DEBRE MARKOS UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

PREVALENCE, ANTIMICROBIAL SUSCEPTIBILITY PATTERN, AND ASSOCIATED FACTORS OF BACTERIAL UROPATHOGENS, AMONG PREGNANT WOMEN, ATTENDING ANTENATAL CARE AT DEBRE MARKOS COMPREHENSIVE SPECIALIZED HOSPITAL, NORTHWEST ETHIOPIA

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A THESIS SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCES, COLLEGE OF HEALTH SCIENCES, DEBRE MARKOS UNIVERSITY FOR THE PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN MEDICAL MICROBIOLOGY

JUNE, 2021

DEBRE MARKOS, ETHIOPIA

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APPROVAL SHEET

DEBRE MARKOS UNIVERSITY

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I, the undersigned, declared that this is my original work, has never been presented in this or any other University, and that all the resources and materials used for the thesis work, have been fully acknowledged.

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This thesis has been submitted for examination with my approval as the student thesis work advisor.

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This thesis examination result is correct and based on the given evaluation criteria

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ABBREVIATIONS AND ACRONYMS

AIDS	Acquired Immune Deficiency Syndrome
ANC	Antenatal Care
ASB	Asymptomatic Bacteriuria
AST	Antimicrobial Susceptibility Test
ATCC	American Type Culture Collection
BW	Birth Weight
CFU	Colony Forming Units
CLED	Cysteine Lactose Electrolyte Deficient
CLSI	Clinical and Laboratory Standards Institute
CoNS	Coagulase Negative *Staphylococcus*
DMU	Debre Markos University
DMCSH	Debre Markos Comprehensive Specialized Hospital
DMT	Debre Markos Town Administration
GBS	Group B *streptococci*
HIV	Human Immune Deficiency Virus
MDR	Multidrug Resistance
MGS	Meta-Genomic Sequencing
SOP	Standard Operational Procedure
UPEC	Uropathogenic *Escherichia coli*
UTI	Urinary Tract Infection

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ABSTRACT

Background: Urinary tract infection is one of the most common causes of morbidity among pregnant women. It affects millions worldwide and if untreated it can result in a serious obstetric complication. The increment of antimicrobial resistance in bacterial uropathogen is becoming a therapeutic challenge. In Ethiopia, the prevalence of urinary tract infection among pregnant women ranges from 9.5% to 35.1% and the prevalence of MDR was 60.65% to 96.0%.

Objective: This study aimed to assess the prevalence, antimicrobial susceptibility pattern, and associated factors of bacterial uropathogens among pregnant women attending antenatal care at Debre Markos Comprehensive Specialized Hospital, Northwest Ethiopia.

Method: Institutional based cross-sectional study was conducted among 205 pregnant women attending antenatal care at Debre Markos Comprehensive Specialized Hospital from April 5-May 11/2021. Data were collected using a questionnaire by interview. Urine specimens were collected and processed using standard procedures. Eighteen antimicrobial discs were used for the antimicrobial susceptibility test. Data were entered into Epi-data version 3.1 and analysis was performed by using SPSS version 26. Logistic binary regression was applied and variables with P-value less than 0.05 was considered as statistically significant and adjusted odds ratio were used to assess the strength of associated factors with bacterial uropathogens.

Results The overall prevalence of urinary tract infection among pregnant women was 19% (39/205) and predominant bacterial isolates were *E. coli* 19 (48.7%), *S. aureus* 9 (23.1%), and *K. pneumonia* 5 (12.8%). Factors associated with urinary tract infection were history of urinary tract infection (AOR: 3.836, 95% CI: 1.716-8.574, P-value = 0.001) and history of hospitalization (AOR: 4.825, 95% CI: 2.117-10.996, P-value = <0.001). The level of resistance against the tested drugs was 58.3% for CoNS, 55.6% for *P. aeruginosa*, and 42.3% for *P. mirabilis.* The prevalence of multi-drug resistance was 69.2% (27/39).

Conclusions and recommendations: Relatively high prevalence of bacterial urinary tract infection and multi-drug resistance for standard antimicrobials were observed. History of urinary tract infection and history of hospitalization were factors associated with urinary tract infection. Treatment of bacterial infections should be based on results from antimicrobial susceptibility tests and there should be also strong drug regulatory activities.

Keywords: Pregnant women, Prevalence, associated factors, urinary tract infection, AST.

1. INTRODUCTION

1.1 Background

Urinary tract infection (UTI) is the invasion and multiplication of microbial pathogens in the entire structure of the urinary system of the infected individual [1]. Infections can occur in any part of the urinary tract including the urethra (urethritis), bladder (cystitis), ureters, and the kidneys (pyelonephritis) [2, 3]. Ascending infection via the urethra to the bladder and then to the kidneys, hematogenous descending route, and lymphatic system are routes by which the microorganisms invade and infect the urinary tract. Untreated UTI may cause long-term complications like chronic pyelonephritis leading to hypertension or chronic renal failure and fatal consequences like septicemia, urosepsis and eventual death [4].

UTIs are classified based on clinical symptoms, laboratory data, and microbiological findings as uncomplicated and complicated UTIs [5]. Uncomplicated UTI mostly affects healthy individuals with no structural or neurological urinary tract abnormalities. These infections are differentiated into lower UTIs and upper UTIs [6]. Complicated UTI occurs in the presence of an abnormal urinary tract or other factors that increases susceptibility to further infection [7].

UTI is the commonest infectious disease especially in underdeveloped countries [8]. UTI is the third most common infection in humans, next to respiratory and gastrointestinal tract infections [9]. The annual incidence of UTI globally is estimated to be 250 million [8]. In the United States, there were an estimated 10.5 million visits for UTI symptoms [10]. Both sex and all age groups including children to older age persons are affected by UTIs [11]. But the incidence of UTI is more commonly seen in women than men due to shorter urethra, large bacterial load in urothelial mucosa, obstruction in the urinary tract, sexual activity, and pregnancy [12].

UTI can be caused by microorganisms like bacteria, fungi, viruses, and parasites but > 95% of UTI cases are caused by bacteria [13]. More than 50% of UTIs are caused by *E. coli* [14]. Other gram-negative bacterial pathogens that cause UTI include *Citrobacter, Enterobacter, Enterococcus, Klebsiella, Proteus, Pseudomonas,* and *Serratia* [15]. *Staphylococcus* and *Streptococcus* species are the most common gram-positive bacteria responsible for UTIs [16]. The other less frequent organisms include *Chlamydia* species *Candida albicans* and *Gardnerella vaginalis* [17].

UTI is among the most frequent infections in pregnant women which contributes 25% of all infections [18] and it is the common reason for admission [19]. UTI among pregnant women usually begins in the first six weeks of conception and reaches the peak at 22nd to 24th week of the pregnancy [20]. Untreated UTI causes about 20% of complications during pregnancy and it is responsible for the majority of antepartum admissions [21]. Complications include pyelonephritis, sepsis, septic shock, hypertensive disease of pregnancy, anemia, acute and chronic renal failure, and increased cesarean delivery [18, 22].

There are many factors associated with UTI among pregnant women. Some of them include history of UTI, HIV/AIDS, diabetes [23], age, residence, educational status, occupation, income, history of catheterization [24], lack of genital hygiene [25], kidney problems [18], gestational period [26], presence of genitourinary abnormalities [15], multiparity, anemia [27], sickle cell disease, use of contraceptive and previous use of third-generation cephalosporin [28].

According to many global data, there is increasing resistance of uropathogens to conventional, latest, and more potent antimicrobial drugs worldwide [29, 30]. It is more severe in developing countries [31]. There is also increasing colonization with multi-drug resistance (MDR) bacteria which is more difficult to treat. The conditions are even more challenging with the increasing prevalence of UTI as well as the reduced discovery of new antibiotics [32].

Appropriate investigation of uropathogens and factors associated with UTI as well as determining antibiotic susceptibility patterns (AST) is essential for the selection of suitable drugs for effective treatment of bacterial uropathogens. It helps also to minimize complications and adverse effects of UTI on both the mothers and children. However, data on the prevalence, associated factors, and antimicrobial susceptibility pattern of bacterial uropathogens among pregnant women are scarce in the Debre Markos area. Therefore, this study aimed to assess the prevalence, AST pattern, and associated factors of bacterial uropathogens among pregnant women at Debre Markos Comprehensive Specialized Hospital (DMCSH), Northwest Ethiopia.

1.2 Statement of the Problem

UTI is a commonest infectious disease, especially in underdeveloped countries. There was an estimated 250 million annual incidence of UTI globally [8] resulting in a cost of 6 billion USD for health care expenditure. Next to respiratory and gastrointestinal tract infections, UTI is the most common infection in humans [9]. In the United States, there were an estimated 10.5 million visits (0.9% of all ambulatory visits) for UTI symptoms [10]. In England and Wales, the burden of urinary tract infections was found to be approximately 315 per 10, 000 persons [33]. UTIs are the most frequent bacterial infection in women [34]. They occur most frequently between the ages of 16 and 35 years with 10% of women getting an infection yearly and more than 40–60% having an infection at some point in their lives [35]. Pregnant women have a 4 times higher rate of developing UTI compared to non-pregnant women [36, 37].

In pregnant women, UTI is among the most frequent clinical bacterial infections and contributes about 25% of all infections [18]. UTI is the common reason for admission and 20% of females have been affected with UTI in their gestational period. 28% of pregnant women with UTI have signs and symptoms of UTI while 71.9% have no symptoms [19]. UTI is one of the most common health problems in pregnancy having a prevalence of 3% to 35% worldwide in which the highest prevalence is predominantly seen in developing countries mainly Sub-Saharan Africa, the Middle East, and Asia [38]. The overall prevalence of UTI among pregnant women in developing countries in Africa and Asia was 13.5% [39].

Pregnancy-related changes in women that include physiological, anatomical, and hormonal make them susceptible to UTI [18]. Up to 70% of women during pregnancy develop glycosuria which encourages bacterial growth in the urine. In addition, some maternal defense mechanisms are less effective during pregnancy [40]. Dilatation of the urethra increased bladder volume and decreased bladder and ureteral tone leads to increased urinary stasis and vesicoureteric reflux. These changes are partly due to increased levels of progesterone and estrogen and also due to pressure created by the growing uterus [18].

Untreated UTI causes about 20% of complications during pregnancy and it is responsible for the majority of antepartum admissions [21]. Complications include pyelonephritis, sepsis, septic shock, hypertensive disease of pregnancy, anemia, acute and chronic renal failure, and increased

cesarean delivery [18, 22]. Acute pyelonephritis has been associated with chronic renal disease, sepsis, anemia, and pre-eclampsia [41, 42]. Twenty to thirty percent of pregnant women without treatment who have asymptomatic bacteruria (ABU) will develop symptomatic urinary tract infection [43]. Women with bacteriuria during their pregnancy may have more chance to have intrauterine growth restriction, premature delivery, low-birth-weight, fetal mortality, postpartum complications which increase neonatal mortality and morbidity [22, 44].

Resistance of bacterial uropathogens to conventional, latest, and more potent antimicrobial drugs is increasing worldwide that reduce therapeutic potential [29, 30]. The condition is more severe in underdeveloped countries [31]. Antimicrobial resistance of bacterial uropathogens depends on the use and misuse of antimicrobial drugs, along with the geographical location [45]. UTI treatment in several countries is mainly focused on the use of antibiotics including β-lactams [46]. Antibiotic resistance to β-lactam drugs by bacteria due to β-lactamase production by various gram-negative bacteria, especially by *Enterobacteriaceae* has a high prevalence globally [47, 48]. Nowadays, the development of MDR in bacterial uropathogens is increasing and colonization with MDR bacteria can lead to an infection that is more difficult to treat. The condition is even more challenging with the increasing prevalence of UTI as well as the reduced discovery of new antibiotics [32]. *Acinetobacter, Escherichia*, and *P. aeruginosa* are known for their intrinsic capability of showing multidrug resistance (MDR) [30].

In Ethiopia, the prevalence of UTI among pregnant women ranges from 9.5% to 35.1% [18, 49-52]. There was also 60.65% to 100% of MDR to antimicrobial drugs by uropathogens [50, 53]. Among *S. aureus*, 36.36% isolates were Methicillin resistance *S. aureus* (MRSA) and the prevalence of coagulase-negative *staphylococcus* (CoNS) for methicillin resistance was 47.06% [50].

According to the report from DMCSH in 2012, there were 2887 annual cases of UTI and the actual practice of treatment of UTI among pregnant women is empirical which has the potential of increasing antibiotic resistance. However, to the best of the investigator's knowledge, there is no published study on the prevalence, antimicrobial susceptibility patterns, and associated factors of bacterial uropathogens among pregnant women at DMCSH, Northwest Ethiopia. Therefore, this study aimed to explore prevalence, AST pattern as well as factors associated with bacterial uropathogens among pregnant women at DMCSH, Northwest Ethiopia.

1.3 Rationale of the study

Pregnant women are more susceptible to develop UTI and have a greater risk of serious complications to both the mother and her child. It is also becoming a great therapeutic challenge due to the development of multi-drug resistance (MDR).

Appropriate investigation of uropathogens and factors associated with UTI as well as determining antibiotic susceptibility patterns is useful for identifying causative agents of UTI and selection of appropriate drugs for an effective treatment that minimizes complication and adverse effects of UTI on both the mothers and children. It also helps to minimize the development of MDR.

But there was no published data on the prevalence of bacterial uropathogens and antimicrobial susceptibility pattern of isolates among UTI suspected pregnant women at Debre Markos Comprehensive Specialized Hospital. As a result, conducting this research fills this gap.

1.4 Significance of the Study

Valuable information and recommendation would be generated on the prevalence of bacterial uropathogens, drug susceptibility of identified bacterial isolates, and associated factors among pregnant women at DMCSH. This would be useful for policymakers, health sector organizations and their leaders, non-governmental institutions, and other concerned bodies to manage, reduce, prevent, and control UTI among pregnant women.

The finding of this research would contribute much to DMCSH on the management of UTI among pregnant women by identifying predominant bacterial isolates causing UTI in pregnant women and through determining the right choice of drugs for the isolated bacterial uropathogens. It also contributes for DMCSH to take action on factors associated with UTI among pregnant women which have been identified by this research.

During the actual research conducting period, pregnant mothers gained additional laboratory tests including gram staining, culture, and antimicrobial susceptibility test. Similarly, the clinicians have got also valuable results from the tests performed during the research that help them to decide on the mothers for the best treatment.

Moreover, this study would serve as a baseline source of information for anybody who wants to conduct further study.

2. LITERATURE REVIEW

2.1. Prevalence of UTI and Predominant Bacterial Isolates

The majority of urinary tract infections for all syndromes and all age groups were 74.4% among outpatients regardless of age group, 65% of hospital-acquired infections, and 47% of healthcare-associated infections. UTIs have an incidence of 50−60% in adult women. [54]. In England and Wales, the burden of urinary tract infections was found to be approximately 315 per 10, 000 persons [33], whereas, in Italy the there was a prevalence of 2.4% from 450, 000 people in a cohort study at primary care [55].

According to a cross sectional study from Mexico conducted on 6199 pregnant women, 6.37% were positive for UTI and predominant isolates were *E. coli* (81.2%), *Klebsiella* (12.8%), *Proteus* (4.9%), and CoNS (10.9%) [56]. On the other hand, a systematic literature search conducted between January 2005 and November 2016 in developing countries in Africa and Asia on 24,248 urine specimens from pregnant women showed 13.5% of the overall prevalence of UTI. *E. coli* accounted for half of the UTI cases followed by *Klebsiella* and *S. aureus* [39].

