Bacterial Characterization, Antimicrobial Sensitivity Pattern and Associated Risk Factors of Bacteriuria among Pregnant Women Attending Selected Health Centers in Adama City, Ethiopia

Hiwot Teshome

A Thesis Submitted to the Department of Applied Biology,
School of Applied Natural Sciences

Presented in Partial Fulfillment of the Requirement for the Degree of Master's in Applied Biology (Specialization in Applied Microbiology)

Office of Graduate Studies

Adama Science and Technology University

June, 2024
Adama, Ethiopia

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Declaration

I hereby declare that this Master Thesis entitled “Bacterial Characterization, Antimicrobial Susceptibility Pattern and Associated Risk Factors of Bacteriuria among Pregnant Women Attending Selected Health Centers in Adama City, Ethiopia” is my work. That is, it has not been submitted for the award of any academic degree, diploma, or certificate in any other university. All sources of materials that are used for this thesis have been duly acknowledged through citation

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Recommendation of Advisor

I, the advisor of this thesis, hereby certify that I have read the revised version of the thesis entitled “Bacterial Characterization, Antimicrobial Susceptibility Pattern and Associated Risk Factors of Bacteriuria among Pregnant Women Attending Selected Health Centers in Adama City, Ethiopia” prepared under my guidance by Hiwot Teshome submitted in partial fulfillment of the requirements for the degree of Masters of Science in Applied Biology (Specialization in Applied Microbiology).

Therefore, I recommend the submission of the revised version of the thesis to the department following the applicable procedures.

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Approval Page

I, the advisor of the thesis entitled "Bacterial Characterization, Antimicrobial Susceptibility Pattern and Associated Risk Factors of Bacteriuria among Pregnant Women Attending Selected Health Centers in Adama City, Ethiopia" developed by Hiwot Teshome, hereby certify that the recommendation and suggestions made by the board of examiners are appropriately incorporated into the final version of the thesis.

_____	_____	_____
Name of Advisor	Signature	Date

We, the undersigned, members of the Board of Examiners of the thesis by Hiwot Teshome have read and evaluated the thesis entitled "Bacterial Characterization, Antimicrobial Susceptibility Pattern and Associated Risk Factors of Bacteriuria among pregnant women attending selected health facilities in Adama City, Ethiopia" and examined the candidate during the open defense. This is, therefore, to certify that the thesis is accepted for partial fulfillment of the requirement of the degree of Master of Science in Applied Microbiology.

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Abbreviations/ Acronyms

APHRRL	Adama Public Health Research and Referral Laboratory
AOR	Adjusted odd ratios
AMR	Antimicrobial resistance
ASB	Asymptomatic bacteriuria
AST	Antimicrobial susceptibility testing
ATCC	American Type Culture Collection
CDC	Center for Disease Control and Prevention
CI	Confidence interval
CLSI	Clinical and Laboratory Standard Institute
CRO	Crude odd ratios
ESS	Ethiopian statistical service
GBD	Global burden of disease
H_2S	Hydrogen sulfide
MCH	Mother and Child Health
MDR	Multiple drug-resistant
MIC	Minimum inhibitory concentration
NSS	National Statistical Services
SPSS	Statistical Package for the Social Sciences
UPEC	Uro-pathogenic *Escherichia coli*
UTI	Urinary tract infection

Abstract

Urinary tract infection (UTI) is caused by the presence and growth of microorganisms anywhere in the urinary tract. Pregnant women are quite vulnerable to UTI-causing bacterial pathogens which can be associated with adverse outcomes for both the mother and fetus. This study aimed to isolate and characterize bacterial pathogens causing Urinary tract infections in pregnant women, to determine antimicrobial sensitivity patterns, and to assess risk factors associated with the infections at Adama City, Ethiopia. A health institution-based cross-sectional study design was conducted and midstream urine sample was collected from180 pregnant women suspected of UTI attending antenatal clinics at Adama Health Center, Biftu Health Center, and Hangatu Health Center in Adama City from March 2024 to May 2024. Using a structured questionnaire, all pregnant women were interviewed face to face to obtain the social demographic profiles. The well-mixed and non-centrifuged urine samples were inoculated into 5% sheep blood agar and MacConkey agar plates. It was incubated at 37°C for 24 hours and bacterial colonies were characterized by gram stain and biochemical properties of isolates. The prevalence of UTI among pregnant women in this study was 15.6%. *E. coli* was the predominant predictor of UTI, nine pathogenic bacteria were isolated, and 85.5% of the bacteria were gram-negative. Pregnant women who had a history of catheter insertion and history of abortion were found more susceptible to UTI-causing bacteria. Most of the isolated bacteria were susceptible to Ceftazidime and Meropenem. 85.7% of the bacterial isolates have antibiotic resistance for at least two antibiotics. 34.2% of the isolated bacteria showed multi-drug resistance. The study area will need further analysis of UTIs using modern and sensitive diagnostic methods including molecular methods.

Keywords: Antimicrobial Susceptibility testing, Pregnant women, Risk factors, Urinary tract infection

1. Introduction

1.1. Background of the Study

Urinary tract infection (UTI) is a comprehensive term that includes a range of infectious diseases that affect the urinary tract everywhere from the urethra to the kidney (Al Lawati et al., 2023).UTI occurs usually due to bacteria from the digestive tract which ascent the opening of the urethra, and begin to reproduce to cause infection. When bacteria from the rectal area get into the urinary tract via the urethra to the bladder and multiply in the urine, an infection occurs. In many cases, bacteria first travel to the urethra (Komala & Bhowmik, 2013). Females are three times more likely to get UTI than males, due to women's shorter urethra which opens nearer to the anus, the nature of sexual activity, pregnancy, easy contamination of the urinary tract with fecal flora, and hormonal changes that occur very quickly (Belete & Saravanan, 2020). The infection can develop in 40% − 50% of women and 5% of men (Westergaard et al., 2019). Pregnant women are more likely infected with UTIs with pathogenic bacteria than non-pregnant women and can be associated with antagonistic consequences for both the mother and fetus (Abate et al., 2020; Musa, 2020).

The risk of UTI begins in the 6th week of pregnancy. It reaches its highest during the 22–24th weeks due to numerous reasons including urethral dilatation and reduced urethral tone which results in high urinary stability. In addition, glycosuria develops in about 70% of pregnant women during pregnancy; these factors mutually enable urinary bacterial development (Belete & Saravanan, 2020). The instrumental agents of urinary tract infections are more complicated and influenced by a variety of factors, including the vaginal biological process, Lactobacillus spp., and behavioral factors (Thangavelu et al., 2022). UTI can be either symptomatic or asymptomatic (Demilie et al., 2012). Treatment of UTI is crucial in achieving the goal of a safe motherhood initiative; that women safely go through the pregnancy period, and childbirth and produce healthy babies (Gessese et al., 2017). UTI is a common health problem reported amongst 20% of pregnant women and a common cause of admission in obstetrical wards (Balakrishnan et al., 2016a).

Globally, UTIs affect about 150 million people annually, resulting in more than 6 billion dollars in healthcare cost crises (Zeng et al., 2022). Nowadays, the emergence of antimicrobial resistance in the treatment of UTI infection is a serious public health concern worldwide.

Particularly in developing countries, where healthcare services are limited the problem is more severe (Abate et al., 2020). In most developing countries' hospitals, routine urine culture is not conducted for antenatal mothers. Most patients are treated empirically without culture analysis and antimicrobial susceptibility testing (AST) (Onyango et al., 2018). UTI is categorized by its broad spectrum of symptoms ranging from mild to severe pain until death (Abate et al., 2020).

Globally, the prevalence of UTI during pregnancy varies between 13%-33% with symptomatic bacteriuria happening in 1% - 18% while asymptomatic cases are observed in 2% - 10% of pregnant women. Relatively, there is a high frequency of UTIs among pregnant women in Africa and Asia (Onyango et al., 2018). In Ethiopia, the frequency of infection with UTI among pregnant women varies from 9.8% to 26.6% (Getaneh et al., 2021). Screening for UTI in pregnancy is not considered an important part of antenatal care in most developing countries including Ethiopia (Demilie et al., 2012).

1.2. Statement of the problem

Pregnancy causes hormonal, physical, and functional changes in women's urinary tract. This increases urinary retention and the ascending of microbially contaminated urine from the bladder into the ureters, resulting in urinary tract infections (Vicar et al., 2023). UTIs are considered a common health problem during pregnancy all over the world, mainly in developing countries like Ethiopia (Mollick et al., 2016).

Failure to treat the UTI may result in low birth weight, premature babies, intrauterine fetal death, intrauterine growth hindrance, preterm labor, and increased prenatal mortality. It also results in severe morbidity such as maternal complications including septicemia, anemia, renal failure, preeclampsia, and adult respiratory syndrome (Ejerssa et al., 2021).

Usually, in Ethiopia, the management of UTIs is empirical without susceptibility testing or a urine culture to guide therapy. In Adama, there are limited reports regarding the analysis of UTIs caused by bacterial pathogens in pregnant women. So, this study was initiated to isolate and characterize bacterial pathogens causing UTIs among pregnant women and determine their antimicrobial susceptibility patterns to commonly used antibiotic regimens.

