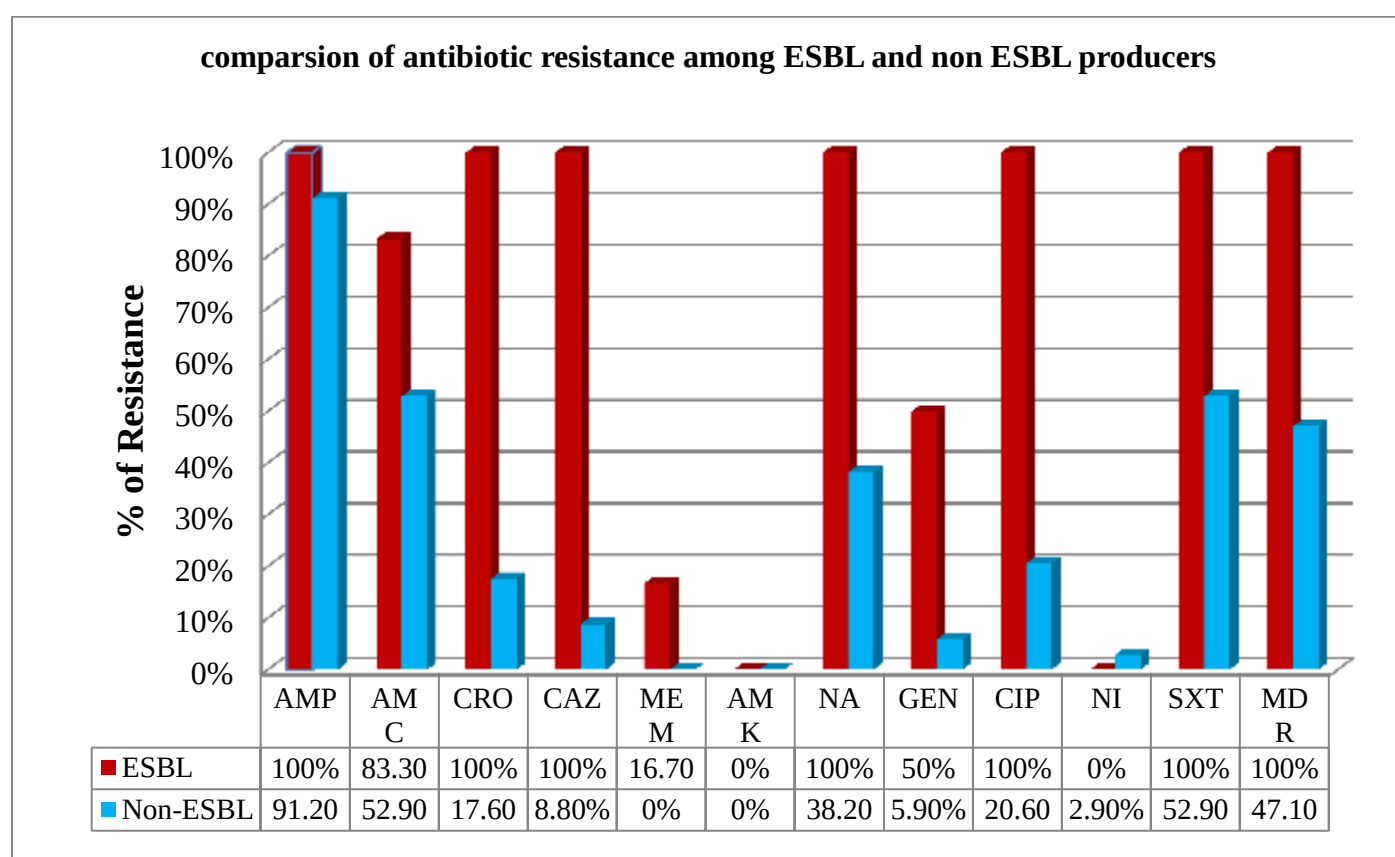
E.coli isolates, which accounted for 66.7% of ESBL-producers, were 100% resistant to ampicillin, ceftriaxone, ceftazidime, nalidixic acid, ciprofloxacin, and trimethoprim-sulfamethoxazole. However, they were sensitive to meropenem, nitrofurantoin, and amikacin, with sensitivity ranging from 75%-100%. Likewise, *K.pneumoniae*, another ESBL producers were also found 100% resistant to ampicillin, amoxicillin-clavulanic acid, ceftriaxone, ceftazidime, nalidixic acid, ciprofloxacin, and trimethoprim-sulfamethoxazole (Table 11).

Table 12: Antimicrobial susceptible pattern of ESBL and carbapenemase producing *Enterobacteriaceae* isolated from pregnant women with symptomatic UTI attending ANC at FHCSH and TGSTH, Bahir Dar, north-west Ethiopia, 2026.

Bacteria l isolates	Patte rn	Antimicrobial agents No (%)										
		AMP	AMC	CRO	CAZ	MEM	AMK	NA	GEN	CIP	NI	SXT
<i>E.coli</i> (n=4) ESBL positive	S	0 (0)	1(25)	0 (0)	0 (0)	3(75)	4(100)	0 (0)	2(50)	0 (0)	4(100)	0 (0)
	R	4 (100)	3(75)	4 (100)	4 (100)	1(25)	0(0)	4 (100)	2(50)	4 (100)	0(0)	4 (100)
<i>K. pneumo niae</i> (n=2) ESBL positive	S	0(0)	0(0)	0(0)	0(0)	2(100)	2(100)	0(0)	0(0)	0(0)	2(100)	0(0)
	I	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(50)	0(0)	0(0)	0(0)
	R	2(100)	2(100)	2(100)	2(100)	0(0)	0(0)	2(100)	1(50)	2(100)	0(0)	2(100)
Total (n=6) ESBL positive	S	0(0)	1 (16.7)	0(0)	0(0)	5 (83.3)	6 (100)	0(0)	2 (33.3)	0(0)	6 (100)	0(0)
	I	0(0)	0(0)	0(0)	0(0)	0 (0)	0 (0)	0(0)	1 (16.7)	0(0)	0 (0)	0(0)
	R	6(100)	5 (83.3)	6(100)	6(100)	1 (16.7)	0 (0)	6(100)	3 (50)	6(100)	0 (0)	6(100)
<i>E.coli</i> (n=1) CPE	S	0(0)	0(0)	0(0)	0(0)	0(0)	1(100)	0(0)	0(0)	0(0)	1(100)	0(0)
	R	1(100)	1(100)	1(100)	1(100)	1(100)	0(0)	1(100)	1(100)	1(100)	0(0)	1(100)

Abbreviations: S=sensitive, I= intermediate, R= resistant, AMP=ampicillin, AMC=amoxicillin-clavulanic acid, CRO=ceftriaxone CAZ=ceftazidime, MEM=meropenem, AMK= amikacin, NA=nalidixic acid, GEN- gentamicin, CIP=ciprofloxacin, NI=nitrofurantoin, SXT=trimethoprim-sulfamethoxazole, ESBL=Extended Spectrum Beta Lactamase, CPE=Carbapenemase Producing Enterobacteriaceae.

Comparison of the antibiotic resistance by ESBL and non-ESBL producers is shown in Figure 5. Both ESBL (100%) and non-ESBL producer (91.2%) were found resistant to ampicillin. However, ESBL-producing isolates were more resistant to amoxicillin-clavulanic acid (83.3% vs. 52.9%), ceftriaxone (100% vs. 17.6%), ceftazidime (100% vs. 8.8%), nalidixic acid (100% vs. 38.2%), gentamicin (50% vs. 5.9%), ciprofloxacin (100% vs. 20.6%), and trimethoprim-sulfamethoxazole (100% vs. 52.9%) than non-ESBL-producers. Nitrofurantoin, amikacin, and meropenem were found effective antimicrobial agents for both ESBL and non-ESBL-producers. Regarding MDR status, ESBL-producers were 100% MDR, while non-ESBL were 47% MDR (Figure 5).



Abbreviations: AMP=ampicillin, AMC=amoxicillin-clavulanic acid, CRO=ceftriaxone
 CAZ=ceftazidime, MEM=meropenem, AMK= amikacin, NA=nalidixic acid, GEN- gentamicin,
 CIP=ciprofloxacin, NI=nitrofurantoin, SXT=trimethoprim-sulfamethoxazole

Figure 5: Comparison of antibiotic resistance among ESBL and non-ESBL producer isolates from pregnant women attending ANC at FHCSH and TGSTH, Bahir Dar, north-west Ethiopia.

5.7. Factors associated with significant bacteriuria

Bivariable and multivariable logistic regression analyses were applied to determine the association between SBU and independent variables. Assumptions of logistic regression, including no multicollinearity between independent variables, were checked. The analysis showed that all assumptions were fulfilled and there was no multicollinearity between independent variables.