Based on a prospective study conducted in both pregnant women visting outpatient, and pregnant department during the period of 01-02-2014 to 31-05-2014 in Kerala, India, there was a 13.4% prevalence of UTI out of 210 urine samples and 10.47% were asymptomatic and 1.9% were symptomatic. Prevalence among 1st, 2nd, and 3rd trimesters was 8.5%, 11.9%, and 13.88% respectively [57]. Ninety nine percent (99%) of isolates were gram-negative and predominant isolates were *E. coli* (56.79%), *Klebsiella* (19.9%), *Pseudomonas* (6.3%), *Proteus* (5.8%), and *Enterococcus* (0.9%) [58]. On the contrary, the prevalence of UTI among pregnant women in Pakistan was 42.66% out of 375 samples and 64.37% and 35.62% were symptomatic and asymptomatic bacteriuria respectively. The predominant isolates were 25% of *Staphylococcus* [59], 54.2% of *E. coli*, 16.7% of *K. pneumonia*, 12.5% of *Pseudomonas*, and 8.3% *Enterococcus* [60]. On the other hand, the prevalence of UTI among pregnant women in Baghdad, Afghanistan was 31.2 % from 1140 pregnant women during September 2016 - August 2017. The highest prevalence was observed in the 21 to 30 years old age group. The predominant species includes *E. coli* (34%), *S. aureus* (22.2 %), *Klebsiella* (14.6 %), CoNS (11.5 %), *Proteus* (4.5%), *Pseudomonas* (3.7%), *Acinetobacter* (2.8 %), and *Citrobacter* (2.8%) [61].

A cross-sectional study conducted between February and September 2018 among a total of 420 pregnant women from Nigeria showed 22.9% prevalence of UTI among pregnant women. Among the study participants 51.4%, 39.3%, and 9.3% were in their 3rd, 2nd, and 1st trimester of pregnancy respectively. *E. coli* (50%), *S. aureus* (18.8%), *S. saprophyticus* (10.4%), *Klebsiella* (8.3%), *Enterococcus* (6.3%), and *P. aeruginosa* (4.2%) were predominant bacterial isolates [62]. On the other hand, a cross sectional study conducted among 400 pregnant women during January 2018 to January 2019 in Egypt revealed 45.5% prevalence of bacteriuria among pregnant mothers and 43% and 57% were symptomatic and asymptomatic UTI respectively. *E. coli* (34%), *K. pneumoniae* (26%), *S. saprophyticus* (23%) *Streptococci* (5%), and *Pseudomonas* (3%) were predominant bacterial isolates [63]. On the contrary, a cross sectional study conducted in 3 hospitals during January to April 2015 from Cameroon showed a 9.9 % prevalence of asymptomatic UTI among pregnant women from 354 participants. Bacterial isolates with higher prevalence include *E. coli* (48.6%), *K. pneumoniae* (14.3%), *Enterobacter cloacae* (11.4%), and *S. aureus* (8.6%) [64]. According to a retrospective analysis performed at 6 public sector hospitals among 5971 pregnant women at Durban, South Africa during 2011 – 2016, *E. coli* (54.2%), *K. pneumoniae* (12.9%), and *P. mirabilis* (10.4%) were among gram-negative organisms. *E. faecalis* (4.2%) and GBS (4.0%) were among gram-positive organisms [65].

Retrospective analysis of 200 mid-stream urine specimens conducted between January 2007 to December 2009 from Tanzania showed a 21% prevalence of Urinary tract infection from 400 pregnant women. *E. coli* (33%), *Klebsiella* (4%), CoNS (16.7%), *S. aureus* (14.3%), *Proteus* (7.1%), and *Enterococcus* (7.1%) were predominant bacterial isolates [66]. Similarly, 21% of significant bacteriuria was found in Hargeisa, Somaliland from the total 376 pregnant mothers and 73.4% and 26.6% were Gram-negative and Gram-positive bacteria respectively. The commonest isolates were *E. coli*, (45.6%), *K. pneumonia* (20.3%), and *S. aureus* (11.4%). The proportion of symptomatic and asymptomatic UTI was 69.6% and 30.4% respectively [67].

Researchers conducted many studies in different parts of Ethiopia and the results showed the following findings among pregnant women. Institution based cross-sectional study design conducted during January to April 2014 among 169 pregnant women attending ANC at Bale Zone showed 26% prevalence of UTI. Symptomatic and asymptomatic UTI was 35.3% and 22% respectively. *E. coli* (27.3%), *K. pneumonia* (20.5%), and *Serratia marcescens* (9.1%) were

isolates with the highest prevalence [18]. On the other hand, research from Ambo showed 18.7% prevalence of UTI among pregnant women and 20.4% and 17.8% were symptomatic and asymptomatic UTI respectively [53]. In Harar, a health facility-based comparative cross-sectional study conducted among 651 women showed 14.1% of the prevalence of UTI and predominant bacterial isolates were *E. coli* (28.8%) and *S. aureus* (14.3% [24]. Based on a cross-sectional study conducted at Adigrat General Hospital, Northern Ethiopia from 1 January to 30 April 2018, among 259 pregnant mothers, 21.2% were positive and *E. coli* (34.6%), *S. aureus* (18.2%), *Klebsiella* (18.2%), *S. saprophyticus* (12.7%), and *P. mirabilis* (9.1%) were the predominant bacterial isolates [26].

Studies conducted in the Amhara region showed the following results. Based on a cross-sectional study conducted on 222 pregnant women at the University of Gondar Teaching Hospital from January 1 to May 31, 2016, the prevalence of UTI among pregnant women was 35.1% out of 134 participants and the bacterial isolates with higher prevalence were *E. coli* (51.1%), *S. aureus* (34%), *K. pneumoniae* (4.3%), and CoNS (4.3%) [52]. On the contrary, a hospital-based cross-sectional study conducted at Dessie, Northeastern Ethiopia region, among 323 pregnant women showed 15.5% prevalence of UTI and 11.8% and 26.6% were asymptomatic and symptomatic UTI respectively. The predominant isolates were *E. coli* (33.3%), CoNS (30.0%), *S. aureus* (27.5%), *K. pneumoniae* (3.9%), *S. agalactiae* (3.9%) and *Enterobacter* species (2.0%) [51]. Similarly, a hospital-based cross-sectional study conducted among 384 pregnant women from March to May 2017 at Bahrdar, Northwest showed a 15.9% prevalence of UTI among pregnant women. The prevalence of symptomatic and asymptomatic UTI was 31.1% and 8.8% respectively. *E. coli* (49.2%), CoNS (27.9%), and *S. aureus* (18%) were predominant bacterial isolates [50].

2.2. Factors Associated with UTI among Pregnant Women

Research from America showed sexual activity, condom/diaphragm/spermicide use, vaginal infection, trauma, diabetes mellitus, obesity genetic susceptibility, and anatomic abnormalities were identified factors associated with UTI [10]. Based on a study from India factors associated with UTI among pregnant include age, parity, diabetes mellitus, and personal hygiene [57]. Based on the study from Saudi Arabia age, occupation, family income and previous treatment for UTI during current pregnancy were factors associated with UTI among pregnant women [68].

A study from Cameroon revealed the history of UTI, multigravida, and low salaries as factors associated with UTI among pregnant women whereas, a high level of education was protective [64]. A study from Uganda identified a history of the previous diagnosis of UTI, living with diabetes mellitus, and HIV/AIDS as factors associated with UTI [23]. Based on a study from Hargeisa Somaliland, previous history of UTI, monthly income, educational status, and having dysuria were found to be factors associated with UTI among pregnant women [67].

According to a study from Goba, southeast Ethiopia factors associated with UTI among pregnant women were low educational status and kidney problems [18]. A study from Harar eastern Ethiopia, identified residence, age, income, educational status, occupation, history of UTI, current symptoms of UTI, and history of the catheterization as factors associated with UTI [24]**.** In a study from Addis Ababa, age occupation, marital status, residents, and educational status were identified as factors associated with UTI among pregnant women [69]. Monthly income, eating raw meat, and the previous history of UTI were identified factors associated with UTI among pregnant women from Ambo, Ethiopia [53]. A study from Adigrat, northern Ethiopia revealed age, income level as factors associated with UTI among pregnant women [26]. Another study from the same location showed the history of previous UTI and catheterization as factors associated with UTI [51].

On the other hand, a study from Gondar, revealed residence, gestational age, and previous history of diabetes mellitus as factors associated with UTI among pregnant women [52]. History of UTI, anemia, sexual activity, and family monthly income were the identified factors associated with UTI among pregnant women from Bahir Dar, Northwest Ethiopia [70].

2.3. Antimicrobial Susceptibility of Bacterial Uropathogens

Based on researches conducted in Mexico *E. coli, Klebsiella* and *Proteus* showed a 97%, 100%, and 20% resistance respectively to the treatments used that include ampicillin, amoxicillin, trimethoprim-sulfamethoxazole, and nitrofurantoin [56]. From countries in Asia and Africa, uropathogens had resistance to ampicillin (67.2%), ciprofloxacin (71.2%), nitrofurantoin (65%), and ceftriaxone (74.1%) [39].

According to research from India, *E. coli* showed higher sensitivity to amikacin, imipenem, tazobactam + piperacillin, and gentamicin but showed 100% resistance to ampicillin. *klebsiella* was 100% sensitive to imipenem, tazobactam+ piperacillin, amikacin, cefepime, gentamicin, ciprofloxacin, and norfloxacin but it was 100% resistant to ampicillin. *S. aureus* was sensitive to cotrimoxazole, cefoxitin, and tetracycline [57]. Research from Pakistan showed piperacillin-tazobactam had a sensitivity of 89.6% followed by meropenem (87.5%), nitrofurantoin (87.5%), and Fosfomycin (81.3%). Cefixime, cefotaxime, and amoxicillin/clavulanic acid had overall sensitivities above 50% [60]. From Baghdad, Afghanistan**,** bacterial isolates showed 85.6%, 72.2%, and 71.3% of resistance against ampicillin, cotrimoxazole, and tetracycline respectively. They showed moderate resistance to ceftazidime (52.2%), ciprofloxacin (49.4%), amoxicillin+clavulanic acid (47.2%), and ceftriaxone (42.7%). Imipenem was the most active antibiotic against isolated uropathogens with a lower resistance prevalence (3.1 %) [61].

Studies from Nigeria showed most of the isolates were resistant to ampicillin, augmentin, gentamicin, and cotrimoxazole. Antimicrobials with the lowest resistance were imipenem 2.7%, nitrofurantoin 23.8%, and ciprofloxacin 34.3%. *P. aeruginosa* was resistant to almost all tested drugs except imipenem [62]. *E. coli* indicated high resistance against sparfloxacin (60.3%), amoxicillin (84.5%), pefloxacin (77.6%), and streptomycin (70.1%). But it was highly susceptible to chloramphenicol (70.1%), ciprofloxacin (87.9%), and augmentin (91.4%). *K. pneumonia* showed high susceptibility to chloramphenicol (78.1%), sparfloxacin (62.5%), and ciprofloxacin. *S. aureus* showed 100.0% susceptibility to ampiclox but it showed 66.7% resistance to amoxicillin. CoNS showed 100.0% resistance to gentamycin but it was susceptible to pefloxacin (87.5%), ampiclox (75.0%), zinnacef (87.5%), and ciprofloxacin (87.5%) [71].

Based on a study from South Africa, the susceptibility of *E. coli* to trimethoprim-sulfamethoxazole was 34.9%. It also showed 61.7% and 72.7% susceptibility to the first- and second-generation cephalosporin respectively. *E. faecalis* showed 93% and 100% susceptibility to ampicillin/amoxicillin and amoxicillin-clavulanic acid respectively [65]. According to a study from Egypt, *E. coli* was susceptible to meropenem (96%), nitrofurantoin (74%), tobramycin (72%), aztreonam (68%), cefepime (63%), and ceftriaxone (55%). *K. pneumoniae* showed 80-100% resistance rates to most tested antimicrobial agents and aztreonam showed the highest susceptibility. Among the total gram-negative isolates, 95% were MDR [63].

Based on a study from Tanzania bacterial isolates such as *E.coli, S. aureus, Klebsiella* species, *Proteu*s species, and CoNS were sensitive to nitrofurantoin (81.3 %), ampicillin (42.3%), cotrimoxazole (61.5 %), ciprofloxacin (86.4 %), and amikacin (95 %) [66]. In Hargeisa, Somaliland, gram-negative isolates displayed resistance against ampicillin (87%), amoxicillin (85%), cefotaxime (57%), and cephalexin (52%). But nitrofurantoin was effective against the majority of the isolates. *S. aureus* displayed 100% sensitivity to amoxicillin-clavulanic acid, ceftriaxone, nitrofurantoin, ceftazidime, norfloxacin, cefotaxime, and cephalexin. Almost all isolates of *Citrobacter* species were found sensitive for the tested antimicrobials except ampicillin and cephalexin [67].

Based on studies from Ethiopia, various bacterial uropathogens showed a different result of AST against various antimicrobials. In Adigrat, gram-negative isolates were 86.8%, 77.1%), 76.3%, and 76.3% sensitive to ceftriaxone, ciprofloxacin, amoxicillin+clavulanic, and gentamycin respectively. But they were resistant to ampicillin (100%). Gram-positive isolates were sensitive to vancomycin (100%), ceftriaxone (88.2%), ciprofloxacin (82.3%), and nitrofurantoin (76.5%). *E. coli* was 100% and 47.4% of resistant against ampicillin and cotrimoxazole respectively. *Klebsiella* was also 100% resistant against ampicillin and 30% resistant against nitrofurantoin. *P aeruginosa* showed 1005% resistance against ampicillin and cotrimoxazole and 66.7% resistance against ciprofloxacin and amoxicillin. *P. mirabilis* also showed 100% resistance against ampicillin [26].

In Gondar, uropathogen isolates were susceptible to amikacin (100%), norfloxacin (86.5%), nitrofurantoin (80.8%), and ceftriaxone (69.2%). *K. pneumoniae* isolates were (100%) resistant to amoxicillin and ampicillin. *E. coli* isolates were (58.3%) and (41.7%) resistant to amoxicillin and trimethoprim-sulfamethoxazole respectively. *S. aureus* was (75%), (50%), (68.8%), and (47.7%) resistant to trimethoprim-sulfamethoxazole, tetracycline, amoxicillin, and ampicillin respectively. CoNS isolates were resistant to tetracycline, amoxicillin, ampicillin, trimethoprim-sulfamethoxazole, gentamicin, and ciprofloxacin at a rate of 50%. Gram-negative and gram-positive bacterial isolates displayed 54.5% and 95.8% of MDR respectively [52].

A study in Bahir Dar showed that 3.28% of isolates were sensitive to all antibiotics used. Isolates were resistant for two, three, and five of the antibiotics at a rate of 22.95%, 26.23%, and 31.15% respectively. *E. coli* was 90%, 83.33%, and 56.7% resistant to ampicillin cephalothin and augmentin respectively. There was 60.65% of MDR among uropathogens. The prevalence of MDR against symptomatic and asymptomatic participants was 65.8% and 52.2% respectively. CoNS and *E. coli* isolates showed 76.47% and 56.67% of MDR respectively [50].