1.3. Objectives

1.3.1 General Objective

To isolate and characterize bacterial pathogens causing urinary tract infections in pregnant women, to determine antimicrobial sensitivity patterns, and to assess risk factors associated with the infections.

1.3.2 Specific Objectives

- To isolate and characterize bacterial pathogens causing urinary tract infections in pregnant women.
- To determine the antimicrobial sensitivity pattern of the isolates causing UTI in pregnant women.
- To determine major risk factors associated with the infections.

1.4. Significant of the study

The output of this study helps to provide current information about the type of bacteria, risk factors responsible for UTIs, and their susceptibility patterns to common antibiotics. It also serves as an input for health centers to understand the prevalence of UTI cases in Adama. It may also support policymakers to plan and take action in controlling UTI-related problems.

1.5. Scope of the Study

The study scope is spatially limited to the three selected health centers (Adama, Hangatu, and Biftu Health Centers). The study population is also limited to the pregnant women attending the selected health centers during the study period.

1.6. Operational Definitions

Antimicrobial resistance (AMR): is the ability of microorganisms to persist or grow in the presence of drugs to inhibit or kill them.

Antimicrobial susceptibility testing (AST): is a procedure used to identify which antimicrobial regimen is specifically effective for individual patients.

Asymptomatic bacteriuria (ASB): is a significant growth of the pathogen ($\geq 10^5$ bacteria/ml) in the absence of clinical manifestation.

Bacteriuria: is the presence of bacteria in the urine.

Gestational age: is the age of the fetus estimated by computing from the first day of the last menstrual period (time that precedes conception) until the day of consultation.

Mid-stream urine specimen: a specimen obtained from the middle part of urine flow: clean catch urine specimen.

Intermediate: bacterial isolates with antimicrobial agent minimum inhibitory concentrations that usually approach attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates.

Multiple drug-resistant: refers to bacterial isolates resistant to three or more antimicrobials belonging to different structural classes.

A pathogenic microorganism: is a microbe that is capable of causing disease.

Proteinuria: is the presence of excess proteins in the urine.

Resistant: bacterial isolates not inhibited by the usually achievable concentrations of the agent with a normal dosage schedule.

Sensitive: bacterial isolates inhibited by the usually achievable concentration of antimicrobial agent.

Symptomatic UTI: a patient whose urine is yielding positive cultures of ($\geq 10^5$ CFU/ml) and who has symptoms referable to the urinary tract.

Urinary tract infection (UTI): significant bacteriuria in a culture that grew $\geq 10^5$ colony-forming units (CFU/mL) in a single voided 10-20 ml midstream urine.

Uro-pathogenic: is related to, or being a pathogen of the urinary tract.

2. Literature Review

The urinary system comprises the kidneys, ureters, bladder, and urethra, and its main purpose is to filter blood by eliminating waste products and surplus water (Mancuso et al., 2023).UTI is caused by the presence and growth of microorganisms anywhere in the urinary tract. UTI generally occurs due to bacteria from the digestive tract that climb the opening of the urethra and initiate the multiplication of bacteria to cause infection (Gessese et al., 2017). UTIs are among the most common bacterial infections and encompass a broad spectrum of diseases, ranging from uncomplicated cystitis to life-threatening. UTIs are common infections caused mainly by uro-pathogenic *E.coli* (UPEC), which cause approximately 80% of the UTIs globally (Klein & Hultgren, 2020).

2.1. Pathogenesis of UTI

There are three ways through which the microorganisms may infect the urinary tracts. Urinary tract attack of the bacteria is largely determined by bacteria virulence factor, bacteria features, the inoculum size, and the insufficiency of host-defined mechanisms. UTI commences when uropathogens inhabit the urethra and subsequently ascend through the urethra into the bladder by the action of specific adhesins. If the host's inflammatory response fails to eliminate all bacteria, start multiplying, producing toxins and enzymes that promote their survival. The spread of UTIs is mainly related to the effectiveness of several strategies that the urinary pathogens have developed to adhere and invade host tissues (Mancuso et al., 2023).

2.2. Clinical Presentations

UTIs correspond to the growth and reproduction of bacteria inside the urinary tract, it can be either symptomatic or asymptomatic. Burning during urination is one of the most important symptoms in pregnant women with symptomatic cystitis. Additional symptoms include feeling the urge to urinate, feeling like the bladder is full, urinating before reaching the toilet, suprapubic pain, hematuria, and pyuria in the absence of systemic symptoms. The usual complaints of increased frequency and suprapubic pressure nocturia are not principally helpful as most pregnant women experience such cases as a result of increased burden from the growing uterus, increasing blood volume, increased glomerular filtration rate, and amplified renal blood flow (Getaneh et al., 2021).

Asymptomatic bacteriuria is a disorder that is characterized by the occurrence of bacteria in clear-voided midstream urine samples which results in positive cultures ($\geq 10^5$cfu/ml) of the uro-pathogenic but without conventional symptoms of UTI (Geerlings, 2016). Whereas symptomatic patients are considered by the existence of bacteria in clear-voided midstream urine specimens that have positive cultures ($\geq 10^5$cfu/ml) of the pathogens in the urinary tract and have at least two symptoms referable to the UTI (dysuria, urgency, frequency, incontinence, supra-pubic pain, flank pain or costovertebral angle tenderness, fever (temp ≥ 38°C) and chills) (Thangavelu et al., 2022).

2.3. Transmission of UTI

When bacteria from the rectal area enter the urinary tract via the urethra to the bladder and multiply in the urine, an infection occurs. In many cases, bacteria first travel to the urethra. When bacteria multiply in the urethra an infection can occur. An infection in the urethra causing inflammation is called urethritis. A bladder infection called cystitis occurs when bacteria move to the bladder and multiply. If such infection is not treated on time, the bacteria further may multiply and travel up the ureters to infect the kidneys, called pyelonephritis (Komala & Bhowmik, 2013).

2.4. Risk factors

Urinary tract infection is among the common health-related issues that occur in pregnant women. UTI usually happens from the 6th week of pregnancy and peaks from 22 to 24th weeks mainly in association with the changes that occur during pregnancy like increased bladder volume, urethral dilatation, and decreased urethral tone along with decreased bladder character (Ejerssa et al., 2021; Getaneh et al., 2021). Socioeconomic are also highly related to the incidence of UTI in pregnancy (Emiru et al., 2013). Untreated Asymptomatic bacteriuria (ASB) is a risk factor for acute cystitis (40%) and pyelonephritis (25–30%) in pregnancy and could lead to adverse obstetric outcomes such as prematurity, low birth weight, and higher fetal mortality rates (Gessese et al., 2017).

The emergence of antibiotic resistance in the management of UTIs is a serious public health issue. Particularly in the developing world where there is a high level of poverty, illiteracy, and

poor hygienic practices, there is also a high prevalence of fake and spurious drugs of questionable quality in circulation (Seifu & Gebissa, 2018).

2.5. Epidemiology of UTI

Urinary tract infection prevalence increases with age. In the USA, the prevalence of UTI in women aged over 65 years is about 20%, compared with nearly 11% in the total population (Medina & Castillo-Pino, 2019). Globally, the number of individuals infected with UTIs approximately increased from about 252 million to more than 404.5 million between the years1990 and 2019 (Zeng et al., 2022). Previous studies showed that about 2 to 10% of antenatal women suffer from different forms of UTI. These cases of infections are complicated in pregnant women up to 20%. The occurrence of asymptomatic forms of UTIs remained constant across the countries, and a majority of the recent studies reported comparable rates, varying from 2 to 10% similar to women without pregnancy (Mollick et al., 2016; Thangavelu et al., 2022; Zeng et al., 2022).

A study carried out in Bangladesh on clinically suspected UTI patients showed that pathogens accountable for UTI presented increasing resistance to drugs prescribed commonly which in turn limits an alternative possibility for the treatment of UTIs. According to the findings, mainly Gram-negative bacteria were responsible for the UTI and the bacteria most commonly identified from women's urinary tract infections were *E. coli* (Majumder et al., 2022).

A study conducted at Al Samawa City; Iraq discovered that the infection degree in women with a history of pregnancies was 50.2% more than women with no pregnancy. The UTI rate was 61.3% indicating the highest risk of UTI among pregnant women than nonpregnant. The study revealed that 210 pregnant and 55 non-pregnant women were suspected of UTI, which shows that pregnant women were more affected by UTI, compared to non-pregnant women. Moreover, out of 210 pregnant women, 181 (86%) women were affected by UTI, while 15 (27%) out of 55 non-pregnant women were infected by UTI (Nahab et al., 2021).

A cross-sectional analysis conducted in the northern part of Ghana on 560 women with pregnancy reported a 39.8% prevalence of UTI among pregnant women from which symptomatic cases cover about 33.1% and 41.8% were asymptomatic. Most of the bacterial

isolates found from the urine samples of the pregnant women were Gram-negative bacteria, *E. coli* being the most common bacteria isolate. Generally, about 90% of the isolated bacteria were resistant to at least one antibiotic, and about 68% of the isolated bacteria were multidrug-resistant (Vicar et al., 2023).