In bivariable logistic regression analysis, variables like age, residence, marital status, direction of cleaning genitalia after defecation or urination, gestational period, history of UTI, history of catheterization, and history of hospitalization were found to be candidates for multivariable analysis with a p-value less than 0.2.

In multivariable logistic regression analysis, age, gestational period, and history of UTI showed significant association (p value <0.05) with SBU. Study participants who were in the age group of 15-24 years had 3.5 times higher odds of SBU than those who were in the age group 35-45 years (AOR=3.5, 95% CI: 1.114- 10.768, P=0.032).

Pregnant women who were on their third trimester gestational period were 3.4 times more likely to have SBU compared with those who were on their first trimester (AOR=3.4, 95% CI: 1.441- 8.141, P=0.005). The odds of SBU were 3.1 times higher among pregnant women who had a history of UTI compared with those who had no history (AOR=3.1, 95% CI: 1.203- 7.845, P=0.019) (Table 12).

Table 13: Bivariate and multivariate logistic regression analyses of factors associated with SBU among pregnant women with symptomatic UTI attending ANC at FHCSH and TGSTH, Bahir Dar, north-west Ethiopia, 2026.

Variables	Culture results		COR(95%CI)	P-value	AOR(95%CI)	P-value
	SBU No (%)	No SBU No (%)				
Age (years)						
15-24	16 (32.7)	33 (67.3)	1.807 (0.739-4.419)	0.195	3.46 (1.114-10.768)	0.032*
25-34	28 (17.6)	131 (82.4)	0.797 (0.365-1.739)	0.568	1.067 (0.431-2.641)	0.889
35-45	11 (21.2)	41 (78.8)	1		1	
Residence						
Urban	34 (18.4)	151 (81.6)	1		1	
Rural	21 (28)	54 (72)	1.727 (0.923-3.232)	0.087	1.725 (0.849-3.502)	0.132
Marital status						
Single	5 (50)	5 (50)	1		1	
Married	45 (19.2)	189 (80.8)	0.238 (0.066-0.858)	0.028	0.292 (0.067-1.268)	0.1
Divorced	5 (31.3)	11 (68.8)	0.455 (0.089-2.318)	0.343	0.694 (0.106-4.553)	0.704
Direction of Cleaning genitalia after defecation or urination						
Forward	8 (32)	17 (68)	1		1	
Backward	47 (20)	188 (80)	0.531 (0.216-1.305)	0.168	0.765 (0.265-2.206)	0.620
Gestational period						
First trimester	12 (13.2)	79 (86.8)	1		1	
Second trimester	13 (31)	29 (69)	2.951 (1.209-7.205)	0.017	2.659 (0.992-7.125)	0.052
Third trimester	30 (23.6)	97 (76.4)	2.036 (0.979-4.235)	0.057	3.426 (1.441-8.141)	0.005*
History of UTI						
Yes	13 (36.1)	23 (63.9)	2.449 (1.147-5.229)	0.021	3.072 (1.203-7.845)	0.019*
No	42 (18.8)	182 (81.3)	1		1	
History of catheterization (in last3 months)						
Yes	5 (45.5)	6 (54.5)	3.317 (0.973-11.30)	0.055	2.724 (0.675-10.990)	0.159
No	50 (20.1)	199 (79.9)	1		1	
History of hospitalization (in last3 months)						
Yes	8 (42.1)	11 (57.9)	3.002 (1.144-7.878)	0.026	2.437 (0.782-7.598)	0.125
No	47 (19.5)	194 (80.5)	1		1	

Abbreviation - COR= Crude odd ratio; AOR=Adjusted odd ratio; CI= Confidence interval, 1= reference group, * P-value < 0.05 statically significant

6. DISCUSSION

UTIs caused by bacteria are among the most prevalent infections that can occur in any region of the urinary system. Pregnant women have a high rate of developing UTIs because of physiological changes that occur in this period (Silverman and Turrentine, 2023). Antibiotic resistance is a rising global problem, in which the emergence of ESBL-carbapenemase-producing uropathogens is an urgent area of research (Husna *et al.*, 2023). Therefore, this study was aimed at determining extended spectrum beta lactamase and carbapenemase profile of uropathogens isolated from pregnant women in Bahir Dar, Northwest Ethiopia.

The overall proportion of symptomatic SBU among pregnant women was 21.2%, which showed that UTI remains a common problem in pregnant women in the study area. This finding is comparable with previous studies conducted in Bahir Dar (18.9%) (Demilie *et al.*, 2012), Harar (20.9%) (Ejerssa *et al.*, 2021), and Nekemte (21%) (Abebe *et al.*, 2025). On the other hand, it was lower than studies conducted in different parts of Ethiopia; Dessie (26.6%) (Belete, 2020), Gondar (31.1%) (Biset *et al.*, 2020), and Bale Zone (35.3%) (Taye *et al.*, 2018), and abroad; Uganda (35%) (Johnson *et al.*, 2021) and Iraq (31.68%) (Taha, 2024). Moreover, it was higher than similar studies conducted in Iran (9.8%) (Omidifar *et al.*, 2020) and the United Arab Emirates (17.9%) (Dube *et al.*, 2022).

The observed variation might be explained by differences in socioeconomic status, hygiene practices, geographic location, and the clinical characteristics of study participants. Altogether, these findings emphasize the importance of regular culture-based screening of SBU in pregnant women for early detection and to minimize complications associated with it.

A total of 55 bacterial uropathogens were isolated, and the majority of them were GNRs at 40 (72.7%). *E. coli* was the predominant uropathogen isolated, accounting for 56.4% of the isolated bacteria. This showed that Gram-negative bacteria of enteric origin, including *E. coli*, remain the primary causes of UTIs in the study area that might be associated with hygiene related issues.

Similarly, a predominance of *E. coli* was observed in studies conducted in Ethiopia, such as in Gondar (49.2%) (Biset *et al.*, 2020), Harar (45.2%) (Ejerssa *et al.*, 2021), and Hawassa (47.8%) (Tula *et al.*, 2020), as well as reports from other countries, including Iraq (56.45%) (Taha, 2024) and South Africa (49.9%) (Orji *et al.*, 2022). UPEC is a commensal of the bowel and possesses virulence factors like pili, which help the bacteria attach to uroepithelial cells and protect them from being flushed out during micturition. This and other similar virulence factors explain its predominance in UTI.

Physicians' options for treating UTIs are limited due to the increase in antibiotic resistance in bacterial uropathogens. In this study, the antibiotics that showed the highest efficacy against Gram-negative isolates were meropenem (97.5% sensitivity), amikacin (97.5% sensitivity), and nitrofurantoin (97.5% sensitivity). This indicates that these drugs remain effective against these uropathogens in the study area. The possible reasons for the observed high rate of sensitivity might be attributed to the infrequent prescription of the drugs in the area. The high susceptibility of Gram-negative isolates to these antibiotics aligns with findings from similar studies conducted in Harar (amikacin 96.4%, nitrofurantoin 90.3%) (Ejerssa *et al.*, 2021) and India (meropenem 100%, nitrofurantoin 87.09%) (Patil *et al.*, 2024). Collectively, these findings highlight the ongoing usefulness of these antibiotics for the treatment of UTIs. In particular, nitrofurantoin remains a drug of choice to treat UTIs in pregnant women because of its high urinary concentration and good safety profile, and it is also recommended by WHO and national guidelines as first-line therapy for UTIs (WHO, 2022, Deneke, 2014).

Conversely, Gram-negative isolates showed a relatively high level of resistance to ampicillin (92.5%), trimethoprim-sulfamethoxazole (60%), and amoxicillin-clavulanic acid (57.5%). The result demonstrates the limited efficacy of commonly used antibiotics in the study area, which might be attributed to prolonged use for many years and empirical prescription. Those results are in line with studies conducted in Gondar (90.9% resistance to ampicillin, 57.6% to amoxicillin-clavulanic acid) (Biset *et al.*, 2020) and Bahir Dar (82.6% resistance to ampicillin, 56.5% to trimethoprim-sulfamethoxazole) (Demilie *et al.*, 2012). These findings emphasize the necessity of conducting antimicrobial susceptibility testing prior to treatment, particularly in pregnant women, for whom the antibiotic choice should consider both efficacy and safety.

Gram-positive bacteria (*S. aureus* and *CoNS*) were resistant to penicillin (100%) and trimethoprim-sulfamethoxazole (80%). This finding is in line with studies conducted in Dessie (93.5% resistance to penicillin, 79.3% to trimethoprim-sulfamethoxazole) (Belete, 2020) and Gondar (89.3% resistance to penicillin, 75% to trimethoprim-sulfamethoxazole) (Biset *et al.*, 2020). The reported high level of drug resistance may be due to the fact that these antibiotics are readily available and are frequently used over the counter by society.