2.4. Conceptual Framework

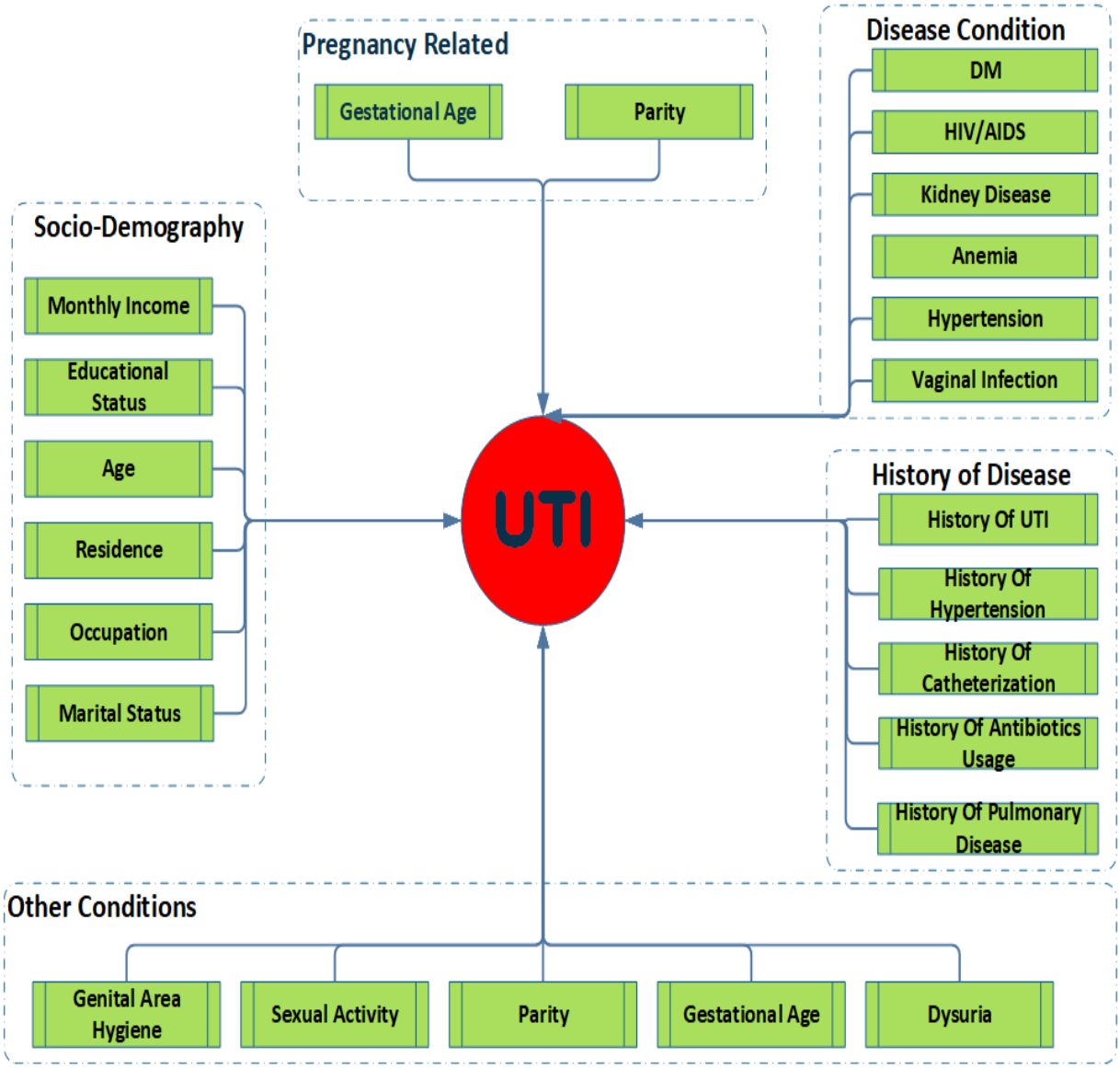


Figure 1: Conceptual framework

3. OBJECTIVES

3.1 General Objective

The general objective of this study was to assess the prevalence, antimicrobial susceptibility pattern, and associated factors of bacterial uropathogens among pregnant women attending antenatal care at Debre Markos Comprehensive Specialized Hospital, Northwest Ethiopia.

3.2 Specific Objectives

- To determine the prevalence of bacterial uropathogens among pregnant women, Northwest Ethiopia.
- To identify factors associated with UTI among pregnant women, Northwest Ethiopia.
- To determine antimicrobial susceptibility pattern of bacterial isolate among pregnant women, Northwest Ethiopia.

4. MATERIALS AND METHODS

4.1. Study Area and Period

The study was conducted among pregnant women at DMCSH which is found in Debre Markos town, the capital of the East Gojjam zone. The town is approximately 300 km far from Addis Ababa which is the capital city of Ethiopia in the northwest direction and is also 265 km far from Bahir Dar, the capital of Amhara Regional State.

According to the estimation from Central Statistical Agency of Ethiopia (CSA), Debre Markos has an estimated population of 262,497 in 2012 E.C. Among those 129,921 are men and 132,576 are women [72]. Based on data from Debre Markos town administration health office, Debre Markos town has 3 governmental health centers, 1 governmental comprehensive specialized hospital, 18 private clinics, and 25 private drug stores. The health service coverage of Debre Markos town is estimated to be 100%.

Debre Markos Comprehensive Specialized Hospital is the only specialized hospital found in Debre Markos town which was established in 1957 E.C. DMCSH gives many services to both east and west Gojjam zone population. One of the services is ANC which gives different services to mothers. The study was conducted from April 6 to May 11, 2021.

4.2. Study Design

An institutional-based cross-sectional study was conducted among pregnant women at DMCSH, ANC unit.

4.3. Population

4.3.1. Source Population

- All pregnant women who came to DMCSH for ANC service.

4.3.2. Study Populations

- All pregnant women who came to DMCSH, ANC clinic during the study period and fulfill the inclusion criteria.

4.4. Inclusion and Exclusion Criteria

4.4.1. Inclusion criteria

- Pregnant women attending ANC and that gave consent.

4.4.2. Exclusion criteria

- Pregnant women who were on antibiotic therapy during the past 14 days.

4.5. Sample Size Determination and Sampling Technique

4.5.1. Sample Size Determination

The sample size was calculated based on a single population proportion, using prevalence P= 15.9% from the study conducted at Bahir Dar, Northwest Ethiopia [50]. This was due to there is an assumption of population similarity and geographical proximity.

$$N = \frac{\left(\frac{Z\alpha}{2}\right)^2 pq}{d^2}$$

Where N= required sample size, $Z\alpha/2^2$= at 95% confidence interval = 1.96 (at alpha=0.05), d^2, marginal error= 5%

$$N = \frac{\left(\frac{Z\alpha}{2}\right)^2 pq}{d^2}$$

$$N = \frac{(1.96)^2 \text{x } 0.159(1-0.159)}{(0.05)^2}$$

$$N = \frac{3.8416 \text{ x } 0.133719}{0.0025}$$

$$N = 205$$

4.5.2. Sampling Technique

A convenient sampling technique was applied to select study participants. Consecutively pregnant women were sampled and sampling continues until the sample size was satisfied.

4.6. Variable

4.6.1. Dependent Variable

- Bacterial uropathogens

4.6.2. Independent Variables

Socio-demographic related variables

- Age
- Occupation
- Residence
- Income
- Marital status
- Educational status

Pregnancy and clinical related variables

- Sexual activity
- Parity
- Stage of pregnancy
- Symptoms of UTI
- History of hospitalization
- History of catheterization
- History of antibiotics usage
- History pulmonary TB
- Anemia
- Hypertension
- Diabetes mellitus
- Kidney disease
- HIV/AIDS
- Sexual activity
- Cleaning genital area
- History UTI

4.7. Operational Definition

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

UTI: - refers to participants whose urine is yielding positive cultures (≥ 10^5 CFU/ml).

Asymptomatic bacteriuria: refers to participants whose urine is yielding positive cultures (≥ 10^5 CFU/ml) without urinary symptoms.

Symptomatic bacteriuria: refers to participants whose urine is yielding positive cultures (≥ 10^5 CFU/ml) with urinary symptoms.

Multidrug resistance: Bacterial isolates showing resistance to at least one agent in three or more antimicrobial categories [73].

Significant bacteriuria: a urine sample containing greater than 10^5 colonies/ml of urine in pure culture [74].

4.8. Data Collection and Processing

4.8.1. Socio-Demographic and Related Data Collection

A Structured questionnaire was prepared in the English language and it was translated into the local language for data collection and re-translated back to the English version. Socio-demographic data and all other variable responses were collected using a pretested structured questionnaire by interview. Four trained DMCSH staff were included for data collection. The principal investigator checked the completeness of the data daily.

4.8.2. Specimen Collection

15-30 ml of clean catch mid-stream urine specimens were collected from each study participant using sterile, wide-mouthed screw-capped urine caps. The sample container was labeled with the patient's code, and time of urine collection. Study participants were oriented on how to collect clean catch mid-stream urine samples. Specimens were analyzed immediately after collection at DMCSH, microbiology laboratory. Specimens that were not processed within 2 hours were kept refrigerated at 2-8 °C until it has been processed for a maximum of 4 hours.

4.8.3. Bacterial Isolation and Identification

Using calibrated wire loop (0.001ml) specimen was inoculated into CLED agar. After 24-48 hours of incubation at 37 °C, colony counts with bacterial growth of $\geq 10^5$ CFU/ml were considered as significant bacteriuria. Significant growth has been sub-cultured into MacConkey and blood agar plates for 24 hours at 37 °C. Bacterial isolates were identified by colony characteristics, gram reaction, and biochemical tests through standard procedure. Biochemical tests like KIA, indole test, citrate, coagulase, catalase, motility, and LDC were used.

4.8.4. Antimicrobial Susceptibility Test

Antibiotic susceptibility testing was performed among all significant bacteriuria based on the criteria of the Clinical and Laboratory Standards Institute (CLSI) [75]. Modified Kirby Bauer disc diffusion method was used to determine the antimicrobial susceptibility of isolates. Individual culture colony of 24-hour growth was suspended in 5 ml normal saline and its turbidity was compared with 0.5 McFarland standards to prepare a standardized suspension of the correct inoculum size.

A sterile cotton swab was dipped into the tube and the excess fluid was removed by rotating the swab across the wall of the tube. Finally, the suspension was evenly inoculated over the entire surface of Muller Hinton Agar (MHA). The antibiotic discs were placed on MHA by using sterile forceps. The inoculated MHA plates were incubated at 37 °C for 24 hours. The antimicrobial discs that were used for antimicrobial susceptibility tests against gram-negative bacteria were ampicillin, gentamycin, amikacin, augmentin, ceftazidime, ceftriaxone, ciprofloxacin, imipenem, meropenem, nitrofurantoin, norfloxacin, tetracycline, cotrimoxazole, piperacillin, and tobramycin. On the other hand, antimicrobial discs used for antimicrobial testing against gram-positive bacterial isolates were gentamycin, ciprofloxacin, nitrofurantoin, norfloxacin, cotrimoxazole, doxycycline, oxacillin, and penicillin. The diameter of the zone of inhibition around the antimicrobial discs was measured using a ruler and the result was interpreted based on the CLSI guideline [75].

4.8.5. Laboratory workflow

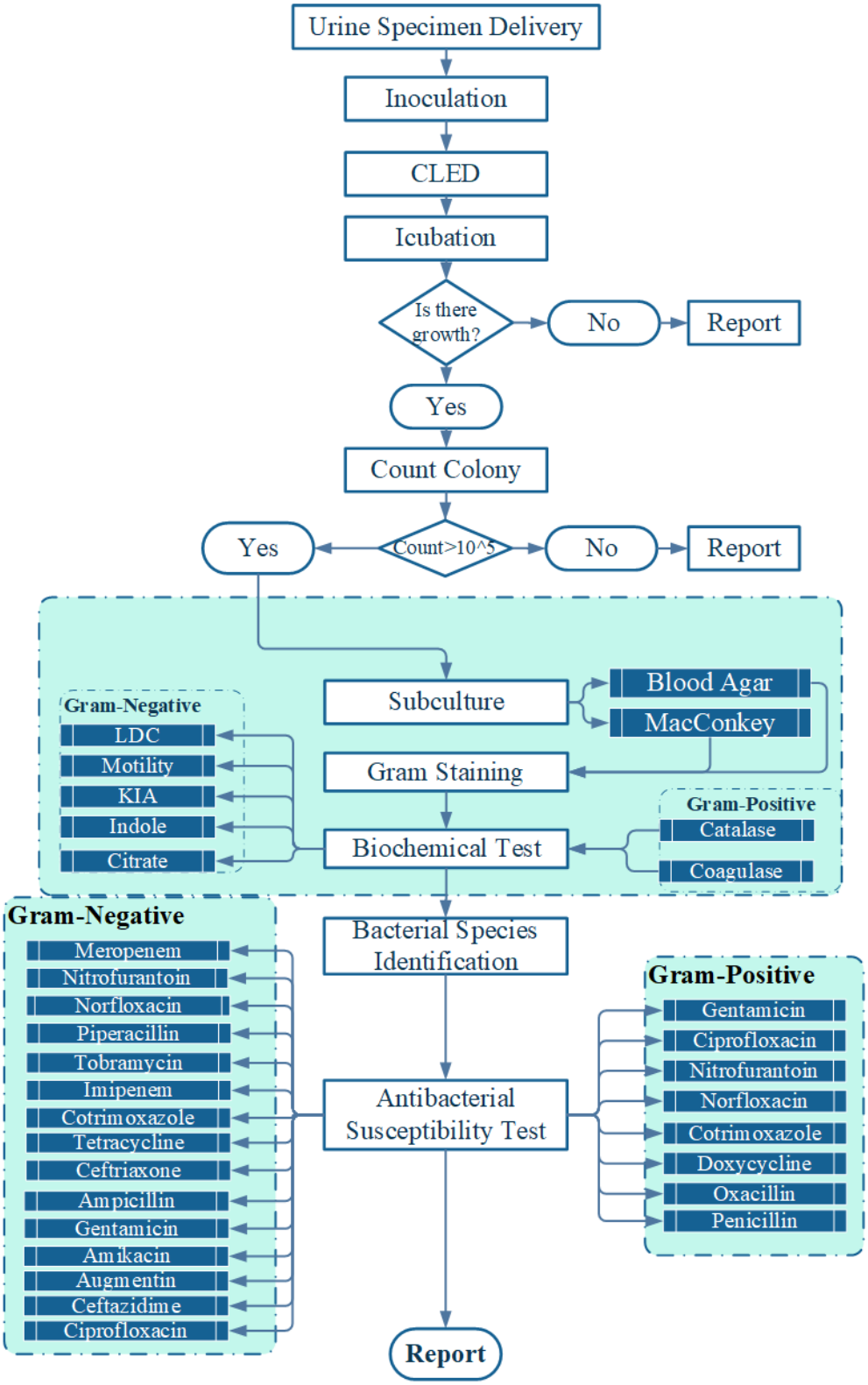


Figure 2: Laboratory workflow

4.9. Data Analysis and Interpretations

After the data was collected, it was entered into Epi-data version 3.1 for cleaning and exported into SPSS version 26 for analysis. Primarily, bivariable logistic regression was done and variables with P-value $\leq$ 0.25 were considered as eligible for multivariable logistic regression analysis. Backward stepwise binary logistic regression was applied to identify factors associated with UTI among pregnant women. Hosmer-Lemeshow test and classification adequacy were performed to assess the fitness of the model and interpreted accordingly. P-values less than 0.05 were considered as statistically significant and adjusted odds ratios were used to assess the strength of associated factors with UTI.

4.10. Quality Assurance

4.10.1. Data Collection Quality Control

Before the actual data collection, a pretest of questionnaires was performed on 11 (5%) pregnant women attending ANC at DMCSH, and amendment measures were taken as necessary based on the feedback from the pretest. The training was given to data collectors before they have started to collect the data. The collected data was checked out daily for accuracy and clarity by the principal investigator. When there were errors in data collection, amendments were made before the next data collection.