In a study conducted in the Northwestern part of Ethiopia at Bahir Dar town on 367 pregnant women, 37 were symptomatic and the remaining 330 were asymptomatic. Bacteriological screening from the collected urine samples revealed the growth of bacteria in about 8.5% of symptomatic pregnant women and 18.9% of asymptomatic women with pregnancy with a general prevalence of 9.5% (Demilie et al., 2012).

A study conducted in Dire Dawa at Dill Chora Referral Hospital found a significant number of bacterial isolates from pregnant women resistant to the usually prescribed drugs. The average prevalence found among pregnant women was 14%. UTI prevalence in pregnant women with symptoms was higher than those without symptoms. Several bacterial isolates identified found high resistance to the most commonly prescribed drugs (Balakrishnan et al., 2016b).

In a study conducted at Saint Paul's Hospital Millennium Medical College in Addis Ababa among 290 pregnant women, 16.9% were found positive for ASB with 95 CI. The predominantly identified bacteria were *Escherichia coli* (43%) and *S. aureus* (20%). The multidrug resistance (MDR) isolates proportion was 57.1%. ASB is prevalent in the study area indicating the importance of screening ASB and possible treatment to prevent its consequences (Wabe et al., 2020a).

A study conducted in Eastern Ethiopia among 200 pregnant women on the urine samples showed a UTI prevalence of 15.5%. Most of the isolated uropathogens were Gram-negative bacteria, and the most frequent isolated bacteria was *Escherichia coli*. Most of the bacterial isolates were susceptible to gentamicin, amikacin, and nitrofurantoin and had a high resistance rate to ampicillin, cotrimoxazole, and amoxicillin-clavulanate. In the multidrug resistance assessment of the bacterial isolate, about half of the isolated bacteria indicated multiple drug-resistant features (Abate et al., 2020).According to previous studies, the prevalence of UTI among pregnant women is higher than among nonpregnant women. The current study is expected to show the

prevalence of UTI in selected health centers and isolate and characterize UTI-causing pathogenic bacteria in the study area.

3. Materials and Methods

3.1. Description of the study area

The study area, Adama town, is located in the Main Ethiopian Rift Valley, East Showa Zone of Oromia National Regional State. The town is 100 kilometers away from Addis Ababa (the capital city of Ethiopia) along the road that connects Addis Ababa to Djibouti. According to the climatic zones of Ethiopia, the study area is located at an altitude ranging from 1500 to 2000 m above sea level and is classified as a sub-tropical climate. According to the Ethiopian Statistics Service report, the population of Adama is estimated at 480,175 (Ethiopian Statistical Service, 2023). In Adama, there are eight governmental health centers and five hospitals (One public and four private). The current study was conducted at three selected health centers. According to the Adama Health Bureau report, the selected health facilities, Adama Health Center, Biftu Health Center, and Hangatu Health Center provide services to approximately 42,496, 52,448, and 25,000 people per year respectively (Gebreselassie & Rao, 2021).

3.2. Study Population

All pregnant women who attended the antenatal care clinic of the Adama, Biftu, and Hangatu health centers during the study period (March to May 2024) participated.

3.3. Inclusion Criteria

Antenatal women who attended the selected health centers and volunteered to consent and who did not acquire antibiotics participated in the study.

3.4. Exclusion Criteria

Antenatal women who acquired antibiotics in the past two weeks were excluded from the study.

3.5. Study Design and Sampling Methods

The health institution-based cross-sectional study was conducted on urine samples collected from pregnant women who attended antenatal clinics at three selected health facilities. Adama Health Center, Biftu Health Center, and Hangatu Health Center were selected by random sample

selection method from governmental health centers considering they represent other health institutions in the town.

3.6. Sample Size Determination and Data Collection

A cross-sectional study was conducted from March 2024 to May 2024 to examine the bacterial isolates that cause UTI among pregnant women attending three health institutions to determine associated risk factors and drug susceptibility of isolates from urine samples of pregnant women with and without symptoms of UTI at selected health institutions.

A formula known as a single population equation (Equation 1) was used to estimate the sample size of the study participants.

$$n = \frac{Z^2 p(1-p)}{d^2} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

For the sample size calculation, the prevalence rate of a previous study conducted on UTI among pregnant women in Eastern Ethiopia (Ejerssa et al., 2021)(15.5%) was used. The margin of error(d) = 5.5%, Z score for 95% confidence interval, with a 10%non-response rate; where Z= Z score for 95% confidence interval Z= 1.96, P =15.5%.

$Z^2 = (1.96)^2 = 3.8416$, P=0.155, (1-p) =1-0.155=0.845, $d^2 = (0.055)^2$=0.003025

n =166, 10% non-response rate =14, total=180

A total number of 180 antenatal women who attended Adama, Biftu, and Hangatu Health Center were included in the study.

3.7. Method of Data Collection

For data related to socio-demographic characteristics, a structured questionnaire was used and translated into the local languages (Afan Oromo and Amharic) (Appendix A). Data collection from women attending antenatal care on signs and symptoms of UTI was conducted by trained health professionals. Orientation was given properly to the study participants before sample collection about the required procedure of urine sample collection. Participants who fulfilled the

required criteria were considered to join the study until the required sample size was accomplished.

3.7.1. Urine Sample Collection

The urine samples were collected from 180 volunteer pregnant women attending Adama, Biftu and Hangatu health centers 102, 36, and 42 urine samples were collected respectively from March to May 2024. Urine specimens were collected by mid-stream clean catch method in a sterile container. About 10 to 20 ml urine specimens were collected from each pregnant woman in a sterile, capped plastic cup. The cup was labeled with a unique sample number and date of collection. To maintain the quality of the samples, the collected urine samples were stored in the refrigerator of the health centers until the samples were sent all together within two hours of collection to the bacteriology department of Adama Public Health Research and Referral Laboratory (APHRRL) for culture and antimicrobial susceptibility testing. The samples were stored in the cold box during transportation to the laboratory.

3.7.2. Bacterial Culture Isolation

The well-mixed and non-centrifuged urine samples were inoculated by a wire loop that can carry 0.001 mL of urine specimen into 5% sheep blood agar and MacConkey agar by using streak plate method and then incubated at 37°C for 24 hours aerobically. The bacterial colonies were counted and multiplied by 100 to estimate the number of bacteria present per milliliter of urine. Colony counts yielding bacterial growth of $\geq 10^5$cfu/ml are regarded as significant bacteriuria but specimens that produced $< 10^5$colonies/ml of urine are considered insignificant or due to contamination (Tille, 2014). Culture media were sterilized based on the manufacturer's instructions. Then the sterility of culture media was checked by incubating 3–5% of the batch at 35 – 37°C overnight and observed for bacterial growth. Those media that showed growth were discarded. Pure isolates of bacterial pathogens were characterized by colony morphology and gram stain. Gram staining was done for all isolates following the standard procedures and the smears were examined microscopically for their morphology and staining reactions (Cheesbrough, 2005)

3.7.3. Biochemical Characterization of the Isolates

Biochemical tests for glucose, sucrose, lactose, oxidase, catalase, indole, citrate, H_2S production, and motility were used as biochemical assays. The gram-negative bacteria were identified by indole production, motility test, H_2S and gas production in SIM, citrate utilization, urease test, oxidase, carbohydrate utilization tests in TSI, and LIA test. The gram-positive bacteria were identified using catalase. The catalase test was used to differentiate species of *Staphylococcu*s from *Streptococcus* (Tille, 2014). The suspected bacteria were placed on the 3% hydrogen peroxide and the formation of the bubble was observed for a positive sample. The urease test is a test for identifying urease-producing bacteria, which break down urea, to give ammonia and carbon dioxide through hydrolysis. The ammonia changes the media to alkaline and pink coloration was considered positive for urease. Oxidase test was used to determine if a bacterium is producing the enzyme cytochrome oxidases (Appendix C).

3.7.4. Antimicrobial Susceptibility Test

Antimicrobial Susceptibility testing was performed on isolates according to the Clinical and Laboratory Standards Institute criteria using the Kirby–Bauer disc diffusion method on Muller-Hinton Agar (CLSI, 2023). Colonies from sheep blood agar were used to prepare a suspension. A single colony of bacteria was taken from a pure culture, transferred to a tube containing 5 ml of normal saline, and mixed gently until it formed a homogenous suspension. The turbidity of the suspension was then adjusted to the density of a McFarland 0.5 to standardize the inoculum size. The sterile cotton swab was used to inoculate suspensions on Muller Hinton Agar. The swab was dipped into the suspension and then used to distribute the bacteria evenly over the entire surface of the Mueller-Hinton agar medium. The antibiotic disks were placed after 15 minutes of inoculation on Muller Hinton agar 15mm from the edge and 24mm distance from each other with the aid of a sterile needle (Coyle, 2005). CLSI M100 guideline was used to select an antibiotic disk and interpret AST results. The diameter of the zone of inhibition around the disc was measured using a sliding metal caliper the isolates were classified as sensitive, intermediate, and resistant according to the standardized table (Appendix E).