Cefoxitin resistance was observed in 40% of Gram-positive bacteria. This is inconsistent with a study conducted in Gondar, Ethiopia, which reported 42.8% resistance to cefoxitin (Biset *et al.*, 2020). This may indicate that there is a possible rise in the prevalence of methicillin-resistant *S. aureus* and *CoNS*, which may be due to the extensive use of beta-lactam and other broad spectrum antibiotics that create selective pressure on the bacterial population, resulting in the proliferation of resistant strains. Infections caused by these bacteria are usually acquired in health care settings and might cause UTIs, usually following catheterization or any urological procedure (Kumar *et al.*, 2025). Overall, the finding highlights the necessity for robust infection prevention measures and ongoing surveillance for methicillin or cefoxitin resistance among uropathogens.

Among the total isolates (n=55), MDR was observed in 32 (58.2%) of uropathogens. The finding is comparable with the studies from Harar (52%) (Ejerssa *et al.*, 2021) and Gondar (60.65%) (Biset *et al.*, 2020). Nevertheless, it was lower than studies from Kenya (96%) (Onyango *et al.*, 2018), Addis Ababa (80%) (Getie *et al.*, 2023), and Nekemte (77.3%) (Abebe *et al.*, 2025). But it was higher than the report from South Africa (4%) (Zwane *et al.*, 2021). Differences in antibiotic use patterns and infection prevention and control practices may contribute to the observed difference. Altogether, these data imply that MDR uropathogens are prevalent in pregnant women, which makes treatment difficult. Poor infection prevention and control strategies and misuse and overuse of antibiotics in the study setting may have contributed to the reported MDR level in our study.

In this study, 16.2% of Gram-negative uropathogens were found to be ESBL-producers, and a single *E. coli* isolate (2.5%) was found to be carbapenemase-producing. The observed proportion of ESBL production is in line with studies conducted in Dessie (15.8%) (Belete, 2020), Gondar (18.2%) (Biset *et al.*, 2020), and India (19.3%) (Patil *et al.*, 2024). On the other hand, it was lower than studies conducted in Addis Ababa (50%) (Getie *et al.*, 2023), Somaliland (32%) (Mohamoud *et al.*, 2021) and global pooled averages (25%) ((Getie *et al.*, 2023, Mansouri *et al.*, 2019). Moreover, the proportion of carbapenemase-production is lower than a report from Nigeria (34%) (Ogban *et al.*, 2024), but it was higher than a report from India (0%) (Patil *et al.*, 2024). Factors such as, healthcare settings that trend to use carbapenems and broad-spectrum antibiotics as treatment options, policy variations in antibiotic usage, and clinical characteristics of study participants could be the causes of the variation. (Getie *et al.*, 2023).

ESBL-producing isolates exhibited a high level of resistance (100%) to ampicillin, third-generation cephalosporins, nalidixic acid, trimethoprim-sulfamethoxazole, and ciprofloxacin. In the present study, they were found susceptible to limited antibiotics; these were nitrofurantoin (100%), amikacin (100%), and meropenem (83.3%). Those findings are supported by studies conducted in Romania (Ene *et al.*, 2026), Turkey (Ipekci *et al.*, 2014), and Gondar (Biset *et al.*, 2020). The high level of drug resistance among ESBL-producing isolates for the aforementioned drugs than non-ESBL producing one might be due to the genes encoding ESBL frequently found on plasmids that carry AMR genes conferring resistance against various antimicrobial agents. Thus, many ESBL-producing bacteria are also resistant to non- β -lactam antibiotics (Husna *et al.*, 2023). This highlights the compounded challenge of UTI treatment caused by those pathogens among pregnant women. In our study, the isolated carbapenemase-producing *E. coli* was found 100% resistant to 9 out of 11 antimicrobial agents tested. Since only a single isolate was identified, it was difficult to draw a meaningful conclusion regarding the antimicrobial susceptibility profile of carbapenemase-producing *E. coli*.

Overall, the present study and other similar studies showed that there is a rise in the prevalence of ESBL-carbapenemase-producing uropathogens, which are one of the significant public health concerns due to their high level of drug resistance. The absence of strict guidelines in antibiotic selection, empirical prescription of drugs, poor infection control strategies, and poor patient compliance with prescribed drugs contribute to the emergence and spread of these bacteria.

The MDR nature of these uropathogens makes the therapy challenging and often needs the use of expensive and not easily accessible drugs, which could be difficult in countries like Ethiopia with scarce medical resources. This suggests the need for systematic screening of ESBL-carbapenemase production, rational use of antibiotics guided by antimicrobial susceptibility results, robust infection control measures, as well as strengthening the antimicrobial stewardship programs to combat the spread of those superbugs and AMR.

Different factors increase the occurrence of SBU in pregnant women. Among the investigated factors, age was significantly associated ($P=0.032$) with SBU. Pregnant women who were in the age group between 15 and 24 years had 3.5 times higher odds of SBU than those who were in the age group between 35 and 45 years. This is in agreement with a study done in Adigrat (Tadesse *et al.*, 2018). This might be due to women in this age category are most likely to engage in frequent sexual activity, which could push the bacteria in the perineum to the urinary tract and make them more susceptible to UTIs.

Gestational period was another predictor variable that was significantly associated ($P=0.005$) with SBU. Pregnant women with a gestational age of the 3rd trimester had a 3.4 times higher risk of SBU compared with women with the gestational age of the 1st trimester. This finding is supported by studies conducted in Yemen (Al-Badaai *et al.*, 2023), South Africa (Orji *et al.*, 2022), and Pakistan (Mudassir A, 2019). This might be due to anatomical and physiological changes that occur in this period. Progesterone, which reaches its peak in the 3rd trimester, relaxes smooth muscle of the urinary tract, and the growing uterus in this trimester compresses the bladder, leads to urine stasis, which prevents any bacteria that enter the urinary tract from being flushed out (Habak *et al.*, 2026, Wanjiku *et al.*, 2023).

The odds of SBU were 3.1 times higher among pregnant women who had a history of UTI compared with those who had no history. The finding is in line with studies conducted in Dessie (Belete, 2020) and Somaliland (Mohamoud *et al.*, 2021). This might be due to the primary infection in pregnant women with a history of UTIs may not have been completely resolved due to poor diagnosis and unsuccessful treatment, which allowed some of the uropathogens to persist in the uroepithelial cells of the urinary tract and recur (Sgarabotto *et al.*, 2025).

7. LIMITATIONS OF THE STUDY

Limitations of the study

- ❖ Convenient sampling technique was used; this may reduce the generalizability of the study findings and introduce selection bias.
- ❖ Due to the unavailability of novobiocin antibiotic discs, *S. saprophyticus* isolates were not differentiated from other *CoNS* species, but standard urine culture interpretation and contamination rejection rules were strictly followed.

8. CONCLUSION

The current study showed that the proportion of SBU among pregnant women was relatively high and the majority of the isolates were Gram-negatives. The predominant isolate was *E. coli*. The findings of this study indicate that Gram-negative bacteria showed a relatively high level of resistance to the commonly prescribed antibiotics in the study area, including ampicillin, amoxicillin-clavulanic acid, and trimethoprim-sulfamethoxazole. Likewise, Gram-positive isolates were also found resistant to penicillin and trimethoprim-sulfamethoxazole.

Furthermore, a high level of MDR and an alarming proportion of ESBL and carbapenemase production were observed. ESBL-producing isolates showed co-resistance to the majority of tested antibiotics. However, nitrofurantoin, amikacin, and meropenem were found effective antimicrobial agents for both ESBL and non ESBL-producers. The current study revealed that age, third-trimester gestation, and history of UTIs were factors significantly associated with SBU.

9. RECOMMENDATIONS

Based on the findings of this study the following points are recommended.

- ❖ Due to the high resistance rates observed in this study, ampicillin, amoxicillin-clavulanic acid, and trimethoprim-sulfamethoxazole are not recommended for empirical therapy in the study settings. Alternatively, nitrofurantoin could be considered the treatment of choice due to its low rate of resistance and good safety profile, except in the near term.
- ❖ Continuous surveillance and molecular or genotypic characterization of ESBL-carbapenemase-producing uropathogens should be carried out to monitor their spread and confirm the study findings.
- ❖ The high levels of drug resistance, including MDR observed in this study highlight the need for concerned stakeholders to strengthen the antimicrobial stewardship program to combat antimicrobial resistance.
- ❖ In pregnant women, particularly in those having possible risk factors such as, a previous history of UTIs, an age category found between 15 and 24 years, and a 3rd trimester gestational period, regular and continuous culture-based screening of SBU should be considered.
- ❖ A more comprehensive study should be carried out with a large sample size in multiple study sites to generate more representative data.