4.10.2. Laboratory Quality Assurance

Pre-analytical

Quality control activities that have been done at the pre-analytical stage includes appropriate storage of all supplies including reagents and culture media, proper identification and orientation of the study participants, proper labeling of the specimen, storage and transportation of specimen, preparation of test algorithm, and SOP preparation of the media based on the manufacturer's manual. All equipment was checked for their functionality and performance by using standard procedures. Sterility and performance of culture media were tested before the actual work by incubating 5% of the prepared media and observe for any growth of the bacteria. There should not be any growth to use the media.

Analytical

All the laboratory tests were performed based on SOP. For the quality control of media, *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), and *P. aeruginosa* (ATCC 27853) strains were used.

Post analytical

The result of the tests was appropriately recorded and documented. All the wastes from the laboratory were disposed of following safety precautions.

4.11. Ethical Considerations

The research was conducted after ethical clearance was obtained from the ethical review committee of the College of Health Sciences, Debre Markos University. A letter of permission was written to DMCSH to continue the study. Each study participant was included after they give written consent. All the information obtained from each participant was kept confidential. Withdrawal of participants from the study was assured at any time when they didn't want to continue in the study. There was no activity of coercion and deception. Patient names were not used rather codes were used to ensure confidentiality of study participant's information.

5. RESULTS

5.1. Socio-demographic Characteristics

In this study, a total of 205 participants were included. The age of the study participants ranges from 18 to 44 and the mean with standard deviation was 27.9 $\pm$ 4.7. Among those participants, 161(78.5%) were from urban and 137(66.8%) were within the age group of 18-29. One hundred ninety-six (95.6%) were married, 49(23.9%) were illiterate, 74(36.1%) had above diploma occupational status, and 86 (42.0%) were housewives (Table 1).

Table 1: Socio-demographic characteristics of pregnant women.

Variables		UTI		
		Positive	**Negative**	**Total**
Residence	Rural	5	39	44(21.5%)
	Urban	34	127	161(78.5%)
Age	18-29	26	111	137(66.8%)
	30-39	13	52	65(31.7%)
	40-49	0	3	3(1.5%)
	Total	39	166	205(100%)
Marital status	Married	37	159	196(95.6%)
	Single	2	3	5(2.4%)
	Divorced	0	3	3(1.5%)
	Widowed	0	1	1(0.5%)
	Total	39	166	205(100%)
Educational status	Illiterate	4	45	49(23.9%)
	Primary school	7	32	39(19.0%)
	Secondary school	11	32	43(21.0%)
	Diploma and above	17	57	74(36.1%)
	Total	39	166	205(100%)
Monthly income	<1000	15	83	98(47.8%)
	1000-5000	12	63	75(36.6%)
	5001-10000	12	18	30(14.6%)
	>10000	0	2	2(1.0%)
	Total	39	166	205(100%)
Occupational status	Housewife	11	75	86(42.0%)
	Governmental employee	15	34	49(23.9%)
	Farmer	1	8	9(4.4%)
	Daily laborer	1	6	7(3.4%)
	Private Employee	1	17	18(8.8%)
	Student	1	2	3(1.5%)
	Merchant	9	24	33(16.1%)
	Total	39	166	205(100%)

5.2. Pregnancy and Clinical Related Factors

Among those participants, 63 (30.7%) had symptoms of UTI, 57 (27.8%) had a previous history of UTI, and 53 (25.9%) has a history of hospitalization. The gestational age of the study participants ranges from 8 to 39 weeks and the mean with a standard deviation of gestational was 21.25 ±10.017. Of the total participants, 85 (41.5%) were in the second trimester and 187 (91.2%) had < 4 children. Sixteen 16 (7.8%) had hypertension, 113 (55.1%) had sexual activity, and 156 (76.1%) clean their genital area (Table 2).

Table 2: Pregnancy and clinical related conditions of pregnant women.

Variables		UTI			Variables		UTI		
		Positive	Negative	Total			Positive	Negative	Total
Parity	0-3	38	149	187(91.2%)	Anemia	No	33	148	181(88.3%)
	4-6	0	16	16(7.8%)	Hypertension	Yes	9	7	16(7.8%)
	7-10	1	1	2 (1.0%)		No	30	159	189(92.2%)
Gestational age	0-13 months	11	45	56(27.3%)	Diabetes mellitus	Yes	1	1	2(1.0%)
	14-26 months	19	66	85(41.5%)		No	38	165	203(99.0%)
	27-40 months	9	55	64(31.2%)	Parasitic infection	Yes	11	10	21(10.2%)
Symptoms of UTI	Yes	22	41	63(30.7%)		No	28	156	184(89.8%)
	No	17	125	142(69.3%)	Kidney disease	Yes	5	9	14(6.8%)
Previous history UTI	Yes	23	34	57(27.8%)		No	34	157	191(93.2%)
	No	16	132	148 72.2%	Liver disease	Yes	1	2	3(1.5%)
History of hospitalization	Yes	3	8	11(5.4%)		No	38	164	202(98.5%)
	No	36	158	194(94.6%)	HIV AIDS	Yes	6	9	15(7.3%)
History of antibiotic	Yes	9	26	35(17.1%)		No	33	157	190(92.7%)
usage	No	30	140	170(82.9%)	Sexual activity	Yes	23	90	113(55.1%)
History of pulmonary TB	Yes	0	1	1(0.5%)		No	16	76	92(44.9%)
	No	39	165	204(99.5%)	Cleaning genital area	Yes	27	129	156(76.1%)
Anemia	Yes	6	18	24(11.7%)		No	12	37	49(23.9%)

5.3. Prevalence of UTI

Among the total 205 participants, 39 were positive and 166 were negative for significant bacterial growth that made the prevalence of UTI among pregnant women 19% (Figure 3). Of the total UTI-positive study participants, 22 (56.4%) had symptoms of UTI. Of the total participants, 63(30.7%) had symptoms of UTI and the prevalence of symptomatic and asymptomatic UTI was 22 (34.9%) and 17(11.9%), respectively (Figure 4).

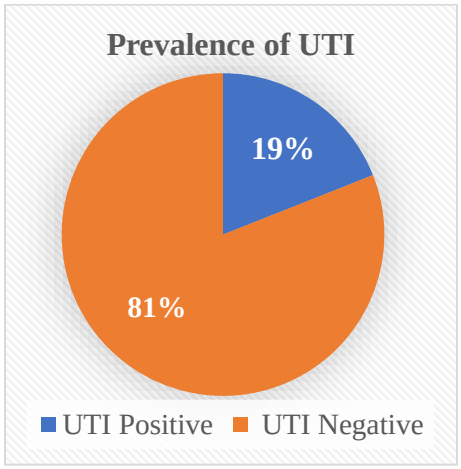


Figure 3: Prevalence of UTI among pregnant women.

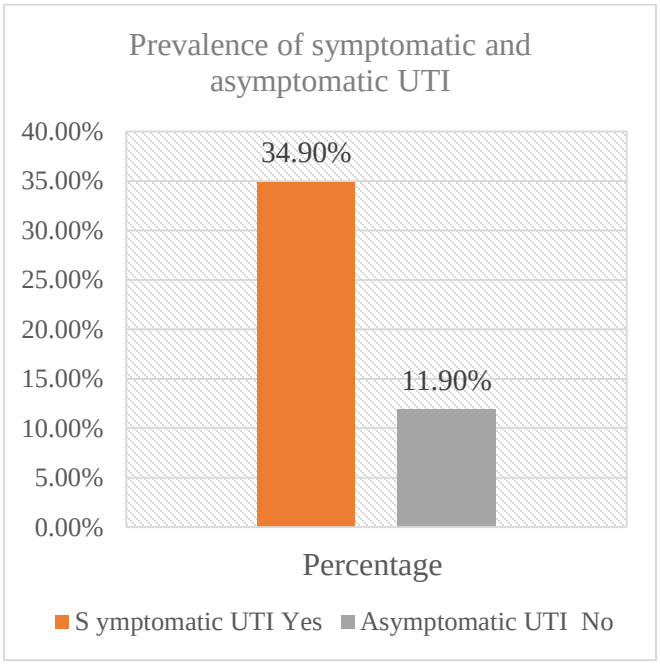


Figure 4: Prevalence of symptomatic and asymptomatic UTI among pregnant women.

5.4. Bacterial Uropathogens

Six bacteria were isolated from urine culture that includes *E. coli* 19 (48.7%), *S. aureus* 9 (23.1%), *K. pneumonia* 5 (12.8%), CoNS 3 (7.7%), *P. aeruginosa* 1 (2.6%), and *P. mirabilis* 2 (5.1%) (Fig 5). Among those isolates, 27 (69.2%) were gram-negative whereas, 12 (30.8%) were gram-positive bacteria (Figure 5).

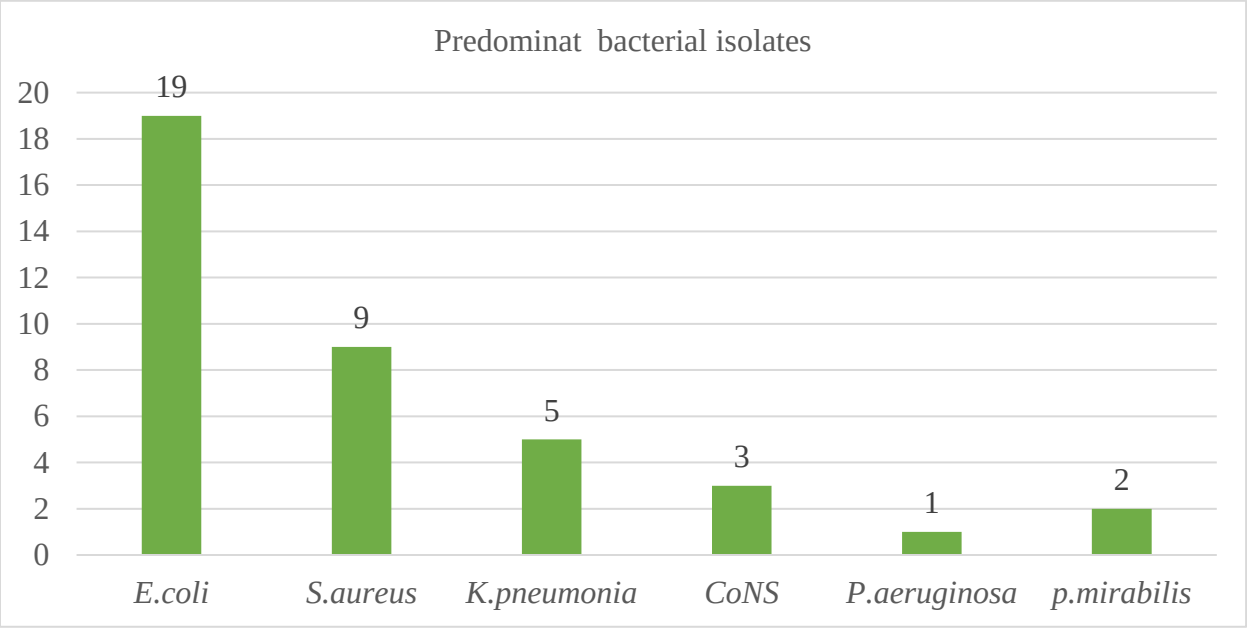


Figure 5: Bacterial isolates among pregnant women.

5.5. Associated Factors of UTI

In the mono-variable variable logistic regression analysis, 22 variables were evaluated. Among those only 9 variables had P-value $\leq$ 0.25 and considered for multivariable logistic regression. In the multivariable logistic regression, only 2 of the variables were significantly associated with UTI having a p-value $\leq$ 0.05. Factors associated with urinary tract infection were history of urinary tract infection (AOR: 3.836, 95% CI: 1.716-8.574, P-value = 0.001) and history of hospitalization (AOR: 4.825, 95% CI: 2.117-10.996, P-value = <0.001) (Table 3).

Table 3: Analysis of socio-demographic, pregnancy, and clinical related factors associated with UTI among pregnant women.

Variables		UTI			COR (95% CI)	P-value	AOR (95% CI)	P-value
		Positive	Negative	Total				
Residence	Rural	5	39	44	0.479 (0.175-1.308)	0.151		
	Urban	34	127	161	1			
Educational status	Illiterate	4	45	49	0.298(0.094-0.948)	0.040		
	Primary school	7	32	39	0.733(0.275-1.956)	0.536		
	Secondary school	11	32	43	1.153(0.481-2.760)	0.750		
	Diploma+	17	57	74	1			
Occupational	Housewife	11	75	86	0.643(0.268-1.541)	0.322		
status	Gov'tal employee	15	34	49	1.934(0.822-4.551)	0.131		
	Private Employee	13	57	9	1			
Gestational age	0-13 months	11	45	56	1			
	14-26 months	19	66	85	1.178(0.512-2.711)	0.701		
	27-40 months	9	55	64	0.669(.255-1.757)	0.415		
Previous history	Yes	23	34	57	5.581(2.660-11.710)	<0.001	3.836(1.716-8.574)	0. .001
UTI	No	16	132	148	1		1	
History of	Yes	3	8	11	7.559(3.537-16.152)	<0.001	4.825(2.117-10.996)	<0.001
hospitalization	No	36	158	194	1		1	
Hypertension	Yes	9	7	16	6.814(2.356-19.708)	<0.001	2.791(0.860-9.054)	.087
	No	30	159	189	1		1	
Kidney disease	Yes	5	9	14	2.565(0.809-8.137)	0.110		
	No	34	157	191	1			
HIV AIDS	Yes	6	9	15	3.172(1.057-9.519)	0.040		
	No	33	157	190	1			

5.6. Antimicrobial Susceptibility Pattern

In this study, *E. coli* showed 31.6% of resistance against the tested antimicrobials. *E. coli* showed 63.2%, 57.9%, and 47.4% of resistance against cotrimoxazole, ampicillin, and ciprofloxacin respectively. *P. aeruginosa* showed 55.6% of resistance against tested drugs and it was 100% resistance for gentamycin, amikacin, ceftazidime, ciprofloxacin, and norfloxacin. *K. pneumonia* showed 30.8% of resistance against the tested antimicrobials. *K. pneumonia* showed 100%, 60%, and 40.0% resistance against cotrimoxazole, tetracycline, and meropenem respectively. *P. mirabilis* showed 42.3% of resistance against tested antimicrobials. It showed 100% resistance to tetracycline, ceftriaxone, and cotrimoxazole (Table 4).

S. aureus showed 40.3% of resistance against the tested antimicrobials. *S. aureus* showed a resistance of 100% to penicillin, 66.7% to cotrimoxazole, and 55.6% to oxacillin. CoNS showed 41.7% of sensitivity and 58.3% of resistance against tested drugs. It showed a resistance of 100% against doxycycline and penicillin (Table 4).

In this study, 18 drugs were used for AST. The level of resistance among antimicrobials was 100% for ampicillin, 68.4% for cotrimoxazole and 58.3% for doxycycline were resistant against the tested isolates. On the other hand, the level of sensitivity was 86.8% for nitrofurantoin, 81.5% for imipenem, 77.8% for meropenem, and 76.9% for norfloxacin against tested bacterial isolates (Table 4).

In this study, all 39 (100%) isolates were resistant at least for one drug and 36 (92.3%) showed resistance for > 2 antimicrobials. The prevalence of MDR (resistance for at least three classes of drugs) against the identified bacterial isolates was 27 (69.2%). In this study 20 (74.1%) gram-negative and 7 (58.3%), gram-positive bacteria showed MDR.

Table 4: Antimicrobial susceptibility test result of bacterial isolate causing UTI among pregnant women.