The following antibiotics were used for the test. Amoxicillin/clavulanic acid (AMC) (30µg Oxoid, UK), Cefepime (FEP), Cefazolin (CZL) Cefotaxime (CTX) (30µg Oxoid, UK), Ceftazidime (CAZ), Ciprofloxacin (CIP) (5µg Oxoid, UK), Gentamicin (G), Nalidixic acid (NA),

Sulfamethoxazole/ trimethoprim (TS) (23µg Oxoid, UK), Piperacillin/ tazobactam (TZP) (100µg Oxoid, UK), Meropenem (MEM) (10µg Oxoid, UK), Tetracycline (T) (30µg) and Penicillin (PG) (10 µg), were used for bacterial isolates. The antimicrobial agents tested were selected based on the availability and the CLSI M100 guideline book (CLSI, 2023).

3.8. Data Analysis

Data from laboratory examination and questionnaire investigation was entered into Microsoft Excel Spreadsheet. The coded data was processed and analyzed using Statistical Package for the Social Sciences (SPSS) version 25. Descriptive statistics was used to summarize the collected data. The prevalence of UTI was calculated during the analysis. Variables with significant association with UTI in the binary logistic regression analysis were reanalyzed on a multinomial logistic regression analysis to find AOR. A chi-square test was done to assess the difference in proportion between culture-positive and culture-negative participants. P- value of <0.05 was considered for statistical significance.

3.9. Ethical Consideration

The study was approved by the examining board assigned by the Department of Applied Biology at Adama Science and Technology University; (Appendix G) a letter of support was written to selected health institutions and Adama Public Health Research and Referral Laboratory from the University for cooperation. The objectives of this study were described to the concerned body of the health institutions. Agreement to conduct the study was secured from selected health facilities. Official permission paper was obtained from the administrator of the selected health institutions. Significant information about the purpose of this study, its actions, and its input were clarified to the participants with the declaration of keeping their confidentiality. After explaining the importance of the study, informed written consent was obtained from study participants. (Appendix B). The name was not stated during the data collection, and documentation was based on the unique identification number given for each questionnaire and corresponding specimen. The analysis result of the study was communicated back to the health centers so that pregnant women could benefit from the study.

4. Results

4.1. Sociodemographic Characteristics of the Study

A total of 180 pregnant women from three selected health centers in Adama were included in this study. The socio-demographic information of pregnant women was collected using a structured questionnaire (Appendix A.) The study participants' ages ranged from 17 to 44 years, and about 99% of the participants were urban dwellers. About 65% of the study population experienced more than one pregnancy history (multiparous) and 50% of them had experienced normal delivery (vaginal delivery). The sociodemographic characteristics of the respondents in this study are summarized in Table 1.

Table 1: Socio-demographics of the respondents from three selected Health Centers in Adama.

Variables	Categories	Frequency	Percent (%)
Age	17-24	77	42.8
	25-34	93	51.7
	35-44	10	5.6
Residence	Urban	178	98.9
	Rural	2	1.1
Marital Status	Married	177	98.3
	Single	2	1.1
	Widowed	1	0.6
Educational Level	Uneducated	5	2.8
	Primary School	66	36.7
	Secondary School	77	42.8
	Higher Education	32	17.8
Occupation	House Wife	96	53.3
	Employee	63	35.0
	Daily laborer	6	3.3
	Merchant	13	7.2
	Farmer	2	1.1
Average Monthly Family Income	≤2500	13	7.2
	2501-4500	85	47.2
	4501-6500	53	29.4
	6501-10000	24	13.3
	>10000	5	2.8
Parity	Primipara	61	33.9
	Multipara	118	65.6
Gestational age	1stTM	55	30.6
	2nd TM	67	37.2
	3rd TM	58	32.2
Mode of delivery	Normal	90	50
	CS	28	15.6
	NA	62	34.4
History of catheter	Yes	29	13.9
	No	151	86.1
History of Abortion	Yes	29	16.7
	No	151	83.3

1st TM= First trimester, 2nd TM= Second trimester, 3rd TM= Third trimester, CS= cesarean section, NA= Not applicable

4.2. Prevalence of UTI and types of bacterial isolates

Among the samples collected from 180 UTI-suspected pregnant women, the prevalence of UTI was 28 (15.6%). Nine different bacteria were isolated. *E. coli* was found the most frequent isolate 14(50%), followed by 4 (14.2%) *Proteus species* and 3 (10.7%) *Staphylococcus aureus*. The frequency of the nine isolated bacteria in this study is summarized in Table 2.

Table 2 : Frequency of bacterial Isolates from urine samples of pregnant women in three selected health centers in Adama

Identified Bacteria	Frequency	Percent (%)
Escherichia coli	14	50
Klebsiella oxytoca	2	7.14
Proteus vulgaris	3	10.7
Corynebacterium urealyticum	1	3.57
Citrobacter diversus	1	3.57
Acinetobacter baumani	1	3.57
Citrobacter freundi	2	7.14
Staphylococcus aureus	3	10.7
Proteus mirabilis	1	3.57

4.3. Morphological Characteristics of Isolates

The morphological characteristics of the bacteria were identified based on form, surface, size, color density, elevation, and gram reaction (Appendix F Figures 1 and 2). The morphological characteristics are shown in Table 3.

Table 3: Morphological Characteristics and gram reaction result

Bacteria species	**Gram stain result**	**Form**	**Surface**	**Size**	**Color**	**Margin**	**Density**	**Elevation**	Gram stain reaction
E. coli	GNR	Circular	Smooth	Small	Pink	Entire	Translucent	Convex	GNR
K. oxytoca	GNR	Irregular	Smooth	Large	Pink	Undulated	Opaque	Convex	GNR
P. vulgaris	GNR	Circular	Rough	Small	Brown	Entire	Translucent	Flat	GPC
Corynebacterium urealatum	GPR	Circular	Glistening	Pinpoint	Gray	Entire	Translucent	Flat	GPR
C. diversus	GNR	Circular	Smooth	Large	Black Pink	Entire	Opaque	Convex	GNR
A. baumani	GNR	Irregular	Rough	Small	Gray	Undulated	translucent	Convex	GNR
S. aures	GPC	Circular	Smooth	Small	Golden yellow	Entire	Opaque	Convex	GNR

C. Freundi	GNR	Circular	Rough	Small	Pink	Entire	Translucent	Convex	GNR
P. vulgaris	GNR	Round	Rough	Medium	Gray	Entire	Opaque	Convex	GNR
P. mirabilis	GNR	Circular	Smooth	Large	Black pink	Entire	Opaque	Convex	GNR

Abbreviation: GNR= Gram-negative rod, GPR= Gram-positive rod, GPC= Gram-positive cocci

4.4. Biochemical characteristics of bacterial isolate

The gram-negative bacteria were isolated using biochemical tests such as TSI, Citrate test, LIA, SIM, Urea, Indole, and oxidase tests (Appendix F Figure 4). The gram-positive bacteria were isolated using catalase tests and Mannitol salt agar (Appendix F Figure 3). The procedure of each biochemical test is written. (Appendix B) and the biochemical characteristics of the bacterial isolates are presented in Table 4.

Table 4: Biochemical Characteristics of Isolated Bacteria from three selected Health Centers in Adama

S. N	Bacteria isolated	TSI	Citrate	LIA	Urea	SIM	motility	Indole	Gas	Oxidase	Catalase	Lactose
1	*Escherichia coli*	A/A	N	N	N	H_2S-	Motile	P	P	N		P
2	*Klebsiella oxytoca*	A/A	P	N	P	H_2S-	Nonmotile	p	P	N		P
3	*Proteus vulgaris*	K/A	P	N	P	H_2S+	Motile	P	P	N		N
4	*Corynebacterium urealyticum*	ND	ND	ND	P	ND	ND	ND	ND	ND	P	N
5	*Citrobacter diversus*	A/A	P	N	N	H_2S+	Motile	P	P	N		P
6	*Acinetobacter baumani*	K/K	N	N	N	H_2S-	Motile	N	N	N	P	N
7	*Citrobacter freundi*	A/A	P	N	N	H_2S+	Motile	N	P	N		P
8	*Staphylococcus aureus*	ND	ND	ND	ND	ND	ND	ND	ND	ND	P	
9	*Proteus mirabilis*	K/A	P	N	P	H_2S+	Motile	N	P	N		N

Abbreviations: A/A= Acid/Acid, K/A= Alkaline/Acid, K/K= Alkaline/Alkaline. N= Negative, P= positive. ND =Not done, TSI =Tripple Sugar Iron, SIM = Sulfide Indole Motility, LIA = Lysine Iron Agar, H_2S+= Hydrogen sulfide produced, H_2S-= No Hydrogen sulfide production

4.1. Antimicrobial susceptibility pattern

The antimicrobial susceptibility test for the isolated bacteria was conducted using thirteen antibiotic discs (11 for gram-negative and 5 for gram-positive bacteria from which 3 were common for both) the zone of inhibition was measured to interpret the antibiotic susceptibility result (Fig 5& 6). Most (95.8%) of the gram-negative bacteria were sensitive to Ceftazidime and Meropenem and 73.6% were sensitive to Ciprofloxacin. All *E. coli* were sensitive to Cefotaxime and Piperacillin. Also, *E. coli* was 92.8% sensitive to Ceftazidime and Meropenem, and 85.7% for Ciprofloxacin. About 54.1% of gram-negative bacteria resisted Sulfamethoxazole and 58.3% to Tetracycline. All *S. aureus* were resistant to Sulfamethoxazole and Penicillin from gram-positive isolates and 66.6% sensitive to Ciprofloxacin. 85.7% of the bacterial isolates have antibiotic resistance for at least two antibiotics. 34.2% of the isolated bacteria showed multi-drug resistance. The susceptibility pattern observed is summarized in Table 5.