10. REFERENCES

- ABEBE, M., NEGASH, A., KEBEDE, S., ALEMU, F., JEMMA, D., AMANSISA, D. & GIZAW, S. 2025. Bacterial agent, antibiotic resistance profile and predictors of urinary tract infection among pregnant women attending antenatal care in Ethiopia. *IJID regions*, 100787.
- ADIGE, D. A., BIRHANU 2023. The Practice of Peri-Urban Land Acquisition by Expropriation for Housing Purposes and the Implications: The Case of Bahir Dar, Ethiopia. *Urban Sci*, 7.
- AKTHER, N., HIRA, F. A., KHATUN, A., SHAWAN, M., ABDULLAH, S. M., SHORNA, E. J., ISLAM, A., ISLAM, K. & HOSSAIN, N. M. 2023. Antibiotic resistance pattern in the bacterial strains of urinary tract infection in Tangail city, Bangladesh.
- AL-BADAIL, F., AL-TAIRI, M., RASHID, A., AL-MORISI, S. & AL-HAMARI, N. 2023. Prevalence, Risk Factors and Antibiotic Susceptibility of Urinary Tract Infections among Pregnant Women: A Study in Damt District Yemen. *Journal of Pure & Applied Microbiology*, 17.
- AL-KOLAIBE, A. & HELMY, E. 2023. Epidemiology of Bacterial Urinary Tract Infection and Antibiotics Sensitivity among Pregnant Women at Taiz City, Yemen. *Journal of Pure & Applied Microbiology*, 6, 282-307.
- AMESHE, A., ENGDA, T. & GIZACHEW, M. 2022. Antimicrobial Resistance Patterns, Extended-Spectrum Beta-Lactamase Production, and Associated Risk Factors of Klebsiella Species among UTI-Suspected Patients at Bahir Dar City, Northwest Ethiopia. *International Journal of Microbiology*, 2022, 8216545.
- ASMARE, Z., AWOKE, T., GENET, C., ADMAS, A., MELESE, A. & MULU, W. 2024. Incidence of catheter-associated urinary tract infections by Gram-negative bacilli and their ESBL and carbapenemase production in specialized hospitals of Bahir Dar, northwest Ethiopia. *Antimicrobial Resistance & Infection Control*, 13, 10.
- BADRAN, Y. A., EL-KASHEF, T. A., ABDELAZIZ, A. S. & ALI, M. M. 2015. Impact of genital hygiene and sexual activity on urinary tract infection during pregnancy. *Urology annals*, 7, 478-481.
- BEGUM, A., JABEEN, A., LAKHO, R., ZAHEEN, Z., BALA, M. & BHUTTO, A. 2022. Prevalence of urinary tract infection during pregnancy at Tertiary Care Hospital: a cross-sectional study. *Pakistan Journal of Medical & Health Sciences*, 16, 851-851.
- BELETE, M. A. 2020. Bacterial profile and ESBL screening of urinary tract infection among asymptomatic and symptomatic pregnant women attending antenatal care of northeastern Ethiopia region. *Infection and Drug Resistance*, 2579-2592.
- BENYAGHUB, E., ALKHUHAIRY, M. K., BENCHAIB, S. M., ZAALAN, A., MEKHFI, Y., TEYEBI, N., RAZNI, D. & BENDADA, F. 2021. Isolation frequency of uropathogenic strains and search for ESBL producing Enterobacteriaceae isolated from patients with UTI in Bechar (Algeria). *Anti-Infective Agents*, 19, 303-316.
- BISSET, S., MOGES, F., ENDALAMAW, D. & ESHETIE, S. 2020. Multi-drug resistant and extended-spectrum β -lactamases producing bacterial uropathogens among pregnant women in Northwest Ethiopia. *Annals of clinical microbiology and antimicrobials*, 19, 25.
- BULABULA, A. N., DRAMOWSKI, A. & MEHTAR, S. 2017. Maternal colonization or infection with extended-spectrum beta-lactamase-producing Enterobacteriaceae in Africa: a systematic review and meta-analysis. *International Journal of Infectious Diseases*, 64, 58-66.
- CAO, J., XUE, D., GAO, D. & ZHANG, G. 2025. Maternal sepsis and other maternal infections: Global Burden from 1990 to 2021. *BMC Pregnancy and Childbirth*, 25, 612.
- CLSI 2025. Performance Standards for Antimicrobial Susceptibility Testing.

- DEMILIE, T., BEYENE, G., MELAKU, S. & TSEGAYE, W. 2012. Urinary bacterial profile and antibiotic susceptibility pattern among pregnant women in North West Ethiopia. *Ethiopian journal of health sciences*, 22.
- DENEKEW, Y. 2014. FOOD, MEDICINE AND HEALTH CARE ADMINISTRATION AND CONTROL AUTHORITY.
- DERESE, B., KEDIR, H., TEKLEMARIAM, Z., WELDEGEBREAL, F. & BALAKRISHNAN, S. 2016. Bacterial profile of urinary tract infection and antimicrobial susceptibility pattern among pregnant women attending at Antenatal Clinic in Dil Chora Referral Hospital, Dire Dawa, Eastern Ethiopia. *Therapeutics and clinical risk management*, 251-260.
- DUBE, R., AL-ZUHEIRI, S. T. S., SYED, M., HARILAL, L., ZUHAIRA, D. A. L. & KAR, S. S. 2022. Prevalence, clinico-bacteriological profile, and antibiotic resistance of symptomatic urinary tract infections in pregnant women. *Antibiotics*, 12, 33.
- EDAE, M., TEKLEMARIAM, Z., WELDEGEBREAL, F. & ABATE, D. 2020. Asymptomatic bacteriuria among pregnant women attending antenatal care at Hiwot Fana specialized university hospital, Harar, eastern Ethiopia: magnitude, associated factors, and antimicrobial susceptibility pattern. *International journal of microbiology*, 2020, 1763931.
- EJERSSA, A. W., GADISA, D. A. & ORJINO, T. A. 2021. Prevalence of bacterial uropathogens and their antimicrobial susceptibility patterns among pregnant women in Eastern Ethiopia: hospital-based cross-sectional study. *BMC Women's Health*, 21, 291.
- EL-KASHIF, M. M. L. 2019. Urinary tract infection among pregnant women and its associated risk factors: A cross-sectional study. *Biomedical and Pharmacology Journal*, 12, 2003-2010.
- EMIRU, T., BEYENE, G., TSEGAYE, W. & MELAKU, S. 2013. Associated risk factors of urinary tract infection among pregnant women at Felege Hiwot Referral Hospital, Bahir Dar, North West Ethiopia. *BMC research notes*, 6, 292.
- ENE, R.-L., POPESCU, R., COBEC, A. E., PUSCASIU, D., ENE, I.-A., VLAD, D. C., COBEC, I. M. & SEROPIAN, P. 2026. Antimicrobial Resistance and ESBL-Associated Predictors Among Uropathogens: A 2019–2024 Isolate-Level Study. *Antibiotics*, 15, 323.
- FARES, R., DEBABZA, M. & MECHAI, A. 2023. Detection and prevalence of extended spectrum β -lactamases production among Enterobacteriaceae isolated from urinary tract infections. *Biosystems Diversity*, 31, 163-169.
- FATIMA, S. S. & MUSSAED, E. A. 2017. Urinary tract infection. *Bacterial Identification and Drug Susceptibility Patterns in Pregnant and Non Pregnant UTI Patients*. Springer.
- GEBEYEHU, E., BANTIE, L. & AZAGE, M. 2015. Inappropriate use of antibiotics and its associated factors among urban and rural communities of Bahir Dar City Administration, Northwest Ethiopia. *PloS one*, 10, e0138179.
- GETANEH, T., NEGESSE, A., DESSIE, G., DESTA, M. & TIGABU, A. 2021. Prevalence of Urinary Tract Infection and Its Associated Factors among Pregnant Women in Ethiopia: A Systematic Review and Meta-Analysis. *BioMed research international*, 2021, 6551526.
- GETIE, M., GEBRE-SELASSIE, S., GETU, Y., BIRARA, S., TIRUNEH, C., ABEBAW, A., AKELEW, Y., ABEJE, G. & ENKOBAHRY, A. 2023. Bacterial profile and extended spectrum beta lactamase screening of urinary tract infection among asymptomatic and symptomatic pregnant women attending antenatal care in ALERT Hospital, Addis Ababa, Ethiopia. *SAGE Open Medicine*, 11, 20503121231197587.
- HABAK, P. J., CARLSON, K. & GRIGGS, J. R. P. 2026. Urinary Tract Infection in Pregnancy. *StatPearls*. Treasure Island (FL): StatPearls Publishing