Bacterial isolates		**AMP**	**GEN**	**AMIK**	**AUG**	**CEFZ**	**CEF**	**CIP**	**IMP**	**MER**
E. coli	S	8	13	11	12	15	13	10	15	15
	I	0			2	1	1			1
	R	11	6	8	5	3	5	9	4	3
K. pneumonia	S	3	3	4	5	3	2	4	4	3
	I	1					2			
	R	1	2	1		2	1	1	1	2
P. mirabilis	S	1	2	1	2	1			2	2
	I									
	R	1		1		1	2	2		
P. aeruginosa	S								1	1
	I									
	R		1	1		1		1		
S. aureus	S		7					8		
	I									
	R		2					1		
CoNS	S		2					1		
	I									
	R		1					2		
Total	S	12(46.2%)	27(69.2%)	16(59.3%)	19(73.1%)	19(70.4%)	15(57.7%)	23(59%)	22(81.5%)	21(77.8%)
	I	1(3.8%)			2(7.7%)	2(7.4%)	3(11.5%)			1(3.7%)
	R	13(50%)	12(30.8%)	11(40.7%)	5(19.2%)	6(22.2%)	8(30.8%)	16(41%)	5(18.5%)	5(18.5)

Table 4: Antimicrobial susceptibility test result of bacterial isolate causing UTI among pregnant women continued.

Bacterial isolates		**NIT**	**NOR**	**TTC**	**COT**	**DOX**	**OXA**	**PEN**	**PIP**	**TOB**	**Total**
E. coli	S	17	15	12	6						162(65.6%)
	I		1		1						7 (2.8%)
	R	2	3	7	12						78(3.6%)
K. pneumonia	S	5	4	2							42(64.6%)
											3(4.6%)
	R		1	3	5						20(30.8%)
P. mirabilis	S	2	2								15 (57.7%)
	I										
	R			2	2						11(42.3%)
P. aeruginosa	S								1	1	4(44.4%)
	I										
	R		1								5(55.6%)
S. aureus	S	7	8		3	5	4				42(58.3%)
	I	1									1(1.4%)
	R	1	1		6	4	5	9			29(40.3%)
CoNS	S	2	1		2		2				10(41.7%)
	I										
	R	1	2		1	3	1	3			14(58.3%)
Total	S	33(86.8%)	30(76.9%)	14(53.8%)	11(28.9%)	5(41.7%)	6(50%)		1(100%)	1(100%)	
	I	1(2.6%)	1(2.6%)		1(2.6%)						
	R	4(10.5%)	8(20.5%)	12(46.2%)	26(68.4%)	7(58.3%)	6(50%)	12(100%)			

AMP ampicillin, GEN gentamycin, AMIK amikacin, AUG augmentin, CEFZ ceftazidime, CEF ceftriaxone, CIP ciprofloxacin, IMP imipenem, MER meropenem, NIT nitrofurantoin, NOR norfloxacin, TTC tetracycline, COT cotrimoxazole, DOX doxycycline, OXA oxacillin, PEN penicillin, PIP piperacillin, TOB tobramycin S, sensitive I, intermediate R, resistant

6. DISCUSSION

In this study the overall prevalence of UTI among pregnant women was 19%. This finding is consistent with the study from India (20.1%) [76], Cameroon (23.5%) [77], Tanzania (21%) [66], Somaliland (21%) [67], Ambo (18.7%) [53], Adigrat (21.2%) [26], Bahirdar (15.9%) [50], and Dessie (15.5%) [51]. On the contrary, this research finding is less than research findings from Pakistan (42.6%) [59], Afghanistan (31.2 %) [61], India (28.0%) [78], Egypt (45.5%) [63], and Bale Zone (26%) [18]. On the other hand, the finding of this study is higher than research findings from India (13.4%) [57], Iran (13.1%) [79], Cameroon (9.9 %) [64], Addis Ababa (11.6%) [80], and Bahir Dar (9.5%) [49]. The observed variation in the above prevalence of UTI might be due to the difference in geographical location, environmental conditions, sample size, sampling method, and socio-demographic characteristics of study participants.

The prevalence of symptomatic UTI in this study was 34.9%. This finding in lines with study findings from India (36.1%) [81], Nigeria (46.5%) [82], Egypt (43%) [63] Gondar (35.1%) [52], Addis Ababa (31.6%) [83], Bale zone (35.3%) [18], Bahirdar (31.1%) [50], Dessie (26.6%) [51], and Southeastern Ethiopia (26%) [84]. On the contrary, our finding is higher than findings from Pakistan (4.3%) [85], India (1.9) [57], Mexico (6.37%) [56], Tanzania (17.9%) [86], Sudan (12.1%) [87], Addis Ababa (20%) [80] and Ambo (20.4%) [53]. On the other hand, our finding is less than findings from India (59.7%) [76], Pakistan (64.37%) [59], and Hargeisa, Somaliland (69.6%) [67].

The prevalence of asymptomatic UTI in this study was 11.9%. This findings is consistent with research findings from India (10.47%) [57], Turkey (8.5%) [88], Tanzania (13%) [86], Cameroon (9.9 %) [64], Dessie (11.8%) [51], Bahir Dar (8.8%) [50], and Gondar (9.0%) [89]. But our finding is higher than findings from, Ghana (5.5%) [90] and Egypt 1(0.0%) [91]. On the contrary, our finding is less than findings from Pakistan (35.62%) [59], Brazil (29.1%) [92], Egypt (57%) [63], Hargeisa, Somaliland (30.4%) [67], Bale zone (22%) [18], and Ambo (17.8%) [53]. The observed variation in the above prevalence of UTI might be due to the difference in geographical location, environmental conditions, sample size, sampling method, and socio-demographic characteristics of study participants.

The most predominant gram-negative bacterial isolate from this study was *E. coli* (48.7%). This finding is consistent with research findings from developing countries in Africa and Asia (50%) [39], India (56.79%) [58], Pakistan (54.2%) [60], Afghanistan (34 %) [61], South Africa (54.2%) [65], Camerron (48.6%) [64], Egypt (34%) [63], Tanzania (33.3%) [66], Somaliland (45.6%) [67], Adigrat (34.6%) [26], Gondar (51.1%) [52], Dessie (33.3%) [51], and Bahirdar (49.2%) [50]. On the contrary, this finding was lower than finding from Mexico (81.2%) [56] and higher than finding from Bale zone (27.3%) [18].

The second most predominant gram-negative bacterial isolate in our study was *K. pneumonia (*12.8%). This finding is consistent with research findings from Mexico (12.8%) [56], India (19.9%) [58], Pakistan (16.7%) [60], Afghanistan (14.6 %) [61], Nigeria (8.3%) [62], Cameroon (14.3%) [64], South Africa (12.9%) [65], Tanzania (4%)[66], Somaliland (20.3%) [67], Bale zone (20.5%) [18], Dessie (3.9%) [51], and Gondar (4.3%) [52].

The most predominant gram-positive bacterial isolate in this study was *S. aureus* (23.1%). This finding in lines with finding from Afghanistan (22.2 %) [61], Nigeria (18.8%) [62], Tanzania (14.3%) [66], Hargeisa Somaliland (11.4%) [67], Adigrat (18.2%) [26], Dessie (27.5%) [51], Gondar (34%) [52], and Bahirdar (27.9%) [50]. But it was higher than study findings from Cameroon (8.6%) [64]. The possible reason for the above differences may be due to the differences in geographical location.

From the multivariable logistic regression, only 2 of the variables showed a statistically significant association with UTI. History of UTI is one of the factors associated with UTI with AOR: 3.836, 95% CI (1.716-8.574), and P-value = 0.001. This implies, those pregnant women having a history of UTI have a 3.836 higher risk of developing UTI as compared to those who have no history of UTI. This finding is consistent with findings from Cameroon (2.183) [64], Uganda (1.98) [23], Ambo (2.29) [53], and Bahir Dar (2.687) [70]. On the other hand, our finding is lower in strength than findings from Saudi Arabia (18.904) [68] and Dessie (8.824) [51]. The association between UTI and previous history of UTI may be possibly due to the re-occurrence of previous bacteria and the presence of resistant bacterial strains from those individuals who had a previous history of UTI as a result of poor diagnosis and ineffective and inappropriate treatment.

History of hospitalization had also an association with UTI among pregnant women with AOR: 4.825, 95% CI (2.117-10.996), and P-value = <0.001). This implies those women who have a history of hospitalization have 4.825 times greater risks of developing UTI as compared to those women who have no history of hospitalization. This might be due to poor medication and care given and the acquisition of nosocomial infection during hospitalization.

In our study, all 39 (100%) bacterial isolates identified showed resistance at least for 1 antimicrobial and 37 (94.8%) showed resistance for ≥ 2 antimicrobials. In this study, 27 (69.2%) bacterial isolates showed MDR and this result is consistent with findings from Bahir Dar (60.65%) [50]. On the contrary, this finding is lower than finding from Kenya (96.0%) [93], Jigjiga (96.0%) [94], Dire Dawa (100.0%) [70], and Gondar (95%) [89]. The above differences might be due to the differences in the type of drugs used for AST, treatment guidelines, and drug regulatory practice. In this study 20 (74.1%) gram-negative and 7 (58.3%), gram-positive bacteria showed MDR. Gram-negative bacteria have a higher level of MDR. This may be due to the presence of an alteration in the outer membrane like changing the hydrophobic properties or mutations in porins and other factors in which gram-positive lacks the above layers.

CoNS showed 58.3% of resistance against tested drugs which made CoNS the highest resistant bacterial isolate in this study. It showed 100% resistance against penicillin. This finding is consistent with findings from Dessie (86.7%) [51] and Bahirdar (88.2%) [50]. On the contrary, our result is greater than findings from Jigjiga (66.7%) [94] and Gondar (0%) [52]. CoNS also showed 100% resistance against doxycycline and this finding is higher than findings from Bahirdar (58.82%) [50].

In this study, the second most resistant bacterial isolate that showed resistance against the tested drugs was *P. aeruginosa* with 55.6% of the resistance. It showed 100% resistance for gentamycin and this finding is consistent with findings from Gondar (100%) [52] and Kenya (100%) [93]. But our finding is greater than studies from Nigeria (33.3%) [95] and Adigrat (33.3%) [96]. *P. aeruginosa* also showed 100% resistance against ciprofloxacin and this result is higher than findings from Kenya (0%) [93], Nigeria (0%) [95], Harar (18.2%) [24], and Gondar (0%) [52]. *P. aeruginosa* showed also 100% resistance against norfloxacin and this finding is higher than findings from Somaliland (0%) [67] and Gondar (0%) [52].

Among the isolates 78 (31.6%) *E. coli* were against the tested antimicrobials. *E. coli* showed 63.2% of resistance to cotrimoxazole and this research finding is greater than findings from Gondar (41.7%) [52], and Dessie (35.3%) [51]. But our finding is lower than the finding from Mexico (99%) [56]. *E. coli* also showed 57.9% resistance against ampicillin and this finding is consistent with findings from Tanzania (57.7%) [66], Gondar (58.3%) [52], and Dessie (64.7%) [51]. On the other hand, our find is lower than findings from Mexico (100%) [56] and Bahir Dar (90%) [50].

7. CONCLUSIONS AND RECOMMENDATIONS

In this study, a relatively higher prevalence of UTI among pregnant women was observed as compared to the other studies in the country. Factors associated with UTI among pregnant women were history of UTI and history of hospitalization. The prevalence of MDR against the identified bacterial isolates was relatively higher. Antimicrobials with a higher level of resistance were ampicillin, cotrimoxazole, and doxycycline.

Treatment of bacterial infections should be based on results from antimicrobial susceptibility tests. Additionally, there should be effective and continuous surveillance to monitor trends in antimicrobial resistance control. To minimize recurrent UTI and the occurrence of resistant organisms there should be appropriate and effective diagnosis as well as treatment of UTI cases both on ambulatory and admitted patients. Moreover, molecular studies will have the advantage in the characterization of resistant strains.

8. LIMITATION OF THE STUDY

The sampling technique of this study was convenient and only those pregnant women who came to the hospital for antenatal care were included which is difficult to generalize to the population. In this study, we didn't perform the extended-spectrum beta-lactamase production status of the bacterial isolates due to the shortage of budget.

9. REFERENCES

1. Foxman B: Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. *Disease-a-month* 2003, 49(2):53-70.
2. Foxman B: The epidemiology of urinary tract infection. *Nature Reviews Urology* 2010, 7(12):653-660.
3. Wagenlehner FM, Johansen TEB, Cai T, Koves B, Kranz J, Pilatz A, Tandogdu Z: Epidemiology, definition and treatment of complicated urinary tract infections. *Nature Reviews Urology* 2020, 17(10):586-600.
4. Das A, Pal SR: Evaluation of Urinary Tract Infections for Microbiological and Antibiogram Studies in The Department of Obstetrics and Gynaecology Of Malda Medical College & Hospital, Malda, West Bengal, India. *IOSR-JDMS* 2020, 19(2):35-44.
5. Grabe M, Bjerklund-Johansen T, Botto H, Çek M, Naber K, Tenke P, Wagenlehner F: Guidelines on urological infections. *European association of urology* 2015, 182.
6. Hooton TM: Uncomplicated urinary tract infection. *New England Journal of Medicine* 2012, 366(11):1028-1037.
7. Sheerin NS: Urinary tract infection. *Medicine* 2011, 39(7):384-389.
8. Parveen S, Reddy SV, Rao M, Rao J: Uropathogens and their drug susceptibility patterns among pregnant women in a teaching hospital. *Ann Biol Res* 2011, 2(5):516-521.
9. Anejo-Okopi A, Okwori A, Eze M, Onaji A, Ali M, Adekwu A, Ejiji I: Prevalence and Antibiotic Resistance Pattern of Urinary Tract Bacterial Infections among Symptomatic Patients Attending University of Maiduguri Teaching Hospital, North East Nigeria. 2015.
10. Foxman B: Urinary tract infection syndromes: occurrence, recurrence, bacteriology, risk factors, and disease burden. *Infectious disease clinics of North America* 2013, 28(1):1-13.
11. Kalpana S, Hegadi SS, Ramesh K: Characterization and antimicrobial susceptibility testing of uropathogens from urinary tract infections. *Intl J CurrMicrobiol App Sci* 2015, 4(2):1010-1016.
12. Sohail M, Khurshid M, Saleem HGM, Javed H, Khan AA: Characteristics and antibiotic resistance of urinary tract pathogens isolated from Punjab, Pakistan. *Jundishapur journal of microbiology* 2015, 8(7):e19272.

13. Delanghe JR, Kouri TT, Huber AR, Hannemann-Pohl K, Guder WG, Lun A, Sinha P, Stamminger G, Beier L: The role of automated urine particle flow cytometry in clinical practice. *Clinica Chimica Acta* 2000, 301(1-2):1-18.
14. Blomgran R, Zheng L, Stendahl O: Uropathogenic Escherichia coli triggers oxygen-dependent apoptosis in human neutrophils through the cooperative effect of type 1 fimbriae and lipopolysaccharide. *Infection and immunity* 2004, 72(8):4570-4578.
15. Nguefack CT, Ebongue CO, Chokotheu CN, Ewougo CE, Njamen TN, Mboudou E: Clinical presentation, risk factors and pathogens involved in bacteriuria of pregnant women attending antenatal clinic of 3 hospitals in a developing country: a cross sectional analytic study. *BMC pregnancy and childbirth* 2019, 19(1):143.
16. Ahmed SS, Shariq A, Alsalloom AA, Babikir IH, Alhomoud BN: Uropathogens and their antimicrobial resistance patterns: Relationship with urinary tract infections. *International journal of health sciences* 2019, 13(2):48.
17. Ferede G, Yismaw G, Wondimeneh Y, Sisay Z: The prevalence and antimicrobial susceptibility pattern of bacterial uropathogens isolated from pregnant women. *Eur J Exp Biol* 2012, 2(5):1497-1502.
18. Taye S, Getachew M, Desalegn Z, Biratu A, Mubashir K: 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* 2018, 11(1):799.
19. Hamdan Z: Ziad Abdel Haliem M, Ali Salah K, Ishag Adam: Epidemiology of urinary tract infections and antibiotics sensitivity among pregnant women at Khartoum North Hospital. *Ann Clin Microbiol Antimicrob* 2011, 10(2):1-5.
20. Le J, Briggs GG, McKeown A, Bustillo G: Urinary tract infections during pregnancy. *Annals of Pharmacotherapy* 2004, 38(10):1692-1701.
21. Lee M, Bozzo P, Einarson A, Koren G: Urinary tract infections in pregnancy. *Canadian Family Physician* 2008, 54(6):853.
22. Mishra PP, Prakash V, Premi HK, Agarwal L, Mog H, Agarwal S, Darbari A, Sharma M: Predisposing factors and aetiology of urinary tract infections in pregnant women. *J Evid Based Medicine Healthcare* 2016, 94556.