Table 5: AST for Gram-negative isolates from three selected Health Centers in Adama Ethiopia

Bacteria species	Antimicrobial Susceptibility test											
	Pattern	AMC	CZL	CTX	CAZ	FEP	CIP	MEM	PTZ	TS	NA	TE
E. coli (n=14)	R	2(14.2)	2(7.1)	0(0)	0(0)	2(14.2)	1(7.1)	1(7.1)	0(0)	7(50)	4(28.5)	8(57.1
	I	3(21.4)	3(21.4)	0(0)	1(7.1)	0(0)	1(7.1)	0(0)	0(0)	2(14.2)	3(21.4)	2(14.2)
	S	9(64.2)	9(64.2)	14(100)	13(92.8	12(857	12(85.7	13(92.8)	14(100)	5(35.7	7(50)	4(28.5
K.oxytoca (n=2)	R	0(0)	1(50)	1(50)	0(0)	0(0)	0(0)	0(0)	0(0)	1(50)	0(0)	1(0)
	I	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(50)	0(0)	0(0)	0(0)
	S	2(100)	1(50)	1(50	2(100)	2(100)	2(100)	2(100)	1(50)	1(50)	2(100)	1(50)
P.vulgaris (n=3)	R	0(0)	1(33.3)	1(33.3	0(0)	2(66.6)	1(33.3	0(0)	0(0)	1(33.3)	1(33.3)	3(100
	I	1(33.3)	1(33.3)	0(0)	0(0)	0(0)	0(0)	0(0)	1(33.3)	1(33.3)	0(0)	0(0)
	S	2(66.6)	1(33.3	2(66.6)	3(100)	1(33.3)	2(66.6	3(100	2(66.6)	1(33.3)	2(66.6)	0(0)
C. diversus (n=1)	R	1(100)	1(100)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	1(100)	1(100)	1(100)
	I	0(0)	0(0)	0(0)	0(0)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
	S	0(0)	0(0)	0(0)	1(100)	0(0)	1(100)	1(100)	1(100)	0(0)	0(0)	0(0)
A.baumannii (n=1)	R	0(0)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(100)	0(0)
	I	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
	S	1(100)	0(0)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	0(0)	1(100)
C.freundii (n=2)	R	0(0)	1(50)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	2(100)	1(50)	0(0)
	I	1(50)	1(50)	1(500	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
	S	1(50)	0(0)	1(50)	2(100)	2(100)	2(100)	2(100)	2(100)	0(0)	1(50)	2(100)
P. mirabilis (n=1)	R	0(0)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(100)	0(0)	0(0)
	I	0(0)	0(0)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(100)	1(100)
	S	1(100)	0(0)	0(0)	1(100)	1(100)	1(100)	1(100)	1(100)	0(0)	0(0)	0(0)
Total	R	3(12.5)	8(33.3)	3(12.5)	0(0)	4(15.7)	2(7.4)	1(4.1)	0(0)	13(54.1)	9(42.8)	14(58.3
	I	5(20.8)	5(20.8)	2(8.3)	1(4.16	1(4.1)	1(7.4)	0(0)	2(8.6)	3(12.5)	5(20.8)	4(16.6)

(n=24)					)							
	S	16(66.6)	11(45.8)	19(70.1)	23(95.8)	19(70.1)	21(87.5)	23(95.8)	22(91.6)	8(33.3)	10(41.6)	9(37.5)

Abbreviations: AMC= Amoxicillin-Clavulanate, CZL= Cefazolin, CTX= Cefotaxime, CAZ = Ceftazidime, FEP = Cefepime, CIP = Ciprofloxacin, GEN = Gentamicin, MEM = Meropenem, PG = Penicillin, PTZ = Piperacillin/Tazobactam, TS = Sulfa/Tri meth, NA = Nalidixic acid, TE = Tetracycline, R = Resistant, I = Intermediate, S = Susceptible

Four antibiotic discs were used for the gram-positive isolates such as Gentamicin, Ciprofloxacin, Sulfamethoxazole, and Tetracycline, 66.6% of S. aureus were sensitive to Ciprofloxacin and Gentamycin, and 33.3% were sensitive to Penicillin and Tetracycline. All *S. aureus* were resistant to Sulfamethoxazole, 66.7 resistant to penicillin and Tetracycline, and 33.3% resistant to Gentamycin.

4.2. Risk factors associated with UTI

Risk factors associated with UTI were identified using logistic regression analysis. Socio-demographic characteristics were analyzed using bivariate logistic regression analysis. History of catheter insertion, history of previous abortion, and gestational age met the cutoff criteria of $P < 0.25$ and the candidate variables for multivariate logistic regression analysis. Binary logistic regression analysis was done for each variable and the detected result is shown in Table 6.

Table 6: Binary logistic regression analysis of risk factors associated with the prevalence of UTI among pregnant women from three selected health centers in Adama

Characteristic		UTI Negative	UTI Positive	Total	p-value (<0.25)	COR
Age	17-24	67	12	79	0.999	
	25-34	78	16	94	0.999	
	35-44	7	0	7	Reference	
	Total	152	28	180		
Educational Status	Higher Education	28	4	32	0.999	
	Primary school	55	11	66	0.999	
	Secondary school	64	13	77	0.971	1.006
	Uneducated	5	0	5	Reference	
	Total	152	28	180		
Occupation	Daily labor	6	0	6	0.999	0.000
	Employee	52	11	63	0.394	2.538
	Farmer	2	0	2	0.999	0.000
	House Wife	80	16	96	0.954	0.989
	Merchant	12	1	13	Reference	
	Total	152	28	180		
Monthly Income	<=2500	11	2	13	0.276	0.273
	2501 - 4500	75	10	85	0.98	0.200
	4501 - 6500	42	11	53	0.337	0.393
	6501 - 10000	21	3	24	0.162	0.214
	> 10000	3	2	5	Reference	
	Total	152	28	180		
Residence	Rural	2	0	2		
	Urban	150	28	178	0.999	0.000
	Total	152	28	180		
Marital Status	Married	149	28	177	0.999	0.000
	Single	2	0	2	1.000	1.000
	Widowed	1	0	1	Reference	
	Total	152	28	180		
History of catheter	No	132	19	151	0.015	3.126
	Yes	20	9	29	Reference	
	Total	152	28	180		
Mode of delivery	CS	21	7	28	0.258	1.810
	NA	55	7	62	0.456	0.691
	Normal	76	14	90	Reference	
	Total	152	28	180		
Gestational age	1st TM	48	7	55	0.55	0.383
	2nd TM	62	5	67	0.05	0.212
	3rd TM	42	16	58	Reference	
	Total	152	28	180		
History of abortion	No	131	20	151	0.057	0.401
	Yes	21	8	29	Reference	
	Total	152	28	180		
Parity	Multipara	97	21	118	0.256	1.701
	Primipara	55	7	62	Reference	
	Total	152	28	180		

Variables that showed significant association with UTI during bivariate logistic regression analysis were reanalyzed by multivariate logistic regression. Finally, history of abortion (AOR: 2.79, 95% CI: 1.011, 7.691, $P = 0.048$) and history of previous catheter insertion (AOR: 3.52, 95% CI: 1.31, 9.47, $P = 0.013$) and ^{2nd} TM gestational age were considered risk factors having a significant association of UTI with p-value < 0.05 The analysis result is shown in Table 7.

Table 7: Multivariate logistic regression analysis of risk factors associated with the prevalence of UTI among pregnant women from three selected health centers in Adama

Characteristic		UTI Negative	UTI Positive	Total	AOR (95%CI)	p-value (<0.05)	95%CI Lower	Upper
History of catheter	No	132	19	151	3.517	0.013	1.307	7.691
	Yes	20	9	29				
	Total	152	28	180				
Gestational age	1st TM	48	7	55	2.598	0.065	0.941	7.171
	2nd	62	5	67	5.051	0.004	1.660	15.367
	3rd TM	42	16	58				
	Total	152	28	180				
History of abortion	No	131	20	151	2.788	0.048	1.011	7.691
	Yes	21	8	29				
	Total	152	28	180				

5. Discussion

Out of 180 urinary tract infection-suspected pregnant women enrolled in this study, the prevalence of bacterial isolates was 28 (15.6%). The results of this finding are in line with previous local reports from the Eastern part of Ethiopia (15.5%) (Ejerssa et al., 2021). On the other hand, the findings of the present study showed a slightly higher prevalence of UTI-associated bacterial reported from the northwestern part of the country (10.4%) (Alemu et al., 2012) and central part of the country (Addis Ababa) (10.9%) (Wabe et al., 2020a). This variation in prevalence might be due to the difference in sample size and personal hygiene practice.