- HUSNA, A., RAHMAN, M. M., BADRUZZAMAN, A., SIKDER, M. H., ISLAM, M. R., RAHMAN, M. T., ALAM, J. & ASHOUR, H. M. 2023. Extended-spectrum β -lactamases (ESBL): challenges and opportunities. *Biomedicines*, 11, 2937.
- IPEKCI, T., SEYMAN, D., BERK, H. & CELIK, O. 2014. Clinical and bacteriological efficacy of amikacin in the treatment of lower urinary tract infection caused by extended-spectrum beta-lactamase-producing *Escherichia coli* or *Klebsiella pneumoniae*. *Journal of Infection and Chemotherapy*, 20, 762-767.
- JOHNSON, B., STEPHEN, B. M., JOSEPH, N., ASIPHAS, O., MUSA, K. & TASEERA, K. 2021. Prevalence and bacteriology of culture-positive urinary tract infection among pregnant women with suspected urinary tract infection at Mbarara regional referral hospital, South-Western Uganda. *BMC pregnancy and childbirth*, 21, 159.
- JUSZCZAK, K., DYBOWSKI, B., HOLECKI, M., HRYNIEWICZ, W., KLIMEK, H., KŁODA, K., SIEROSZEWSKI, P. & DREWA, T. 2024. Summary of guidelines from the Polish Urological Association, Polish Society of Gynaecologists and Obstetricians, and Polish Society of Family Medicine on the diagnosis, therapy, and management of community-acquired lower urinary tract infections. *Central European journal of urology*, 77, 520.
- KARAH, N., RAFEL, R., ELAMIN, W., GHAZY, A., ABBARA, A., HAMZE, M. & UHLIN, B. E. 2020. Guideline for urine culture and biochemical identification of bacterial urinary pathogens in low-resource settings. *Diagnostics*, 10, 832.
- KUMAR, G., SHUKLA, S., AGARWAL, S., SHUKLA, D., SONI, S. R., VERMA, R. K., SINGH, V., KUMAR, A., KUMAR, D. & SONI, E. S. 2025. Methicillin-Resistant *Staphylococcus aureus* in Urinary Tract Infections: A Comprehensive Review With Insights From a North Indian Cohort. *Cureus*, 17.
- MANSOURI, F., SHEIBANI, H., JAVEDANI MASROOR, M. & AFSHARIAN, M. 2019. Extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae and urinary tract infections in pregnant/postpartum women: A systematic review and meta-analysis. *International journal of clinical practice*, 73, e13422.
- MATULAY, J. T., MLYNARCZYK, C. M. & COOPER, K. L. 2016. Urinary tract infections in women: pathogenesis, diagnosis, and management. *Current Bladder Dysfunction Reports*, 11, 53-60.
- MOGES, F., ESHETIE, S., ABEBE, W., MEKONNEN, F., DAGNEW, M., ENDALE, A., AMARE, A., FELEKE, T., GIZACHEW, M. & TIRUNEH, M. 2019. High prevalence of extended-spectrum beta-lactamase-producing Gram-negative pathogens from patients attending Felege Hiwot Comprehensive Specialized Hospital, Bahir Dar, Amhara region. *PloS one*, 14, e0215177.
- MOHAMOUD, H., TADESSE, S. & DERBIE, A. 2021. Antimicrobial resistance and ESBL profile of Uropathogens among pregnant women at Edna Adan Hospital, Hargeisa, Somaliland. *Ethiopian journal of health sciences*, 31.
- MUDASSIR A, I. M., KHANAM S 2019. A Comparative Study of Incidence of Urinary Tract Infections during Three Trimesters in Pregnancy. *Pakistan Journal of Medical and Health Sciences*, 13, 760-761.
- NOWICKI, B. 2002. Urinary tract infection in pregnant women: old dogmas and current concepts regarding pathogenesis. *Current infectious disease reports*, 4, 529-535.
- OGBAN, G., IWUAFOR, A., IDEMUDO, C., BEN, S., USHIE, S. & EMANGHE, U. 2024. Urinary tract infections in pregnancy caused by carbapenem-resistant Enterobacteriaceae in University of Calabar Teaching Hospital, Nigeria: An emerging therapeutic threat: Infections des voies urinaires pendant la grossesse causée par des Entérobactéries résistantes aux carbapénèmes à l'hôpital universitaire de Calabar, Nigéria: une menace thérapeutique émergente. *African Journal of Clinical and Experimental Microbiology*, 25, 160-168.

- OMIDIFAR, N., TAGHI, E., MOHEBI, S. & MOTAMEDIFAR, M. 2020. Distribution and antimicrobial susceptibility pattern of bacterial pathogens causing urinary tract infection in pregnant women in Shiraz, Southwest Iran. *Gene Reports*, 20, 100731.
- ONYANGO, H. A., NGUGI, C., MAINA, J. & KIIRU, J. 2018. Urinary tract infection among pregnant women at Pumwani Maternity Hospital, Nairobi, Kenya: bacterial etiologic agents, antimicrobial susceptibility profiles and associated risk factors. *Advances in microbiology*, 8, 175.
- ORJI, O., DLAMINI, Z. & WISE, A. J. 2022. Urinary bacterial profile and antibiotic susceptibility pattern among pregnant women in Rahima Moosa Mother and Child Hospital, Johannesburg. *Southern African Journal of Infectious Diseases*, 37, 343.
- ÖZTÜRK, R. & MURT, A. 2020. Epidemiology of urological infections: a global burden. *World journal of urology*, 38, 2669-2679.
- PATIL, G., PATIL, D., PATIL, A., SHRIKHANDE, S. & PATIL, A. S. 2024. Microbiological profile and antimicrobial susceptibility pattern of uropathogens isolated from pregnant women attending a tertiary care hospital in Central India. *Cureus*, 16.
- RIBEIRO-DO-VALLE, C. C., BONET, M., BRIZUELA, V., ABALOS, E., BAGUIYA, A., BELLISSIMO-RODRIGUES, F., BUDIANU, M., PUSCASIU, L., KNIGHT, M. & LISSAUER, D. 2024. Aetiology and use of antibiotics in pregnancy-related infections: results of the WHO Global Maternal Sepsis Study (GLOSS), 1-week inception cohort. *Annals of Clinical Microbiology and Antimicrobials*, 23, 21.
- RODRÍGUEZ-BAÑO, J., GUTIÉRREZ-GUTIÉRREZ, B., MACHUCA, I. & PASCUAL, A. 2018. Treatment of infections caused by extended-spectrum-beta-lactamase-, AmpC-, and carbapenemase-producing Enterobacteriaceae. *Clinical microbiology reviews*, 31, 10.1128/cmr. 00079-17.
- ROSANA, Y., OCVIYANTI, D., KARUNIAWATI, A. & AKHMAD, S. R. P. 2016. Comparison of microbial pattern causing urinary tract infection in female out-and hospitalized patients in Jakarta. *Microbiology Indonesia*, 10, 5-5.
- SGARABOTTO, D., ANDRETTA, E. & SGARABOTTO, C. 2025. Recurrent urinary tract infections (UTIs): a review and proposal for clinicians. *Antibiotics*, 14, 22.
- SHER, E. K., DŽIDIĆ-KRIVIĆ, A., SESAR, A., FARHAT, E. K., ČELIKOVIĆ, A., BEĆA-ZEĆO, M., PINJIC, E. & SHER, F. 2024. Current state and novel outlook on prevention and treatment of rising antibiotic resistance in urinary tract infections. *Pharmacology & therapeutics*, 261, 108688.
- SILVERMAN, M. & TURRENTINE, M. A. 2023. Urinary tract infections in pregnant individuals. *Obstet Gynecol*, 142, 435-45.
- TADESSE, S., KAHSAY, T., ADHANOM, G., KAHSU, G., LEGESE, H., G/WAHID, A. & DERBIE, A. 2018. Prevalence, antimicrobial susceptibility profile and predictors of asymptomatic bacteriuria among pregnant women in Adigrat General Hospital, Northern Ethiopia. *BMC research notes*, 11, 740.
- TAHA, A. B. 2024. Bacteriological profile, antimicrobial susceptibility, and factors associated with urinary tract infection in pregnant women. *The Journal of Infection in Developing Countries*, 18, 391-398.
- TAMMA, P. D., AITKEN, S. L., BONOMO, R. A., MATHERS, A. J., VAN DUIN, D. & CLANCY, C. J. 2021. Infectious Diseases Society of America guidance on the treatment of extended-spectrum β -lactamase producing Enterobacterales (ESBL-E), carbapenem-resistant Enterobacterales (CRE), and *Pseudomonas aeruginosa* with difficult-to-treat resistance (DTR-P. *aeruginosa*). *Clinical Infectious Diseases*, 72, e169-e183.
- TAYE, S., GETACHEW, M., DESALEGN, Z., BIRATU, A. & MUBASHIR, K. 2018. Bacterial profile, antibiotic susceptibility pattern and associated factors among pregnant women with Urinary Tract Infection in Goba and Sinana Woredas, Bale Zone, Southeast Ethiopia. *BMC research notes*, 11, 799.