23. Bahati J, Stephen BM, Joseph N, Asiphas O, Musa K, Taseera K: Prevalence and Bacteriology of Symptomatic Urinary Tract Infection among Pregnant women at Mbarara Regional Referral Hospital, South-western Uganda. 2020.
24. Abate D, Marami D, Letta S: Prevalence, Antimicrobial Susceptibility Pattern, and Associated Factors of Urinary Tract Infections among Pregnant and Nonpregnant Women at Public Health Facilities, Harar, Eastern Ethiopia: A Comparative Cross-Sectional Study. *Canadian Journal of Infectious Diseases and Medical Microbiology* 2020, 2020.
25. Seifu WD, Gebissa AD: Prevalence and antibiotic susceptibility of Uropathogens from cases of urinary tract infections (UTI) in Shashemene referral hospital, Ethiopia. *BMC infectious diseases* 2018, 18(1):30.
26. Tadesse S, Kahsay T, Adhanom G, Kahsu G, Legese H, A GW, Derbie A: Prevalence, antimicrobial susceptibility profile and predictors of asymptomatic bacteriuria among pregnant women in Adigrat General Hospital, Northern Ethiopia. *BMC Res Notes* 2018, 11(1):740.
27. Emiru T, Beyene G, Tsegaye W, Melaku S: Associated risk factors of urinary tract infection among pregnant women at Felege Hiwot Referral Hospital, Bahir Dar, North West Ethiopia. *BMC Research Notes* 2013, 6(1):292.
28. Vardi M, Kochavi T, Denekamp Y, Bitterman H: Risk factors for urinary tract infection caused by Enterobacteriaceae with extended-spectrum beta-lactamase resistance in patients admitted to internal medicine departments. *IMAJ-Israel Medical Association Journal* 2012, 14(2):115.
29. Tula M, Iyoha O: Distribution and antibiotic susceptibility pattern of bacterial pathogens causing urinary tract infection in Mubi general hospital, Yola-Nigeria. *Journal of Advances in Medicine and Medical Research* 2014:3591-3602.
30. Gallagher JC, MacDougall C: Antibiotics simplified: Jones & Bartlett Learning; 2016.
31. Zhanel GG, Hisanaga TL, Laing NM, DeCorby MR, Nichol KA, Weshnoweski B, Johnson J, Noreddin A, Low DE, Karlowsky JA: Antibiotic resistance in Escherichia coli outpatient urinary isolates: final results from the North American Urinary Tract Infection Collaborative Alliance (NAUTICA). *International journal of antimicrobial agents* 2006, 27(6):468-475.

32. Geffers C, Gastmeier P: Nosocomial infections and multidrug-resistant organisms in Germany: epidemiological data from KISS (the Hospital Infection Surveillance System). *Deutsches Ärzteblatt International* 2011, 108(6):87.
33. Royal College of General Practitioners, Office of Population Censuses and Surveys: Morbidity Statistics from General Practice, Fourth National Study 1991-1992,. In. Edited by Department of Health, vol. Series MB5,. London, UK,: HMSO; 1995.
34. Gupta K, Hooton TM, Naber KG, Wullt B, Colgan R, Miller LG, Moran GJ, Nicolle LE, Raz R, Schaeffer AJ: International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: a 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. *Clinical infectious diseases* 2011, 52(5):e103-e120.
35. Salvatore S, Salvatore S, Cattoni E, Siesto G, Serati M, Sorice P, Torella M: Urinary tract infections in women. *European journal of obstetrics & gynecology and reproductive biology* 2011, 156(2):131-136.
36. Rosana Y, Ocviyanti D, Karuniawati A, Akhmad SRP: Comparison of microbial pattern causing urinary tract infection in female out-and hospitalized patients in Jakarta. *Microbiology Indonesia* 2016, 10(1):5-5.
37. Vasudevan R: Urinary tract infection: an overview of the infection and the associated risk factors. *J Microbiol Exp* 2014, 1(2):00008.
38. Gilbert NM, O'brien VP, Hultgren S, Macones G, Lewis WG, Lewis AL: Urinary tract infection as a preventable cause of pregnancy complications: opportunities, challenges, and a global call to action. *Global advances in health and medicine* 2013, 2(5):59-69.
39. Belete MA, Saravanan M: A Systematic Review on Drug Resistant Urinary Tract Infection Among Pregnant Women in Developing Countries in Africa and Asia; 2005–2016. *Infection and Drug Resistance* 2020, 13:1465-1477.
40. Okonko I, Ijandipe L, Ilusanya O, Donbraye-Emmanuel O, Ejembi J, Udeze A, Egun O, Fowotade A, Nkang A: Incidence of urinary tract infection (UTI) among pregnant women in Ibadan, South-Western Nigeria. *African Journal of Biotechnology* 2009, 8(23).
41. Wing DA, Fassett MJ, Getahun D: Acute pyelonephritis in pregnancy: an 18-year retrospective analysis. *American journal of obstetrics and gynecology* 2014, 210(3):219. e211-219. e216.

42. Easter SR, Cantonwine DE, Zera CA, Lim K-H, Parry SI, McElrath TF: Urinary tract infection during pregnancy, angiogenic factor profiles, and risk of preeclampsia. *American journal of obstetrics and gynecology* 2016, 214(3):387. e381-387. e387.
43. Smaill F: Asymptomatic bacteriuria in pregnancy. *Best Practice & Research Clinical Obstetrics & Gynaecology* 2007, 21(3):439-450.
44. Smaill FM, Vazquez JC: Antibiotics for asymptomatic bacteriuria in pregnancy. *Cochrane database of systematic reviews* 2019(11).
45. Asmat U, Mumtaz MZ, Malik A: Rising prevalence of multidrug-resistant uropathogenic bacteria from urinary tract infections in pregnant women. *Journal of Taibah University Medical Sciences* 2020.
46. Karam MRA, Habibi M, Bouzari S: Urinary tract infection: Pathogenicity, antibiotic resistance and development of effective vaccines against Uropathogenic Escherichia coli. *Molecular immunology* 2019, 108:56-67.
47. Moland ES, Black JA, Ourada J, Reisbig MD, Hanson ND, Thomson KS: Occurrence of newer β-lactamases in Klebsiella pneumoniae isolates from 24 US hospitals. *Antimicrobial agents and chemotherapy* 2002, 46(12):3837-3842.
48. Chaudhary U, Aggarwal R: Extended spectrum-lactamases (ESBL)-An emerging threat to clinical therapeutics. *Indian journal of medical microbiology* 2004, 22(2):75.
49. Demilie T, Beyene G, Melaku S, Tsegaye W: Urinary bacterial profile and antibiotic susceptibility pattern among pregnant women in North West Ethiopia. *Ethiopian journal of health sciences* 2012, 22(2).
50. Biset S, Moges F, Endalamaw D, Eshetie S: Multi-drug resistant and extended-spectrum β-lactamases producing bacterial uropathogens among pregnant women in Northwest Ethiopia. *Annals of Clinical Microbiology and Antimicrobials* 2020, 19(1):25.
51. Belete MA: 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* 2020, 13:2579.
52. Bitew AM, Tegene BM, Yeshitila BM, Howe R, Dagnew M: Bacterial profile, antibacterial susceptibility pattern and associated factors among women attending antenatal and post-natal health services at the university of Gondar teaching hospital, Northwest Ethiopia. 2019.

53. Gessese YA, Damessa DL, Amare MM, Bahta YH, Shifera AD, Tasew FS, Gebremedhin EZ: Urinary pathogenic bacterial profile, antibiogram of isolates and associated risk factors among pregnant women in Ambo town, Central Ethiopia: a cross-sectional study. *Antimicrob Resist Infect Control* 2017, 6:132.
54. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ: Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nature reviews microbiology* 2015, 13(5):269-284.
55. Galatti L, Sessa A, Mazzaglia G, Pecchioli S, Rossi A, Cricelli C, Schito GC, Nicoletti G, Caputi AP: Antibiotic prescribing for acute and recurrent cystitis in primary care: a 4 year descriptive study. *Journal of Antimicrobial Chemotherapy* 2006, 57(3):551-556.
56. Ordaz-Lopez V, Manzo-Banales H, Garcia Herrera H, Cerda-Rivera P, Ochoa M, Ramirez-Leyva D: Urinary Tract Infection in Pregnancy: A Study of Pathogen and Bacterial Resistance in Mexico. *J Fam Med* 2016, 3(11):1-4.
57. Srinath M, Shajina M, Fasalu Rahiman O: Etiology and prevalence of urinary tract infections among pregnant women in Kerala. *International Journal of Community Medicine and Public Health* 2018, 5(8):3298.
58. Parida B, Projna Paty B, Padhi A, Padhi S, Narasimham M, Sahu S: Prevalence of Urinary Tract Infection in Pregnant Women in a Tertiary Care Hospital of Odisha. *IOSR Journal of Dental and Medical Sciences* 2018, 17(5):47-50.
59. Habib R, Mehmood MD, Noreen S, Anwar H, Gul M, Ayub N, Raza A: Characterization, prevalance and antimicrobial susceptibility pattern of bacterial uropathogens isolated from pregnant women at Lahore general hospital, Lahore, Pakistan. *International Journal of Clinical Obstetrics and Gynaecology* 2018, 2(6):31-42.
60. Yaseen M, Rashid S, Naqvi S: Urinary tract infection in pregnant females attending antenatal clinics among middle socioeconomic settings. *The Professional Medical Journal* 2020, 27(08):1636-1641.
61. Ghaima KK, Khalaf ZS, Abdulhassan AA, Salman NY: Prevalence and Antibiotic Resistance of Bacteria Isolated from Urinary Tract Infections of Pregnant Women in Baghdad Hospitals. *Biomedical and Pharmacology Journal* 2018, 11(4):1989-1994.
62. U Maduakor MO, B Nnedu, U Okongwu, C Uchenna, I Okonkwo, R Salih. : Etiology, Prevalence And Antimicrobial Susceptibility Profile Of Urinary Tract Infection Among

Pregnant Women In Enugu, South East Nigeria . . *The Internet Journal of Gynecology and Obstetrics* 2020, 24(2).

63. Elnagar WSMaWM: Multidrug Resistant Uropathogens among Egyptian Pregnant Women. *American Journal of Infectious Diseases* 2019, 15:115-122.
64. Tchente Nguefack C, Okalla Ebongue C, Nouwe Chokotheu C, Ebong Ewougo C, Nana Njamen T, Mboudou E: Clinical presentation, risk factors and pathogens involved in bacteriuria of pregnant women attending antenatal clinic of 3 hospitals in a developing country: a cross sectional analytic study. *BMC Pregnancy Childbirth* 2019, 19(1):143.
65. P Bhola NRM, Y Balakrishna, K P Mlisana, K Swe Swe-Han,: Antimicrobial susceptibility patterns of uropathogens isolated from pregnant women in KwaZulu-Natal Province: 2011 - 2016. *SAMJ* 2020, 110(9).
66. Moyo SJ, Aboud S, Kasubi M, Maselle SY: Bacterial isolates and drug susceptibility patterns of urinary tract infection among pregnant women at Muhimbili National Hospital in Tanzania. *Tanzania Journal of Health Research* 2010, 12(4):233-236.
67. Mohamoud H, Tadesse S, Derbie A: Antimicrobial Resistance and Extended Spectrum Beta-Lactamase Profile of Uropathogens Among Pregnant Women Attending Antenatal Care at Edna Adan Hospital, Hargeisa, Somaliland. 2020.
68. Elkashif M: Urinary Tract Infection among Pregnant Women and its Associated Risk Factors: A Cross-Sectional Study. *Biomedical and Pharmacology Journal* 2019, 12:2003-2010.
69. Wabe YA, Reda DY, Abreham ET, Gobene DB, Ali MM: Prevalence of Asymptomatic Bacteriuria, Associated Factors and Antimicrobial Susceptibility Profile of Bacteria Among Pregnant Women Attending Saint Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia. *Therapeutics and clinical risk management* 2020, 16:923-932.
70. Derese B, Kedir H, Teklemariam Z, Weldegebreal F, Balakrishnan S: 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* 2016, 12:251.
71. Simon-Oke IA, Odeyemi O, Afolabi OJ: Incidence of urinary tract infections and antimicrobial susceptibility pattern among pregnant women in Akure, Nigeria. *Scientific African* 2019, 6:e00151.

72. https://en.wikipedia.org/wiki/Debre_Marqos,
73. Magiorakos A-P, Srinivasan A, Carey Rt, Carmeli Y, Falagas Mt, Giske Ct, Harbarth S, Hindler Jt, Kahlmeter G, Olsson-Liljequist B: Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical microbiology and infection* 2012, 18(3):268-281.
74. Cheesbrough M: District laboratory practice in tropical countries, part 2: Cambridge university press; 2005.
75. CLSI: Performance standards for antimicrobial susceptibility testing,. In: *CSLSI supplement M100,*. 30th, edn: Clinical and Laboratory Standards Institute Wayne, PA,; 2020.
76. Vaijanathrao CY, Nalini YL, Reddy CM: Antibiotic sensitivity pattern of uropathogens: a comparative study between symptomatic and asymptomatic bacteriuria in pregnant women. *Int J Curr Microbiol App Sci* 2015, 4(6):689-695.
77. Mokube MN, Atashili J, Halle-Ekane GE, Ikomey GM, Ndumbe PM: Bacteriuria amongst pregnant women in the Buea Health District, Cameroon: Prevalence, predictors, antibiotic susceptibility patterns and diagnosis. *PLoS One* 2013, 8(8):e71086.
78. Yasmin T, Sarwar MY, Sen A: Prevalence of urinary tract infection in pregnant women in Katihar district, Bihar. *prevalence* 2018, 2(98):2.06.
79. Rejali M, Ahmadi SS: Prevalence and risk factors of urinary tract infection among pregnant women in Shahrekord, Iran. *International Journal of Epidemiologic Research* 2019, 6(2):55-59.
80. Assefa A AD, Woldeamanuel Y, et al,: Bacterial profile and drug susceptibility pattern of urinary tract infection in pregnant women at Tikur Anbessa Specialized Hospital Addis Ababa, Ethiopia. *Ethiop Med J* 2008, 46:227–235.
81. MP S: Bacteriological Profile of Urinary Tract Infections in Pregnant Women; I. *Indian J Microbiol Res* 2016, 3:17-21.
82. Onoh RC, Umeora O, Egwuatu V, Ezeonu P, Onoh T: Antibiotic sensitivity pattern of uropathogens from pregnant women with urinary tract infection in Abakaliki, Nigeria. *Infection and drug resistance* 2013, 6:225.