Most of the bacterial isolates 24 (85.7%) were Gram-negative. The present study showed a higher proportion of bacterial isolates than the findings of earlier local studies from Gondar Teaching Hospital (67.5%) (Alemu et al., 2012) and Tikur Anbesa Specialized Hospital (60%) (Assefa et al., 2008). The probable reason for more gram-negative isolates than gram-positive may be due to the presence of unique virulence factors in Gram-negative bacteria that assist in attachment to the uroepithelial cells and prevent bacteria from urinary lavage, allowing for multiplication and tissue invasion – resulting in hostile infection and pyelonephritis in pregnancy.

Among the isolates, *E. coli* was the most frequent 14 (50%) bacteria followed by *Proteus* species 4 (14.2%) in this study. The present study reported the dominance of *E. coli* to be associated with urinary tract infection which concurs with previous findings from elsewhere in the country and abroad (Alemu et al., 2012; Getaneh et al., 2021; Onyango et al., 2018; Vicar et al., 2023, 2023; Yismaw et al., 2012). *E.coli* is the most predominant uro-pathogenic perhaps due to several virulence factors specific for colonization and invasion of the urinary epithelium (Alemu et al., 2012; Getaneh et al., 2021; Krzysztof et al., 2021).

Antibiotic sensitive test in the present study showed that 95.8% of the gram-negative bacteria were sensitive to Ceftazidime and Meropenem, 91.6% were sensitive to Piperacillin and 87.5 were sensitive to Ciprofloxacin. This result is agreed with the study conducted in Addis Ababa that reported 72.4% of gram-negative bacteria were sensitive to Ciprofloxacin and 75.9% were sensitive to Meropenem (Wabe et al., 2020b). On the contrary, this finding is higher than a local report (58.1%) of susceptibility against ceftazidime from the Eastern part of Ethiopia (Ejerssa et

al., 2021). Remarkably, all *E. coli* isolates were sensitive to Cefotaxime and Piperacillin and 92.8% were sensitive to Ceftazidime and Meropenem.

In this study, 85.7% of the bacterial isolates have antibiotic resistance for at least two antibiotics. The finding of this study is relatively higher than the study conducted in Goba and Sinana Woredas, Southeast Ethiopia (Taye et al., 2018). In this study, 54.1% and 56.5% of gram-negative bacteria resisted Sulfamethoxazole/Trimeth and Tetracycline respectively.

From the gram-positive isolates found in this study, 66.6% of S. aureus were sensitive to Ciprofloxacin and also to that of Gentamycin. The result was less than the study conducted in Saint Paul's Hospital Millennium Medical College, Addis Ababa (90% sensitive to Gentamycin and 85 % sensitive to ciprofloxacin) (Wabe et al., 2020b). In this finding 66.7 % of the S. aureus was resistant to Penicillin and Tetracycline, the result is comparable with the finding of Web et al. (2020b), 70% resistant to penicillin. 100% of *S. aureus* were resistant to Sulfamethoxazole.

In this study, socio-demographic factors such as age, educational status, family income, marital status, mode of delivery, residence, parity, and occupation have no significant association with UTI. The finding of this study corroborates similar studies from Gondar Teaching University, Shashemen, Hospital, and Harar Hospital (Addis et al., 2021; Seifu & Gebissa, 2018).

Pregnant women with a previous history of abortion (AOR: 2.79,95% CI: 1.011, 7.691, P=0.048) and a history of catheterization (AOR: 3.52, 95% CI: 1.31, 9.47, P=0.013) exhibited higher odds of having UTI compared to those who did not have a history of abortion and catheter insertion. The present study result is in agreement with a study conducted at Saint Paul's Hospital Millennium Medical College in Addis Ababa where previous history of catheterization and history of abortion were significantly associated with UTI (Wabe et al., 2020a).

6. Conclusions and Recommendations

6.1. Conclusions

The UTI prevalence in the present study among pregnant women attending antenatal clinics in three selected health centers in Adama City was 28 (15.6%). Most of the isolates were gram-negative bacteria. Among the bacteria isolates, the highest 14 (50%) prevalence of *E. coli* bacteria was found. Most of the isolated bacteria were found susceptible to Ceftazidime. About 85% of the isolated bacteria resisted more than two antibiotics. Pregnant women who had a history of catheter insertion were 3.52 times more associated with UTI-causing bacteria than pregnant women who had no previous history of catheter insertion. Pregnant women having a history of abortion were 2.79 times more associated with UTI-causing bacteria than pregnant women who had no history of abortion.

6.2. Recommendations

Based on the findings of the current study the following recommendations are made:

- To prevent antibiotic resistance, a urine culture should be made before prescribing the common antibiotics.
- Further analysis of UTI using modern and sensitive diagnostic methods including molecular methods will be needed in the study area to improve the prevalence and antibiotic sensitivity results.

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Appendix A: Questionnaire

Questions related to socio-economic and demographic factors

Characteristics	**Alternatives: Choose/describe/Category**
How old are you?	Age: ---------- year_____
What is your current marital status?	1. Married 2. Single 3. Divorced/separated/ widowed 4. Other/specify----------
What is your residence	1. Rural 2. Urban
What is the level of education you attended?	1. No formal Education 2. Primary school (1-8) 3. Secondary school (9-12) 4. Higher level (Diploma and above)
what is your occupation?	1. Petty trader 2. Employee 3. Daily labor 4. Housewife 5. Other
What is the average monthly income of your household?	(Monthly in Ethiopian birr) Total income _____
Gestational period	1. First trimester 2. Second trimester 3. Third trimester
Do you have a history of catheterization	1. Yes 2.No

History of natural abortion	1. Yes 2. No
Parity (Number of pregnancies)	1. Primipara 2. Multipara

Appendix B: Consent Form of Study Participants

I _____, agree to participate in the research project titled "Bacterial Characterization, Antimicrobial Sensitivity Pattern and Associated Risk Factors of Bacteriuria among Pregnant Women Attending Selected Health Centers in Adama City, Ethiopia", conducted by Hiwot Teshome who have discussed the research project with me.

I have read the information in the questionnaire and fully understand its content. I have had the opportunity to ask questions about this research and received satisfactory answers. I also fully understand the general purposes and methods of this research.

I consent to participate in the research project and the following has been explained to me:

- the research may not be of direct benefit to me.
- my participation is completely voluntary.
- whom I should contact for any complaints about the research.
- I can request the research findings related to me.
- security and confidentiality of my personal information.
- publication of results from this study on the condition that my identity will not be revealed.

Name: _____

Signature: _____

Date: _____

Appendix C. Gram stain and Biochemical Test Procedures

a) Gram stain Procedure

- A thin smear was prepared from the bacterial species on a clean glass slide.
- The smear was dried.
- Heat fixed smear.
- Each smear is covered with crystal violet for 1 minute.
- Washed each slide with water.
- Covered each smear with Gram's iodine solution for 1 minute.
- Gently washed with water.
- Decolorized with 95% alcohol.
- Washed the slide with water and drained.
- Counterstain was applied safranin for 30 seconds.
- Washed with water and blot-dried with absorbent paper.
- The stained slides were air-dried and observed under the microscope.
- Examined the slides microscopically using the oil immersion objective.
- The Gram reaction of the cultures identified and classified based on the morphology and arrangement of cells.
- Those bacteria that appear blue were referred to as Gram-positive and those appearing pink were described as Gram-negative.

b) Citrate test procedure

- Using a sterile technique Simmons citrate agar slant was inoculated with the test organism using a stab and streak inoculation.
- The tubes were incubated at 37°C for 24 hours.
- The tubes were observed for growth and coloration of the medium.

Interpretation: If the color of the medium turns blue, a positive result is indicated.

If the color of the medium remains green, a negative result is indicated.

c) Catalase test Procedure

- A small amount of bacterial colony was transferred to the surface of a clean, dry glass slide using a loop.
- A drop of 3% H_2O_2 was added onto the slide and mixed.
- A positive result is the rapid evolution of oxygen (within 5-10 s) as evidenced by bubbling.
- A negative result is no bubbles or only a few scattered bubbles.

d) Oxidase test procedure

- The test organisms were rubbed over the reagent-impregnated, filter paper disc using sterile applicator sticks.
- The formation of a purple color indicates a positive test. No color changes show a negative test.

e) Urea test Procedure

- The test organism was inoculated in the media using an inoculation loop.
- The tubes were incubated at 37°C for 24-24 hours.
- The Development of a pink color indicator is a positive test and no color change shows a negative test.

f) Indole test procedure

- Kovac's reagent was added to the tryptone broth.
- The formation of a red color ring at the top showed an indole-positive result.
- No red color ring formation showed an indole-negative result.

g) Lysine Iron Agar test procedure (LIA)

- The test organism was stabbed in the butt and streaked on the slant in a fishtail streak.
- The tube was then tightly capped and incubated for 24 hours before reading the results.
- Pink color formation showed a positive result and no color change (remaining purple) showed a negative result.

h) Triple Sugar Iron (TSI) procedure

- TSI agar test was inoculated by first stabbing through the center of the medium to the bottom of the tube and then streaking on the surface of the agar slant.
- The cap was left on loosely and incubated at 35°C in ambient air for 24 hours.