- TEKLE, M. T., SEMA, F. D., WORKU, M. C., JARA, A. G. & AYENEW, W. E. 2025. Dispensing of antibiotics without prescription in community drug retail outlets of Bahir Dar city in Northwest Ethiopia: A simulated client visit before and after educational intervention. *Journal of Global Antimicrobial Resistance*.
- THAPA, R., LAMICHHANE, P., BANJARA, M. R. & ACHARYA, G. P. 2015. Prevalence of extended spectrum beta lactamase producing uropathogens in pregnant women. *PREVALENCE*, 8.
- TIGABIE, M., AYALEW, G., DEMOZE, L., TADESSE, K., GASHAW, Y. & ASSEFA, M. 2025. Extended-spectrum beta-lactamase and carbapenemase-producing Gram-negative bacteria in urinary tract infections in Ethiopia: a systematic review and meta-analysis. *BMC urology*, 25, 11.
- TULA, A., MIKRU, A., ALEMAYEHU, T. & DOBO, B. 2020. Bacterial profile and antibiotic susceptibility pattern of urinary tract infection among pregnant women attending antenatal care at a tertiary care hospital in southern Ethiopia. *Canadian Journal of Infectious Diseases and Medical Microbiology*, 2020, 5321276.
- WANJIKU, A., MWAMBURI, L., NGEIYWA, M. & OBALA, A. 2023. Variation in progesterone levels and urinary tract infections in pregnant women attending moi teaching and referral hospital, Eldoret, Kenya. *Medical Microbiology*, 13, 125-135.
- WHO 2022. *The WHO AWaRe (access, watch, reserve) antibiotic book*, World Health Organization.
- WHO. 2023. *Antimicrobial resistance* [Online]. Geneva: World Health Organization. Available: <https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance> [Accessed 8 November 2025].
- YALLEW, W. W., KUMIE, A. & YEHUALA, F. 2017. Hospital acquired infections and infection prevention practices in teaching hospitals in the Amhara regional state, Ethiopia. *Healthcare and Patient Safety*, 2016, 8.
- YETERA, A. A., NIMA, T. M., ALI, M. M. & ORMAGO, M. D. 2024. Urinary Tract Infection and Fetal Outcomes Among Pregnant Women in Adare General Hospital, Hawassa, Ethiopia. *International Journal of Microbiology*, 2024, 8562296.
- ZWANE, T., SHUPING, L. & PEROVIC, O. 2021. Etiology and antimicrobial susceptibility of pathogens associated with urinary tract infections among women attending antenatal care in four South African tertiary-level facilities, 2015–2019. *Antibiotics*, 10, 669.

11. ANNEXES

Annex I. Participant information sheet and Consent Form (English Version)

Good morning/afternoon dear participant!

My name is _____. I am working as a data collector for the study being conducted in this hospital / health institution on Extended-Spectrum β -Lactamase and Carbapenemase Profile of Uropathogens among Pregnant Women in Bahir Dar, Northwest Ethiopia, by Mr. Eyoel Hunie, who is studying his master's degree at Bahir Dar University, College of Medicine and Health Sciences, School of Medical Laboratory Sciences, Department of Medical Microbiology. I kindly request you to give me your attention to explain about the study and being selected as the study participant.

Title of the project: Extended-Spectrum β -Lactamase and Carbapenemase Profile of Uropathogens isolated from Pregnant Women in Bahir Dar, Northwest Ethiopia

Purpose of the study: The purpose of the study is to assess Extended-Spectrum β -Lactamase and Carbapenemase Profile of Uropathogens among Pregnant Women in Bahir Dar, Northwest Ethiopia. The findings of this study can be important for this hospital to plan and implement activities that based on the outcome of the findings and pregnant women participated in the study will also benefitted from the result of this study. It can also provide important information to policy makers, and program managers to address such issues in the future.

Procedures to be followed: you will stay with me for 10 minutes for interview and after interview you will sign this informed consent form. You will provide 5-10 ml of clean catch mid-stream urine specimen.

Risk and benefits: there is no risk with the collection of clean catch mid-stream urine specimen. There will not be any cash payment or direct benefit for participating, but if your result is clinically significant, we will inform your physician for treatment and this study will contribute to provide information or data for future and further study, and to develop health programs for health policy makers.

Confidentiality: Any information that I am going to collect about you during this research will be kept confidential. Your name and identity on the request paper will be changed to confidentiality code for the purpose of this study.

Withdrawal from the study: Your participation in this study will be voluntary, and you may drop the participation at any time or refuse to answer some of the questions if you feel uncomfortable. You can ask any questions regarding this study, and you have a right to get laboratory results. If you have any questions regarding this study, please contact us at the following address:

Principal investigator: Eyoel Hunie

Institution: Bahir Dar University, College of Medicine and Health Sciences, School of Medical Laboratory Sciences, Department of Medical Microbiology

Mobile no: +251946467509

Email: eyoelhunie9@gmail.com

Consent Form

Principal investigator: Eyoel Hunie

Research title: Extended-Spectrum β -Lactamase and Carbapenemase Profile of Uropathogens isolated from Pregnant Women in Bahir Dar, Northwest Ethiopia

I have read or had this document read to me in a language that I understand, and I understand the purposes, procedures, and risks of this research project as described within it. I understand that at any time I can withdraw from this study without giving a reason. I have had an opportunity to ask questions, and I am satisfied with the answers I have received. I know that there will be no special payment for being a participant in the study. I freely agree to participate in this study, as described. Participant code _____ Signature_____ Date_____ Interviewer's name_____ Signature_____

Annex II. Amharic Version (Participant information sheet and Consent Form)

የተሳታፊው መረጃ ጽሑፍና የተሳታፊነት ምግብ ትመረጋገጫ

እንደምን አደሩ/ እንደምን ዋሉ?

ስሜ-----

እባላለሁ፡ ፡ ፡ ፡ የምርምር መረጃ ሰብሳቢነት ሻ፡ ፡ አሁን እየሰራሁኝ ያለሁት በዚህ ሆስፒታል ማድረግ ወጥና ትመረጃ ሰብሳቢ ሆኜልሁ፡ ፡ ፡ ፡ ለተማሪ ኤድዩኬሽን በባህር ዳርዩ ኒሽርሲቲ በማድካል ማይክሮባዮሎጂ የሚሰጥ ትምህርት ስዲግሪ የመረጃ ቁልፍ የሚሆን ጥናት ለማካሄድ ወ፡ ፡ ፡ ፡ ስለዚህ ስለጥናቱ በተመለከተ መረጃ ለራሪያ እንድትሰጡ ተወስኑ ጊዜ እንዲሰጡ ተከብሮት እጠይቃለሁ፡ ፡

የጥናቱ አጠቃላይ **እና** **ዓላማ**-በእርጉዝ እና ቶችላይ የሽንት ቧን ቧን ሽታ የሚመጡ ባክቴሪያ የተባሉትን ረቂቅ ህዋሳት ስር ጭቅ፡ ለህዋሳቱ ተመራጭ የሆኑት መዳሃኒቶች መምረጥና በሽታውን ሊያስከትሉ የሚችሉ ቅድመ ሁኔታዎች ማጥናት ነው፡ ፡ ፡ የዚህ ጥናት አላማ በዋናነት ትክክለኛ የሚሰጥ ትምህርትን ለማጠናቀቅና የመረጃ ቁልፍ ሁፍለ ማዘጋጀት ይጠቅማል፡ ፡ ፡ በተጨማሪም ጥናቱ ግኝት ለሆነ ፒታሉና በጥናቱ ወስን ተሳታፊዎች እርጉዝ እና ቶች ከፍተኛ ጠቀሜታ ይኖረዋል፡ ፡ ፡ የዚህ ምርምር ግኝቶች የጠፍ ፖሊሲ አወጪዎች እርምጃ እንዲወስዱ እና እቅድ እንዲያወጡ ይጠበቅማል፡ ፡

የጥናቱ ሂደትና ጊዜ፡

በጥናቱ ላይ ለመሳተፍ ቃደኛ ከሆናችሁ የስምዎን ትቅጽ ላይ ትፈርሙ ማለት ሆኖ በሁላችሁ ጥናቱ ጠቃሚ ሚሆን መረጃ ይሰጠኛል፡ ፡ ፡ ለቃ ለምልሰስ ጥያቄዎቹን ለመሙላት 10 ደቂቃ ይፈጃል፡ ፡ ፡ ስለዚህ ጊዜዎን እንዲሰጡ በአክብሮት እጠይቃለሁ፡ ፡ ፡ በጥናቱ በመሳተፍ ቃደኛ ከሆኑ ማቅረብ ለዎትን መጠይቅ ምላሽ ሽከሰ ጠብሯል የሽንትና መፍ እንዲሰጡ ይጠበቃል፡ ፡

ጉዳትና ጥቅም፡ በዚህ ጥናት በመሳተፍ ምክንያት ደወሊዜ በስተቀር የሚደርስ ትጉዳት

የለም፡ ፡ ባለመሳተፍ ምክንያት ምትጸና የለም፡ ፡ በዚህ ጥናት በመሳተፍ ምክንያት ፕላስታክ ትጉዳት ሊኖር ይችላል፡ ፡ ፡ ከፍተኛ ምጥ ለወምት ገር ግን ከጥናቱ የተገኘ ፕላስታክ መረጃ ምንም ዓይነት ምትጸና ሊኖር አይችልም፡ ፡ ፡ ለጥናቱ ላይ ለመሳተፍ ቃደኛ ከሆኑ በመሳተፍ ጉዳት ሊኖራቸው ይችላል፡ ፡ ፡