83. Mengistie Z, Woldeamanuel Y, Asrat D, Adera A: Prevalence of bacterial vaginosis among pregnant women attending antenatal care in Tikur Anbessa University Hospital, Addis Ababa, Ethiopia. *BMC research notes* 2014, 7(1):1-5.
84. Taye S, Getachew M, Desalegn Z, Biratu A, Mubashir K: 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* 2018, 11(1):1-7.
85. Haider G, Zehra N, Munir AA, Haider A: Risk factors of urinary tract infection in pregnancy. *JPMA The Journal of the Pakistan Medical Association* 2010, 60(3):213.
86. Masinde A, Gumodoka B, Kilonzo A, Mshana S: Prevalence of urinary tract infection among pregnant women at Bugando Medical Centre, Mwanza, Tanzania. *Tanzania journal of health research* 2009, 11(3).
87. Hamdan HZ, Ziad AHM, Ali SK, Adam I: Epidemiology of urinary tract infections and antibiotics sensitivity among pregnant women at Khartoum North Hospital. *Annals of clinical microbiology and antimicrobials* 2011, 10(1):1-5.
88. Çelen Ş, Oruç AS, Karayalçin R, Saygan S, Ünlü S, Polat B, Danişman N: Asymptomatic bacteriuria and antibacterial susceptibility patterns in an obstetric population. *International Scholarly Research Notices* 2011, 2011.
89. Alemu A, Moges F, Shiferaw Y, Tafess K, Kassu A, Anagaw B, Agegn A: Bacterial profile and drug susceptibility pattern of urinary tract infection in pregnant women at University of Gondar Teaching Hospital, Northwest Ethiopia. *BMC research notes* 2012, 5(1):1-7.
90. Labi A, Yawson A, Ganyaglo G, Newman M: Prevalence and associated risk factors of asymptomatic bacteriuria in ante-natal clients in a large teaching hospital in Ghana. *Ghana medical journal* 2015, 49(3):154-158.
91. Elzayat MA-A, Barnett-Vanes A, Dabour MFE, Cheng F: Prevalence of undiagnosed asymptomatic bacteriuria and associated risk factors during pregnancy: a cross-sectional study at two tertiary centres in Cairo, Egypt. *BMJ open* 2017, 7(3):e013198.
92. Rahimkhani M, Khavari-Daneshvar H, Sharifian R: Asymptomatic bacteriuria and pyuria in pregnancy. *Acta Medica Iranica* 2008:409-412.

93. Onyango HA, Ngugi C, Maina J, Kiiru J: 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* 2018, 8(03):175.
94. Negussie A, Worku G, Beyene E: Bacterial identification and drug susceptibility pattern of urinary tract infection in pregnant Women at Karamara Hospital Jigjiga, Eastern Ethiopia. *African Journal of Bacteriology Research* 2018, 10(2):15-22.
95. Simon-Oke I, Odeyemi O, Afolabi O: Incidence of urinary tract infections and antimicrobial susceptibility pattern among pregnant women in Akure, Nigeria. *Scientific African* 2019, 6:e00151.
96. Tadesse S, Kahsay T, Adhanom G, Kahsu G, Legese H, Derbie A: Prevalence, antimicrobial susceptibility profile and predictors of asymptomatic bacteriuria among pregnant women in Adigrat General Hospital, Northern Ethiopia. *BMC research notes* 2018, 11(1):1-6.

10. ANNEXES

Annex- I English version of the questionnaire

Debre Markos University College of Health Science

Department of Medical Laboratory Sciences

Student Research Proposal Questionnaire Format

S/no		
Part 1: Socio-demographic related questions		
1	Code no	_____
2	MRN	_____
3	Age	_____
4	Residence	A. Rural ☐ B. Urban ☐
5	Marital status	A, married ☐B, single☐C, divorced ☐D, widowed ☐
6	Educational status	A. Illiterate☐ B. Primary school☐ C. secondary school☐ D. Diploma and above☐
7	Occupation	A. Governmental employee ☐ B. Private Employee ☐ C. Student☐ D. Merchant☐ E. Housewife☐ F. daily laborer ☐ G. Commercial sex worker ☐ I. Soldier ☐ J. Farmer ☐
8	Monthly income	
Part 2: Pregnancy related questions		
9	Parity	
10	Gestational age	

Part 3 Disease and other related condition			
11	Do you have symptoms of UTI?	A. Yes ☐	B. No☐
12	Do you have a previous history of UTI infection?	A. Yes ☐	B. No☐
13	Did you have a history of hospitalization?	A. Yes ☐	B. No☐
14	Do you have a history of catheterization?	A. Yes ☐	B. No☐
15	Do you have a history of antibiotic usage?	A. Yes ☐	B. No☐
16	Do you have a history of pulmonary tuberculosis?	A. Yes ☐	B. No☐
17	Do you have anemia?	A. Yes ☐	B. No☐
18	Do you have hypertension?	A. Yes ☐	B. No☐
19	Do you have diabetes mellitus?	A. Yes ☐	B. No☐
20	Do you have a parasitic infection?	A. Yes ☐	B. No☐
21	Do you have kidney disease?	A. Yes ☐	B. No☐
22	Do you have Liver disease?	A. Yes ☐	B. No☐
23	Do you have HIV/AIDS?	A. Yes ☐	B. No☐
24	Do you have sexual activity?	A. Yes ☐	B. No☐
25	Do you clean your genital area?	A. Yes ☐	B. No☐

Thank you very much!!!

Name of the data collector _____Sign_____ Date _____

Annex 2: Amharic version of the questionnaire

በደብረማርቆስ ዩኒቨርሲቲ የጤና እና ህክምና ሳይንስ ኮሌጅ

የሜዲካል ላቦራቶሪ ሳይንስ ትምህርት ክፍል

የተማሪ ጥናት የቃለ-መጠይቅ ፎርም

ይህ የቃለ መጠየቅ ፎርም የተዘጋጀው በደብረ ማረቆስ አጠቃላይ ስፔሻላይዝድ ሆስፒታል ውሰጥ በነፍሰጡር እናቶች ታካሚዎች ላይ ስለ ማህበረ-ኢኮኖሜያዊ፣ የእርግዝና ሁኔታ፣ ህመሞች እና ሌሎች ተያያዥ ጉዳዮች በተመለከተ ያሉበትን ሁኔታ ለመጠየቅ ነው።

ክፍል አንድ: የማህበረ ኢኮኖሚያዊ ጥያቄዎች		
1	ኮድ ቁጥር	_____
2	ካርድ ቁጥር	_____
3	አድራሻ?	ሀ) ገጠር☐ ለ) ከተማ☐
4	ዕድሜ	_____
5	የጋብቻ ሁኔታ?	ሀ) ያገባ☐ ለ) ያላገባ ☐ ሐ) የፈታ(ች)☐ መ) የሞተባት/የሞተችበት☐
6	የትምህርት ደረጃ?	ሀ) ያልተማረ☐ ለ) የመጀመሪያ ደረጃ☐ ሐ) ሁለተኛ ደረጃ☐ መ) ዲፕሎማና በላይ☐
7	የስራ ሁናቴ?	ሀ) የመንግስት ተቀጣሪ☐ ለ) የግል ተቀጣሪ ☐ ሐ) ተማሪ☐ መ) ነጋዴ☐ ሠ) የቤት እመቤት☐ ረ) የቀን ሰራተኛ☐ ሰ) ሴትኛ አዳሪ☐ ሸ) ሾፌር☐ ቀ) ወታደር☐ በ) ገበሬ☐
8	ወርሀዊ የገቢ መጠን	_____
ክፍል ሁለት: ከእርግዝና ጋር የተያያዙ ጉዳዮች		

9	ስንት ልጆችን ወልደዋል?	_____
10	ካረገዙ ስንት ጊዜ ሆነዎት?	_____

ክፍል ሶስት: የማህበረ ኢኮኖሚያዊ ጥያቄዎች		
11	የሽንት ቧንቧ ህመም ምልክት አለብዎት?	ሀ) አዎ☐ ለ) የለም☐
12	የሽንት ቧንቧ ህመም ታመው ያውቃሉ?	ሀ) አዎ☐ ለ) የለም☐
13	ሆሰፒታል ተኝተው ታክመው ያውቃሉ?	ሀ) አዎ☐ ለ) የለም☐
14	በሽንት ቧንቧዎ ውስጥ ቱቦ ገብቶ ያውቃል?	ሀ) አዎ☐ ለ) የለም☐
15	ለጸረ ተህዋስያን የሚሆን መድሃኒት ወስደው ያውቃሉ?	ሀ) አዎ☐ ለ) የለም☐
16	በሳንባ ነቀርሳ ታመው ያውቃሉ?	ሀ) አዎ☐ ለ) የለም☐
17	የደም ማነስ አለብዎት?	ሀ) አዎ☐ ለ) የለም☐
18	የደም ግፍት አለብዎት?	ሀ) አዎ☐ ለ) የለም☐
19	የስኪር ህመም አለብዎት?	ሀ) አዎ☐ ለ) የለም☐
20	የሆድ ትላትል ህመም አለብዎት?	ሀ) አዎ☐ ለ) የለም☐
21	የኩላሊት ህመም አለብዎት?	ሀ) አዎ☐ ለ) የለም☐
22	የጉብት ህመም አለብዎት?	ሀ) አዎ☐ ለ) የለም☐
23	የኤች. አይ.ቪ/ኤድስ ታማሚ ነዎት?	ሀ) አዎ☐ ለ) የለም☐
24	የግብረ ስጋ ግንኙነት ያደርጋሉ?	ሀ) አዎ☐ ለ) የለም☐
25	ብልትዎን እና አካባቢውን ያፀዳሉ?	ሀ) አዎ☐ ለ) የለም☐

Annex 3: English version of the information sheet and Consent form for study participant

1. Information Sheet

Title of the Research: "Prevalence, antimicrobial susceptibility pattern and associated factors of bacterial uropathogens among pregnant women attending ANC at Debre Markos Comprehensive Specialized Hospital, Northwest Ethiopia, Debre Markos, Northwest Ethiopia"**.**

Name of principal investigator: - Abebaw Admasu (BSc)

Name of the organization: - Debre Markos University

1.1. Introduction

You are invited to participate in research conducted by MSc candidates from Debre Markos University, College of Health Sciences, Department of Medical Laboratory Science. Your participation is voluntary. The research team includes one principal investigator and two advisors. Please take time as you need to read or listen to the information sheet.

1.2.Purpose of the research

We are asking you to take part in this study because we are trying to learn more about the isolation of bacteria profile from urine, associated factors, and drug susceptibility pattern of bacterial uropathogens among pregnant women.

1.3. Procedure

If you are voluntary to participate in this study, you need to understand the purpose of the study and give your consent. Necessary information and the required clinical sample will be collected by a group member of this study and respective analysis will be done to have the result.

1.4. Potential risks and discomforts

There are no anticipated risks with your participation and the sample that you will give us to conduct the study.

1.5.Potential benefits

Pregnant mothers who will participate in the study will gain additional laboratory tests including gram staining, culture, and antimicrobial susceptibility test. Similarly, the clinicians will also get valuable results from the tests performed during the research that help them to decide on the mothers for the best treatment.

After the accomplishment of this study valuable information will be generated on prevalence, associated factors, and antimicrobial susceptibility of bacterial uropathogens among pregnant women attending ANC at Debre Markos Comprehensive Specialized Hospital, and recommendations will be given to the concerned bodies.

1.6. Compensation for participation

You will not get any payment for your participation in this study.

1.7. Confidentiality

Any information that you will give and obtained concerning this study will remain confidential. We will use codes rather than names to collect information and report results.

1.8. Participation and withdrawal

You can choose whether to participate in the study or not. You can also withdraw from the study at any time (any stage of the study) after you have started to participate.

1.9. Person to contact

If you have any questions, you can contact any of the following principal investigator and advisors at any time.

Name of the investigator's	Phone number
1. Abebaw Admasu (BSc)	0913214879
2. Alemayehu Reta (Assistant professor)	0942310734
3. Tebelay Dilnessa (MSc, PhD Fellow)	0912198715

2. Consent form

Serial no_____

First, I would like to thank you for spending your time. I would also like to inform you that this study is approved by Debre Markos University College of health and medical science department of medical laboratory science and ethically cleared by the Institutional Review Committee (IRC) of the college of health science, Debre Markos University. The objective of this study is to assess the prevalence, associated factors, and antimicrobial susceptibility of bacterial uropathogens among pregnant women attending ANC at Debre Markos comprehensive specialized hospital, Northwest Ethiopia. The interview will take about 20 minutes. You have the right not to participate or withdraw in the middle of the interview. All the information you will give us will be used for research purposes only and kept confidential.

Do you agree to participate A. Yes ☐ B. No ☐

Thank you!!!

I_____ hereby give my consent for giving of the requested information to the data collector.

Signature: _____

Date_____

Annex 4: Amharic version of information sheet and Consent form for study participant

1. ለጥናቱ ተሳታፊዎች የተሰጠ ማብራሪያ

የጥናቱ ርእስ፡ ነፍሰጡር እናቶች ላይ በሽንት ቧንቧ ህመም ዉስጥ የሚገኙ የባክቴሪያዎችን ስርጭት፣ ለሽንት ቧንቧ ህመም የሚያጋልጡ ምክንያቶችን እዲሁም በምርመራ የተገኙ ባክቴሪያዎች ለታወቁ ጸረ-ተህዋስያን ያለቸውን ተጠቂነት ለማወቅ የሚደረግ ጥናት ነው።

ጥናቱን የሚያካሒደው ስም - አበባው አድማሱ

የመስሪያ ቤቱ ስም ደብረማርቆስ ዩኒቨርሲቲ

1.1. መግቢያ

በመጀመሪያ እርስዎ በደማርቆስ ዩኒቨርሲቲ፤ የጤና ሳይንስ ኮሌጅ፤ የህክምና ላቦረቶሪ ሳይንስ ትምህርት ክፍል ስር በሚደረግ ጥናት ውስጥ ተሳታፊ እንዲሆኑ ሲጠይቁ ተሳታፊ የሚሆኑት ፍቃደኛ ከሆኑ ብቻ ነው። ይህንን ጥናት የሚያካሒዱት ሰዎች አንድ ዋና ተመራማሪ ሁለት የጥናቱ አማካሪዎችን ያካተተ ነው። እባክዎ ስለጥናቱ የሚሰጠውን መረጃ አዳምጦም ሆነ አንብቦ ለመረዳት ያሻዎትን ጊዜ ይውሰዱ።

1.2. የጥናቱ ዓላማ

በዚህ ጥናት እንዲሳተፉ የጠየቅንበት ዋና አላማ ነፍሰጡር እናቶች ላይ በሽንት ቧንቧ ህመም ዉስጥ የሚገኙ የባክቴሪያዎችን ስርጭት፣ ለሽንት ቧንቧ ህመም የሚያጋልጡ ምክንያቶችን እዲሁም በምርመራ የተገኙ ባክቴሪያዎች ለታወቁ ጸረ-ተህዋስያን ያለቸውን ተጠቂነት ለማወቅ ነው።

1.3. የጥናቱ ቅደም ተከተል

ይህን ጥናት ለማካሄድ ፈቃደኛነትዎን ማግኘት አስፈላጊ ነው። የጥናቱን አላማና ጥቅም በሚገባ ተረድተው ለመሳተፍ ፋላጎት ካለዎት በጥናት ቡድኑ ውስጥ ላሉ ሰዎች አስፈላጊ መረጃዎች የሚሰጡ ሲሆን የሽንት ናሙና የሚሰጡ ይሆናል። በመጨረሻም ተገቢው ሂደት ተክትሎ የምርምሩ ውጤት የሚታወቅ ይሆናል።