- If lactose (or sucrose) was fermented**,** a large amount of acid was produced, which turns the phenol red indicator yellow both in the butt and in the slant.
- If lactose is not fermented but a small amount of glucose is fermented, the butt turns yellow and the slant turns red (alkaline or neutral pH).
- If neither lactose/sucrose nor glucose is fermented**,** both the butt and the slant will be red.
- If H_2S is produced, the black color of ferrous sulfide is seen.

Appendix D. Antimicrobial susceptibility test procedure

- 3 –5 well-isolated colonies were selected from the same morphologic type of culture.
- The top of each colony was taken and transferred into a tube containing 5 ml of normal saline.
- The bacterial suspension was inoculated on a plate.
- A sterile cotton-tipped applicator swab was dipped into the inoculum within 15 minutes of adjusting turbidity and rotated against the tube to remove excess inoculum.
- The entire surface of the agar plate was swabbed three times, rotating the plate approximately 60^{o} between streaking to ensure even distribution. As a final step, swabbed the rim of the agar.
- The inoculated plate is allowed to stand for 3 -15 minutes (no longer than 15 minutes) before applying disks.
- Antibiotic disks were applied to the agar surface using a sterile needle. Pressure was applied to ensure complete contact of the disk with the agar.
- No more than 12 disks on a 150mm plate and no more than 5 disks on a 100mm plate were used.
- The working supply of antibiotic disks was stored in a refrigerator (2 – 8 ^{C}) in a tightly-capped container with desiccant.

- Within 15 minutes of disk application the plate was inverted and incubated for 24 hours at 35 ± 2^{o}C in an ambient air incubator.

Appendix E. Antimicrobial agent selection and result interpretation

a) Antimicrobial agent selection and result interpretation based on CLSI guidelines for *Enterobacteriaceae*

Antimicrobial agent	Disk content/µg	Group	Zone Diameter interpretive criteria			CSF	Blood	Urine	Sputum
			S	I	R				
Ampicillin	10	1st	≥17	14-16	≤16	X	X	X	X
Ceftazidime	30	1st	≥18	15-17	≤14	X	X	X	X
Gentamicin	10	1st	≥18	15-17	≤14	X	X	X	X
Cefazolin	30	1st	≥23	20-22	≤19	-	X	X	X
Amoxicillin-Clavulanate	30	2nd	≥18	14-17	≤13	-	X	X	X
Cefepime	30	2nd	≥25	19-24	≤18	-	X	X	X
Cefotaxime	30	2nd	≥26	23-25	≤22	X	X	X	X
Cefotetan	30	2nd	≥16	13-15	≤12	-	X	X	X
Meropenem	10	3rd	≥23	20-22	≤19	-	X	X	X
Ciprofloxacin	5	2nd	≥26	22-25	≤21	-	X	X	X
Piperacillin	100	2nd	≥25	21-24	≤20	-	X	X	X
Sulfamethoxazole	23.75	2nd	≥16	11-15	≤10	-	X	X	X
Ceftazidime	30	3rd	≥21	18-20	≤17	-	X	X	X
Tetracycline	30	3rd	≥15	12-14	≤11	-	-	X	-
Chloramphenicol	30	3rd	≥18	13-17	≤12	X	X	-	X
Nalidixic Acid	30	U	≥19	14-18	≤13	-	-	X	-

b) Antimicrobial disk selection and result interpretation based on CLSI guidelines for *Acinetobacter spp.*

Antimicrobial agent	Disk content	Group	Zone Diameter interpretive criteria	CSF	Blood	Urine	Sputum

	/ µg		S	I	R				
Gentamicin	10	1st	≥15	13-14	≤12	X	X	X	X
Ceftazidime	30	1st	≥18	15-17	≤14	X	X	X	X
Meropenem	10	1st	≥18	15-17	≤14	X	X	X	X
Ciprofloxacin	5	1st	≥21	16-20	≤15	-	X	X	X
Piperacillin	100	2nd	≥21	18-20	≤17	-	X	X	X
Cefepime	30	2nd	≥18	15-17	≤14	X	X	X	X
Cefotaxime	30	2nd	≥23	15-22	≤14	X	X	X	X
Amikacin	30	2nd	≥17	15-16	≤14	X	X	X	X
Doxycycline	30	2nd	≥13	10-12	≤9	-	-	X	X
Sulfamethoxazole	23.75	2nd	≥16	11-15	≤10	-	-	X	X
Tetracycline	30	U	≥15	12-14	≤11	-	-	X	-

c) Antimicrobial disk selection and result interpretation based on CLSI guidelines for *Staphylococcus spp.*

Antimicrobial agent	Disk content/ µg	Group	Zone	Diameter interpretive	criteria	CSF	Blood	Urine
			S	I	R			
Oxacillin	30	1st	≥22	-	≤21	X	X	X
Penicillin	10	1st	≥29	-	≤28	-	-	X
Sulfamethoxazole	23.5	1st	≥16	11-15	≤10	-	-	X
Tetracycline	30	2nd	≥19	15-18	≤14	-	-	X
Ciprofloxacin	5	3rd	≥21	16-20	≤15	-	-	X
Gentamicin	10	3rd	≥15	13-14	≤12	-	X	X
Vancomycin	30	2nd	≥17	15-16	≤14	X	X	X
Erythromycin	15	2nd	≥23	14-22	≤13	-	X	-

Symbol: X = the disk required for the test; ― = the disk not required for the test

Appendix F: Figures

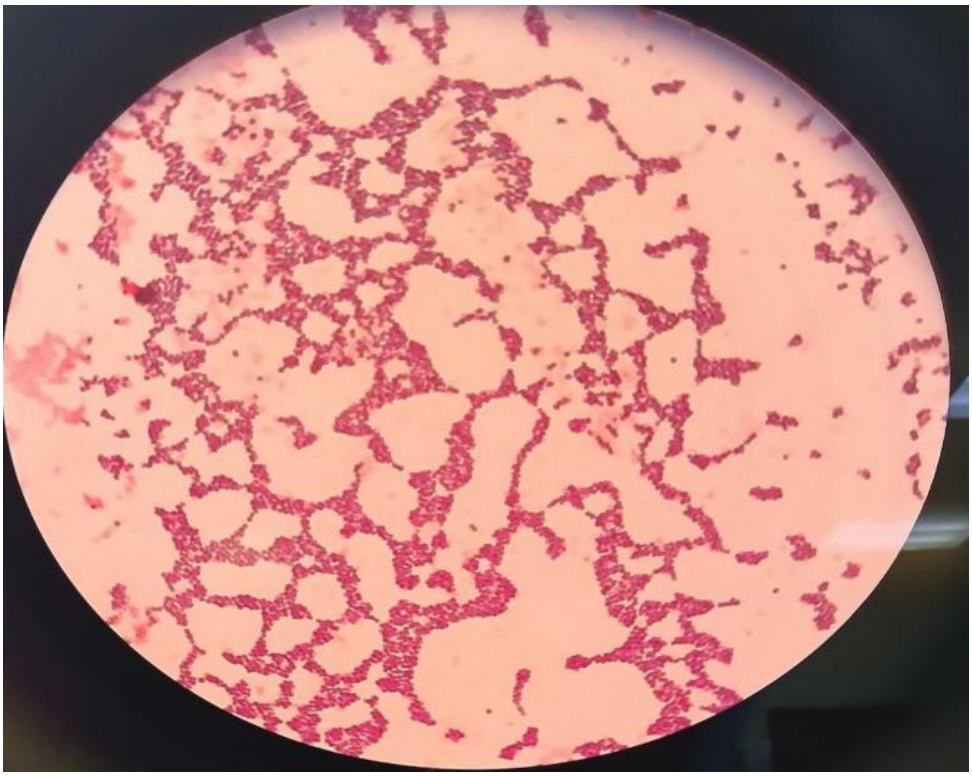

Figure 1: Gram staining results of gram-positive *cocci* from microscopic examination of urine culture

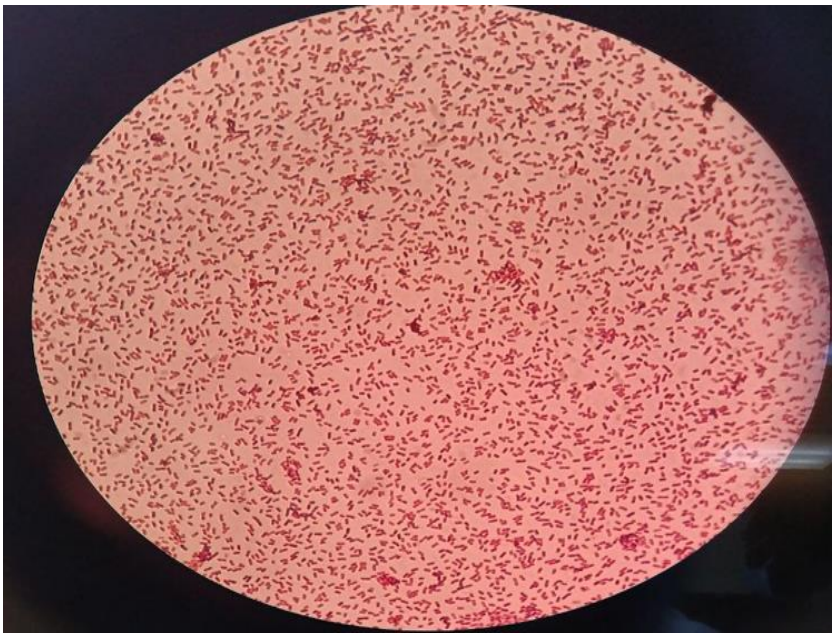

Figure 2: Gram staining results of a gram-negative short rod from urine culture.