ከርስዎ የምናገኘው መረጃ እና ሚዛን ጥራቱ፡-

የእርስዎን ምግብ መጠይቅ ላይ አይጠቀስም፡ ፡ በተጨማሪም ሚዛን ጥራት መረጃ እና በስተቀር ምናልባት ላይ የሚደረግ ወደ ምር መራወጥ ከተባለ ለትጉዳት ወጪ እንደሚወጣ እና ሚዛን ጥራቱን ተጠብቆ እንደሚሆን አረጋግጣለሁ፡ ፡ ፡

የተሳታፊነት መብት፡ በዚህ ጥናት ያለመሳተፍ መብት አለዎት፡ ፡ ፡ ላለመሳተፍ ከፈለጉ ደግሞ ማንኛውም ሊራሰም ከጥናቱ ማግለል ይችላሉ፡ ፡ ፡ ግልፅ ያልሆነ ጥያቄ ካለ ማጠቃለያ ይቻላል፡ ፡ ፡

በዚህ ጥናት ላይ ያለዎትን ጥያቄ በሚሰጡት ሰዓት አድራሻ በማንኛውም ሊጠይቁ ይችላሉ፡ ፡

or phrases where required. Code number ____

I- socio-demographic, clinical , behavioral and pregnancy related data		
1	Age (years)	
2	Residence	A. Urban ☐ B. Rural ☐
3	Marital status	A. Single ☐ B. Married ☐ C. Divorced ☐ D. Widowed ☐
4	Educational level	A. Unable to read and write. ☐ B. Only read and write ☐ C.Primary school☐ D.Secondary school☐ E.College and above☐
5	Type of occupation	A. House wife ☐ B. private business☐ C. Government employee ☐ D.other, specify -----
6	Monthly income (in birr)	
7	Do you have the habit of urination after sexual intercourse	A yes ☐ Bno ☐
8	To what direction do you wash yourgenitals after defecation or urination	A forward☐ Bbackward ☐
9	Gestational period (trimester)	A. Frist ☐ B. Second ☐ C. Third ☐
10	Gravidity	A. Primigravidae ☐B. Multigravidae ☐
11	Parity	A.Nulliparous ☐B.Primiparous ☐ C.Multiparous☐
12	History of UTI	A. Yes ☐ B. No ☐
13	Historyofcatheterization(in last3 months)	A. Yes ☐ B. No ☐
14	History ofDiabetes Mellitus	A. Yes ☐ B. No ☐
15	Historyofhospitalization(in last3 months)	A. Yes ☐ B. No ☐

Annex-IV Questionnaire (Amharic version)

ባህርዳርዩ ኒቨርሲቲ፡ የጤሳይንስኮሌጅ፡ የሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል

በእርጉዝእናቶችላይየሽንት ሲንረጋገጥሽታየ ማጽናት ባክቴሪያየ ተባሉትን ረቂቅ ህዋሳትስርጭት፣ ለህዋሳቱ ተመራጭ የሆኑት መዳሃኒቶች መምረጥና በሽታውን ሊያስከትሉ የሚችሉ ቅድመ ሁኔታዎች የሚጠፍ ጥናት ለማድረግ የተዘጋጁ መጠይቆች

የሚጠፍ ጥናት -----

I-መግለጫ፡ ሕክምና እና እርግዝና -ተያያዥ መረጃ		
1	እድሜ	
2	መኖሪያ ቦታ	ሀ. ገጠር ☐ ለከተማ ☐
3	የጋብቻሁኔታ	ሀ. ያላገባች ☐ ለያገባች ☐ ሐ አግብታየ ፈታች ☐ መባል የሞተባት ☐
4	የትምህርት ሁኔታ	ሀ. ማንበብና መጻፍ የማትችል ☐ ለ. ማንበብና መጻፍ የምትችል ☐ ሐ. መጽሐፍ ያደረጃትምህርት ☐ መሀለተኛደረጃትምህርት ☐ ሠ. ኮሌጅ እና ከዚያ በላይ ☐
5	የስራሁኔታ	ሀ. የቤት እመቤት ☐ ለ. የግል ሰራተኛ ☐ ሐ. የመንግስት ሰራተኛ ☐ መሬት ፡ ይግለጹ -----
6	ወርሃዊ ገቢ (በብር)	
7	ከግብረሰጋግኑኝነት በዋላ ሽንት የመሸናት ልማድ አለሽ	ሀ. አወ ☐ ለየለም ☐
8	ከሽንት ቤት በዋላ ብልት ሽንት ወደየት አቅጣጫ ወደምትታጠብዋለህ	ሀ. ወደፊት (ከብልት ወደፊት ጠገን) ☐ ለ ወደ ወሃ ላ (ፊት ጠገን ወደ ብልት) ☐
9	የእርግዝና ጊዜ	ሀ. የመጽሐፍ 3 ወር ☐ ለሀለተኛ 3 ወር ☐ ሐ. ሶስተኛ 3 ወር ☐
10	የእርግዝና ብዛት	ሀ. አንድ ጊዜ ☐ ለ ከአንድ ጊዜ በላይ ☐
11	የወለድ ብዛት	ሀ. የለም ☐ ለ 1 ☐ ሐ. 2 በላይ ☐
12	ከዚህ በፊት የታከመሽንት ሲንገባኢን ፊክሽን አለ	ሀ. አወ ☐ ለ የለም ☐
13	ከዚህ በፊት የሽንት ሲንገባ ካቴተር መጠቀም ታሪክ አለ (ባለፈው 3 ወራት)	ሀ. አወ ☐ ለ የለም ☐
14	ስኳር በሽታ ታመማሽ ነበር	ሀ. አወ ☐ ለ የለም ☐
15	ባለፈው 3 ወራት ወቅት ሆስፒታል ተገኝተዋል/ታመማሽ	ሀ. አወ ☐ ለ የለም ☐

Annex V-SOP for preparation of culture media, collection, and processing of specimens

Urine sample collection

Mid-stream urine for microbiological examination is collected as follows:

1. Give the patient a sterile, dry, wide-necked, leak proof container and request a 5-10 ml specimen. Wash the hands. Cleanse the area around the urethral opening with clean water and collect the urine with the labia held apart.
→ Important: Explain to the patient the need to collect the urine with as little contamination as possible, i.e. a clean-catch specimen.
2. Label the container with the date, identification number of the patient and time of collection.
3. As soon as possible, deliver the specimen to the laboratory. When immediate delivery to the laboratory is not possible, refrigerate the urine at 4–8 °C.

Laboratory procedures

Gram Stain

Purpose: Used to classify bacteria as gram positive and gram negative based on their gram reaction.

Principle: Gram's stain is that cells are first fixed to slide by heat or alcohol and stained with a basic dye (e.g. crystal violet), which is taken up in similar amounts by all bacteria. The slides are then treated with a Gram's iodine (iodine KI mixture) to fix (mordant) the crystal violet stain on Gram positive bacteria, decolorized with acetone or alcohol, and finally counter stained with Safranin.

Procedure

1. Place the fixed slide on the staining rack.
2. Cover the heat-fixed smear with crystal violet; leave for 10-60 seconds. Wash off with water and drain well.

3. Cover the smear with Gram's iodine; leave for 10-60 seconds. Wash off promptly with water and drain.
4. Holding the slide over the sink at a 45 degree angle, run the decolorizing agent (acetone/alcohol) over the slide and watch the excess run off the bottom edge of the slide.
5. Cover the smear with basic fuchsin or safranin (red counterstain); leave for 10-60 seconds. Wash with water and drain; air-dry in an upright position.
6. Leave until dry and look for a microscope (100 xs)
7. Report as gram positive (blue cocci or rods) and gram negative (red rods or cocci) and others accordingly

Biochemical tests

Catalase test procedure

Principle

Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. An organism is tested for catalase production by bringing it into contact with hydrogen peroxide. Bubbles of oxygen are released if the organism is a catalase producer. The culture should not be more than 24 hours old.

Required

Hydrogen peroxide, 3% H₂O₂ (10 volume solution)

Method

1. Pour 2–3 ml of the hydrogen peroxide solution into a test tube.
2. Using a sterile wooden stick or a glass rod (not a nichrome wire loop), remove several colonies of the test organism and immerse in the hydrogen peroxide solution. — Important: Care must be taken when testing an organism cultured on a medium containing blood because catalase is present in red cells. If any of the blood agar is removed with the organism, a false positive reaction may occur.