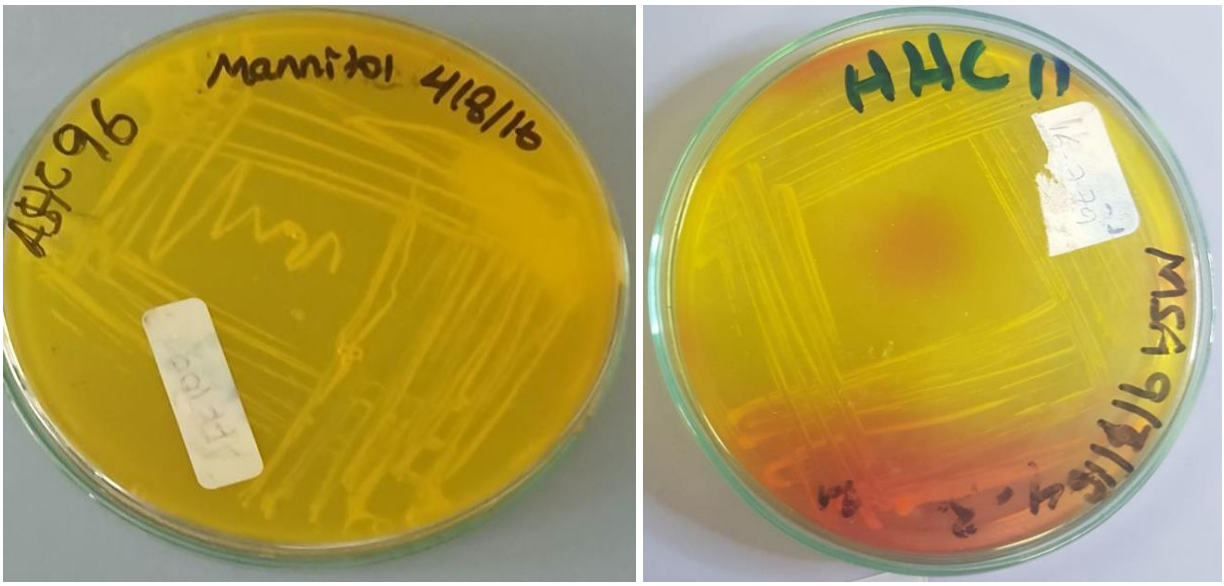

Figure 3: Significant growth of *S. aureus* on Mannitol salt agar from a urine sample.

(a) (b)

Figure 4: Biochemical Tests for G-negative bacteria (a) LIA, TSI, SIM, Urea, and Citrate tests from left to right (b) Citrate, Urea, TSI, SIM, and LIA tests from right to left.

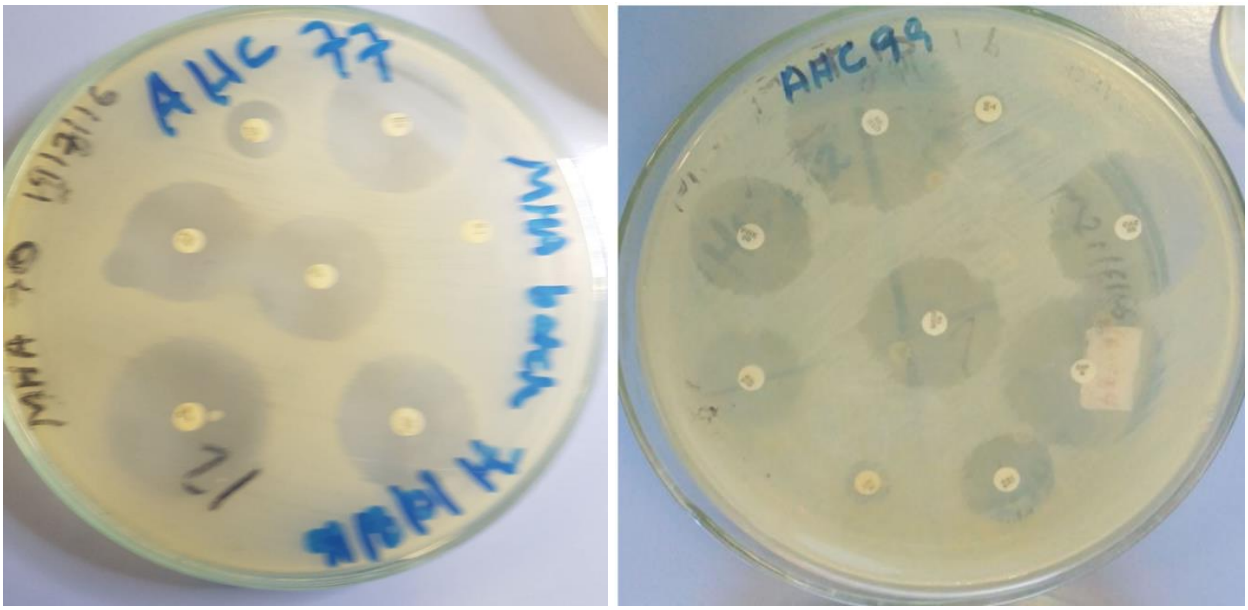

Figure 5: Antimicrobial susceptibility test of *E-coli* from two different urine samples.

(a) (b)

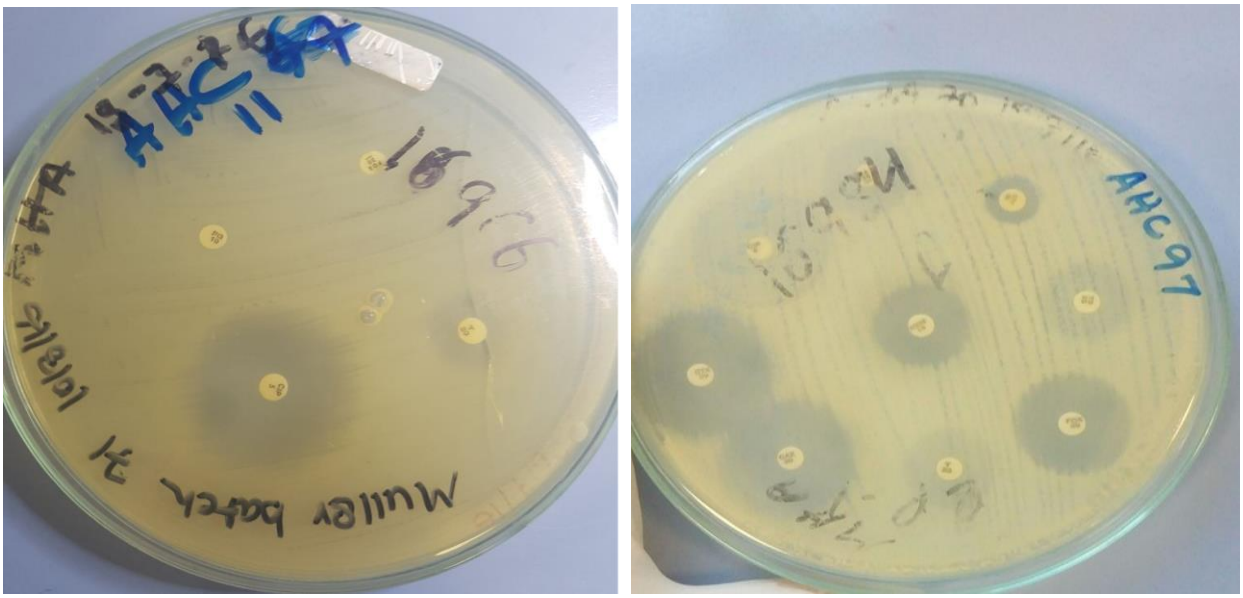

Figure 6 Antimicrobial susceptibility test of *S. aureus* (a) and *P. vulgaris* (b).

Appendix G: Ethical Consideration