3. Look for immediate bubbling.

Results Active bubbling Positive catalase test

No bubbles Negative catalase test

Coagulase test procedure

Principle -Coagulase causes plasma to clot by converting fibrinogen to fibrin. Required EDTA anti- coagulated human plasma or rabbit plasma. The plasma should be allowed to warm to room temperature before being used. Slide test method (detects bound coagulase)

1. Place a drop of distilled water on each end of a slide or on two separate slides.
2. Emulsify a colony of the test organism in each of the drops to make two thick suspensions and mix gently, look for clumping. Note: Colonies from a Mannitol salt agar culture are not suitable for coagulase testing. The organism must first be cultured on nutrient agar or blood agar.
3. Add a loop full (not more) of plasma to one of the organisms within 10 seconds.

.Results Clumping within 10 seconds *S. aureus*

No clumping within 10 seconds no bound coagulase .

Indole test

Testing for indole production is important in the identification of enterobacteriaceae. Most strain of *E. coli*, *P. vulgaris*, *P. rettgeri*, *M. morganii*, and *Providencia species* break down the amino acid tryptophan with the release of indole.

Principle

The test organism is cultured in a medium which contains tryptophan. Indole production is detected by Kovac's or Ehrlich's reagent which contains 4 (p)-dimethylaminobenzaldehyde. This reacts with the indole to produce a red colored compound.

Method

- 1) Using a sterile straight inoculating wire pick bacterial colony, stab the SIM semisolid agar and incubate the inoculated tube.
- 2) Add 3-5 drops of Indole reagent (modified Kovac's reagent).

Results- Positive test: Red color

Negative test: No red color

Urease test (Christensen's (modified) urea broth):

Urea agars were inoculated heavily over the entire surfaces of the slants and then incubated at 37°C for 3-12 hours. A urease-positive culture produces an alkaline reaction in the medium, evidenced by pinkish red color of the Medium. Urease-negative organisms do not change the color of the medium, which is pale yellow-pink.

Triple Sugar Iron (TSI) Agar Slant:

Using a sterile inoculating needle, stab the butt of the TSI slant then streak back and forth along the surface of the agar with the organism. Incubate at 37°C for 18 to 24 h. If acid slant–acid butt (yellow–yellow): glucose and sucrose and/or lactose fermented. If alkaline slant–acid butt (red–yellow): glucose fermented only. If alkaline slant alkaline butt (red–red): glucose not fermented. The presence of black precipitate (butt) indicates hydrogen sulfide production, and presence of splits or cracks with air bubbles indicates gas production.

Citrate utilization test using Simmon's citrate agar:

Simmon's citrate slopes were prepared as recommended by the manufacturer (stored at 2-8°C). And the slopes were then inoculated and incubated at 37°C aerobically for 24 hours. Blue color indicates a positive reaction and if Simmon's citrate agar slopes remained as green in color indicate negative reaction.

Motility Test (using motility agars):

Motility agar was prepared and inoculated with a straight inoculating needle making a single stab about 1-2 cm down into the medium. The motility was examined after 35-37°C for 24 hours.

Motility indicated by the presence of diffuse growth (appearing as coloring of the medium) away from the line of inoculation.

Modified Kirby-Bauer susceptibility testing technique

Method

- 1) Using a sterile wire loop, touch 3–5 well-isolated colonies of similar appearance to the test organism and emulsify in 3–4 ml of sterile physiological saline or nutrient broth.
- 2) Match the turbidity of the suspension to the turbidity standard (mix the standard immediately before use.
- 3) Using a sterile swab, inoculate a plate of Mueller Hinton agar. Remove excess fluid by pressing and rotating the swab against the side of the tube above the level of the suspension. Streak the swab evenly over the surface of the medium in three directions, rotating the plate approximately 60° to ensure even distribution.
- 4) With the Petri dish lid in place, allow 3–5 minutes (no longer than 15 minutes) for the surface of the agar to dry.
- 5) Using sterile forceps, needle mounted in a holder, or a multidisc dispenser, place the appropriate antimicrobial discs, evenly distributed on the inoculated Mueller Hinton agar. Note: The discs should be about 15 mm from the edge of the plate and no closer than about 24 mm from disc to disc. Each disc should be lightly pressed down to ensure its contact with the agar. It should not be moved once in place.
- 6) Within 30 minutes of applying the discs, invert the plate and incubate it aerobically at 37°C for 16–18 h
- 7) After overnight incubation, using a ruler on the underside of the plate, measure the diameter of each zone of inhibition in mm.

SOP for media preparation

Procedure for preparation:

Prepare as instructed by the manufacturer.

1. Suspend certain gram of powder in liter of distilled or deionized water based on manufacturer
2. Heat and boil until completely dissolved with frequent agitation.
3. Sterilize in autoclave at 121⁰C for 15 minutes
4. Cool to 45-50 ⁰C
5. Mix well and dispense (15-20 ml) aseptically into sterile petri dishes.
6. Leave standing to solidify.
7. Perform sterility testing..
8. Label the bottom of each plate with date of preparation and batch number.

Store the culture media plates upside down at 2-8⁰C sealed in plastic bags to reduce chances of contamination. Test Samples for performance, using stable, typical control culture.

Extended spectrum beta-lactamase detection

Isolates that shows Inhibition zone ≤ 22 mm Ceftazidime for and ≤ 25 mm forceftriaxone for were confirmed for their ESBL production by combination disk test based on CLSI recommendation.

Combination disk test

For each test disks containing cephalosporin alone (ceftriaxone and ceftazidime) and in combination with clavulanic acid was applied. The inhibition zone around the cephalosporin disk/tablet combined with clavulanic acid was compared with the zone around the disk/tablet with the cephalosporin alone. The test was considered positive if the inhibition zone diameter is

5 mm and larger with clavulanic acid than cephalosporin alone in all other cases the test result was considered is negative.

Carbapenemase Detection

Isolates, which showed resistance the tested carbapenem, was confirmed for carbapenemase-production by the modified carbapenem inactivation methods

Modified carbapenem inactivation methods

After preparation of bacterial suspension of tested isolates in 2 ml Trypticase soya broth, meropenem disk (10 µg) was added and incubated at $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$ in ambient air for 4 hours $\pm$ 15 minutes. When the time is completed, meropenem disk (10 µg) was placed on a Muller Hinton agar plate previously inoculated with meropenem susceptible *E. coli* ATCC 25922 suspension of 0.5-McFarland turbidity standards and was be incubated overnight (18–24 hrs.) at 37°C . Bacterial isolates having a zone of inhibition 6–15 mm or presence of pinpoint colonies within a 16–18 mm zone and no inhibition of the meropenem-susceptible *E. coli* ATCC 25922 was confirmed as carbapenemase-producing (CLSI, 2025).

Interpretive Categories and Zone Diameter Breakpoints (CLSI, 2025).

Enterobacterales-recommended antimicrobial agents and zones of inhibition

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm		
		S	I	R
Ampicillin	10 µg	≥ 17	14–16	≤ 13
Amoxicillin-Clavulanate	20/10 µg	≥ 18	14–17 [^]	≤ 13
Cefotaxime or Ceftriaxone	30 µg	≥ 26	23–25 [^]	≤ 22
	30 µg	≥ 23	20–22 [^]	≤ 19
Cefoxitin	30 µg	≥ 18	15–17 [^]	≤ 14
Ceftazidime	30 µg	≥ 21	18–20 [^]	≤ 17
Meropenem	10 µg	≥ 23	20–22 [^]	≤ 19
Gentamicin	10 µg	≥ 18	15–17 [^]	≤ 14
Amikacin	30 µg	≥ 20	17–19 [^]	≤ 16
Tetracycline	30 µg	≥ 15	12–14	≤ 11
Ciprofloxacin	5 µg	≥ 26	22–25 [^]	≤ 21
Nalidixic acid	30 µg	≥ 19	14–18	≤ 13

Trimethoprim-Sulfamethoxazole	1.25/ 23.75 µg	≥ 16	11–15	≤ 10
Chloramphenicol	30 µg	≥ 18	13–17	≤ 12
Nitrofurantoin(U)a	300 µg	≥ 17	15–16	≤ 14

Zone Diameter Breakpoints for *Staphylococcus* spp

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm		
		S	I	R
Penicillin	10 units	≥ 29	-	≤ 28
cefoxitin	30 µg cefoxitin (surrogate test for Oxacillin)	≥ 22 (cefoxitin)	-	≤ 21 (cefoxitin)
Gentamicin	10 µg	≥ 15	13–14	≤ 12
Tetracycline	30 µg	≥ 19	15–18	≤ 14
Ciprofloxacin	5 µg	≥ 21	16–20	≤ 15
Clindamycin	2 µg	≥ 21	15–20	≤ 14
Trimethoprim-Sulfamethoxazole	1.25/ 23.75 Mg	≥ 16	11–15	≤ 10
Chloramphenicol*	30 µg	≥ 18	13–17	≤ 12
Nitrofurantoin	300 µg	≥ 17	15–16	≤ 14