1.4. በዚህ ጥናት ውስጥ በመሳተፍዎ ሊከሰቱ የሚችሉ ስጋቶችና የምቾት መጓደሎችን

ጥናቱ ውስጥ በመሳተፍዎ እና ለጥናቱ በሚወሰደው ናሙና ምክንያት ሊከሰትብዎ የሚችል የተለየ ምንም አይነት ችግር የለም።

1.5. ጥናቱ የሚያስገኘው ጥቅም

በጥናቱ ውስጥ የሚሳተፉ ነፍሰጡር እናቶች ለሽንት ቧንቧ ህመም የሚያጋልጡ የባክቴሪያ አይነቶችን ለመለየት የሚያስችሉ ተጨማሪ የላቦራቶሪ ምርምራዎችን ማለትም ባክቴርያዎችን ማሳደግ (ካልቸር)፣ ግራም ሰቴኒንግ፣ ባክቴሪያዎች ለታወቁ ጸረ-ተህዋስያን ያላቸውን ተጠቂነት ምርመራ ያገኛሉ። በተጨማሪም ከጥናቱ የሚገኙ የላቦራቶሪ ውጤቶች ሃኪሞች በሽንት ቧንቧ ለሚከሰቱ ህመሞች ትክክለኛ መድሃኒት እንዲዝዙ የሚያግዙ ይሆናል።

በጥናቱ ነፍሰጡር እናቶች ላይ በሽንት ቧንቧ ህመም ዉስጥ የሚገኙ የባክቴሪያዎችን ስርጭት፣ ለሽንት ቧንቧ ህመም የሚያጋልጡ ምክንያቶችን እዲሁም በምርመራ የተገኙ ባክቴሪያዎች ለታወቁ ጸረ-ተህዋስያን ያለቸውን ተጠቂነት የሚያሳይ ውጤት የሚገኝ ሲሆን ይህንን መነሻ በማድረግ በጉዳዩ ላይ ለሚሰሩ ተቋማት ተገቢ የሆኑ የጥናት ውጤት መረጃዎች የሚሰጡ ይሆናል።

1.6. ከጥናቱ ስለሚገኝ ማካካሻ ወይም ጥቅማጥቅም

እርሰዎን በዚህ ጥናት መሳተፈዎ ምንም አይነት ማካካሻም ሆነ ጥቅማጥቅም አያገኙም።

1.7. ምስጢር አጠባበቅ

በዚህ ጥናት በመሳተፍዎ የሰጡንን እና እኛም ያገኘናቸውን ማንኛውም አይነት መረጃዎች በሚስጢር ይያዛሉ። በተጨማሪም መረጃዎችም የሚሰበሰቡት እና ውጤትም የሚታወቀው ከስም ይልቅ በኮድ ይሆናል።

1.8. በጥናቱ የመሳተፍ ወይም ራስን የማግለል መብት

የእርስዎ በዚህ ጥናት የመሳተፍ መብት በፈቃደኝነትዎ ላይ የተመሰረተ ነው።። ባሻዎት ጊዜ በየትኛውም የጥናቱ ሂደት ላይ ከጥናቱ የመውጣትም ሆነ የማቋረጥ መብት አለዎት።

1.9. መረጃ ማግኘትን በተመለከተ

ተጨማሪ መረጃ ከፈለጉ ዋናውን ተመራማሪ ወይም አማካሪዎች ማነጋገር ይችላሉ ።

የተመራማሪዎች ስም	**ስም ስልክ ቁጥር**
1. አበባው አድማሱ	0913214879

2. አለማየሁ ረታ (ረዳት ፕሮፌሰር) 0942310734
3. ተበላይ ድልነሳ (ኤም.ኤስ.ሲ፣ ፒ.ኤች.ዲ ተከታታይ) 0912198715

2. <u>የጥናት ሳታፊ ለመሆን የስምምነት መጠየቂያ ቅፅ በአማርኛ</u>

መለያ ቁጥር _____

በመጀመሪያ ዉድ ጊዜዎትን ሰዉተዉ ለቃለ-መጠየቅ ስለተባበሩኝ አመሰግናለሁ። ይህ ጥናት በደብረ ማርቆስ ዩንቨርስቲ የጤና እና ህክምና ሳይንስ ኮሌጅ በሜዲካል ላቦራቶሪ ሳይንስ የትምህርት ክፍል እንዲሰራ ጥናቶች እንዲሰሩ ከሚፈቅደው ከኮሌጁ ኮሚቴ ሀጋዊ እዉቅና የተሰጠዉ መሆኑን ልገልፅልዎት አፈልጋለሁ። የጥናቱ አላማም በነፍሰጡር እናቶች ላይ ከሽንት ቧንቧ ኢንፌክሽን ጋር በተገናኘ በሽንት ውስጥ የሚገኙ የባክቴሪያዎችን ስርጭት፣ የሽንት ቧንቧ ተጋላጭነትን የሚጨምሩ ምክንያቶችን ለመለየት እና ባክቴርያዎች ለጸረ ተህዋስያን የሚኖራቸውን ምላሽ ለማወቅ ነዉ። ጥናቱ የሚካሄደዉም በደብረ ማርቆስ አጠቃላይ ስፔሻላይዝድ ሆስፒታል የነፍሰጡር እናቶች ክትትል መስጫ ክፍል ሲሆን ቃለ መጠይቁ 20 ደቂቃ ብቻ ይፈጃል። ይሁን እንጅ በጥናቱ ላይ ያለመሳተፍ መብት ሲኖርዎት ካልተመቸዎት ደግሞ በቃለ-መጠይቁ መሃል ላይም ማቋረጥ ይችላሉ። የሚሰጡት ግለሰባዊ መረጃ የሚዉለዉ ለጥናቱ ብቻ ሲሆን ሚስጥራዊነቱም የተጠበቀ ነዉ።

በጥናቱ ለመሳተፍ ተስማምተዋል? ሀ) አዎ☐ ለ) የለም☐

ከልብ አመሰግናለሁ!!!

እኔ _____ በዚህ ሰዓት የተጠየኩትን መረጃ መረጃ ለሚሰበስበው ሰው ለመስጠት የተስማማሁ መሆኑን እገልጻለሁ።

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ቀን _____

Annex 5: Biography of the Principal Investigator

Abebaw Admasu Debebe, Debre Markos University, Department of Medical Laboratory Sciences, Student of MSc in Medical Microbiology, Tel: +251913214879, Debre Markos, Ethiopia.

Annex 6: Laboratory Procedures

Preparation of CLED Agar

1. Suspend 36 grams of the CLED agar media in one liter of distilled water.
2. Mix well and heat with frequent agitation and boil for one minute until complete to dissolution.
3. Autoclave at 121°C for 15 minutes to keep the sterility of the media.
4. Make the media cool to 50°C,
5. Mix well the media and dispense into plates.
6. When the medium is solidified, invert the plates to avoid excess moisture.
7. Store the prepared media at 8-15°C.

Preparation of MacConkey Agar

1. Mix 49.53 grams of the dehydrated medium of MacConkey in 1 liter of distilled water.
2. Dissolve the medium completely with the help of Heat.
3. Autoclave at 121°C for 15 minutes to keep the sterility of the media.
4. Make the media cool to 45-50°C.
5. Mix well the media and pour into sterile plates.
6. Store the plates at 2-8 ºC.

Preparation of Blood Agar

1. Mix 28 g of nutrient agar powder in 1000 ml of distilled water.
2. Heat this mixture and stir dissolve all components completely.
3. Autoclave the mixture for 15 minutes at 121°C.

4. Allow the media to cool but don't allow solidifying.
5. When the agar has cooled to 45-50 ℃, Add 5% (vol/vol) sterile defibrinated blood that has been warmed to room temperature and mix gently but well.
6. Pour into sterile plates.
7. Store the plates at 2-8 ºC.

Preparation of Muller Hinton Agar

1. Prepare all the necessary materials.
2. Suspend 38 gm of Muller Hinton agar media in one liter of distilled water.
3. Heat and boil for one minute with frequent agitation to completely dissolve the medium.
4. Autoclave the suspension at 121℃ for 15 minutes.
5. Allow cooling to room temperature.
6. Using sterile Petri dishes, pour cooled Mueller Hinton Agar on a level, horizontal surface to give uniform depth.
7. Check for the final pH 7.3 ± 0.1 at 25ºC.
8. Store the plates at 2-8 ºC.

LABORATORY PROCEDURE FOR COLLECTION AND CULTURING OF URINE

Purpose: This procedure provides instructions for the collection of urine, inoculating, reading, and interpreting a culture.

Principle: The culture of urine will be performed quantitatively to distinguish bacterial isolates infecting the urinary tract. Bacterial growth $\geq 10^5$ CFU/ml of urine is considered to be indicative of infections.

Culture media: CLED Agar, Blood agar, MacConkey,

Materials: Calibrated 0.001ml inoculating loop, 70% alcohol, 35-37 ^{0}C incubator, and biological safety cabinet.

Sample Type: Midstream urine specimen: - a specimen obtained from the middle part of urine flow.

Mid-stream urine is collected as follow:

1. Sit on the toilet with your legs spread apart. Use two fingers to spread open your labia using your two fingers.
2. Clean the inner folds of the labia and the opening where urine comes out from the inner to the out.
3. Urinate a small amount into the toilet bowl and stop the flow of urine.
4. Collect the next voided ruining using a sterile wide-mouth screw-capped urine container
5. Finish urinating by voiding the last portion of urination into the toilet.

The procedure of urine culture:

1. Bring the culture media to room temperature
2. Gently swirl the container to mix the sample
3. Take a 0.001ml of urine with the sterile loop from the sample until the top of the loop fills the circle.
4. Inoculate the sample into the media. Cross-streak with a series of very close streak lines in zigzag fashion to cover the entire plate surface of the culture media.
5. Incubate plates for 24-48 hours at 37^0C aerobically
6. If there is no growth after 24 hours of incubation the final report will be, no growth of bacteriuria.
7. If there is growth after 24hr of incubation, count the number of colonies and if the growth is $\geq 10^5$ CFU/ml of urine it is significant bacteriuria.
8. Perform definitive biochemical identification and sensitivity test for those plates with growth $\geq 10^5$ CFU/ml of urine.

Gram staining procedure

Purpose: This procedure provides instructions to perform Gram stain.

Reagents: Crystal violet, Lugol‘s iodine, Acetone solution, and Safranin.

Supplies: Disposable sterile plastic loops, Glass microscope slides, and flame or Bunsen burner.

Principle: Gram-positive bacteria have a thick mesh-like cell wall made of Peptidoglycan (50-90% of the cell wall), which stains purple while Gram-negative bacteria have a thinner layer (10% the dry weight of cell wall), which stains pink. Gram-negative bacteria also have an

additional outer membrane that contains lipids and is separated from the cell wall by the periplasmic space.

1. Labeling the slides clearly with the date, patient‘s name, and laboratory number.
2. Take one colony from the culture, dilute with one drop of sterile normal saline on the slide and spread smear over an area of the size of 1cm x2cm and air dry.
3. Fix the smear
    a) Pass air-dried smears through a flame two or three times. Do not overheat.
    b) Allow the slide to cool before staining.
4. Drying the smears and the slide should be left in a safe place to air-dry. Protect the slide from flies and dust.
5. Flood the prepared slide with crystal violet for one minute.
6. Rinse the slide gently with tap water and flood the slide with Gram‘s iodine for one minute.
7. Rinse the slide gently with tap water.
8. Flood the slide with decolorizes for 30 seconds and rinse with tap water.
9. Flood the slide with Safranin for one minute then rinse the slide gently with tap water.
10. Drain the slide in an upright position. Blot the back of the slide and air dry the slide.
11. Observe slide under a microscope.

Result Interpretation

Gram-positive bacteria and yeast will stain blue to purple.

Gram-negative bacteria will stain pink to red.

Antimicrobial susceptibility test

Muller Hinton agar

Principle: A disk of blotting paper is impregnated with a known volume and appropriate concentration of an antimicrobial agent, and this is placed on a plate of sensitivity testing agar uniformly inoculated with the test organism. The antimicrobial diffuses from the disc into the

medium and the growth of the test organism is inhibited at a distance from a disc that is related to the sensitivity of the organism.

Strains sensitive to the antimicrobial agent are inhibited at a distance from the disc, whereas resistant strains have smaller zones of inhibition or grow up to the edge of the disc.

Procedure

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 to sterile physiological saline or nutrient broth.

2. In a good light match the turbidity of the suspension to the turbidity standard (mix the standard before use)

3. Using a sterile swab, inoculate a plate of Muller 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 60o to ensure even distribution.

4. With the Petri dish lid in place, allow 3 – 5 minutes for the surface of the agar to dry.

5. Using sterile forceps or multi-disc dispenser, place the appropriate antimicrobial discs evenly distributed on the inoculated plate. The discs should be about 15mm from the edge of the plate and no closer than 25mm from disc to disc. No more than 6 discs should be applied in a 90mm dish. 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 35oC for 16 – 18 hours.

7. After overnight incubation, examine the control and the test plates. Using a ruler measure the diameter of each zone of inhibition in mm on the underside of the plate. the endpoint of inhibition is where growth starts.

Result interpretation

1. **Sensitive (S);** the susceptible category implies that isolates are inhibited by the usual achievable concentration of antimicrobial agent when the recommended dosage is used for the infection.
2. **Intermediate (I);** a pathogen reported as intermediately sensitive suggests that the infection it has caused is likely to respond to treatment when the drug is used in larger doses than normal or when the drug is concentrated at the site of infection. Consideration should be given to using other drugs that may provide more optimal therapy.
3. **Resistant (R);** A pathogen reported as 'resistant' implies that the infection it has caused will not respond to treatment with the drug to which it is resistant irrespective of dose or site of infection.

Biochemical Test Procedure

Indole Test

Principle: the test organism is cultured in a medium that contains tryptophan. The generation of Indole is through reductive deamination from tryptophan. When indole is combined with Kovac's Reagent (containing HCL and p-dimethyl-amino-benzaldehyde) the color of the solution turns from yellow to pink to red color.

Reagents Used in Indole Test

Indole Spot Reagent:

- p-Dimethylaminocinnamaldehyde (DMACA) 10.0 gm

- Hydrochloric Acid, 37% 100.0 ml
- Deionized Water 900.0 ml

Indole Kovacs Reagent:

- p-Dimethyl-amino-benzaldehyde 50.0 gm
- Hydrochloric Acid, 37% 250.0 ml
- Amyl Alcohol 750.0 ml

Procedure

1. Inoculate the tube tubes containing 4 ml of tryptophan broth aseptically with isolates from 18 to 24 hours culture.
2. Incubate the tube at 37°C for 24 hours.
3. Add 0.5 ml of Kovac's reagent to the broth culture.
4. Examine the color change on the surface of the tube.

Interpretation

Red surface layer……Positive indole test

No red surface layer……Negative indole test

Citrate usage test

Principle

Some bacteria can obtain energy in a manner other than by the fermentation of carbohydrates by using citrate as the source of carbon. The utilization of citrate by a test bacterium is detected in a citrate medium by the production of alkaline by-products. The medium includes sodium citrate as the sole source of carbon and ammonium phosphate as the sole source of nitrogen. Bacteria that can use citrate can also extract nitrogen from the ammonium salt, with the production of ammonia (NH+), leading to alkalinization of the medium. In the presence of the indicator bromothymol blue, the medium will be converted from green (at pH 6.0) to blue (pH above 7.6).

Procedure

1. Inoculate the media with the well-isolated colony.

2. Incubate aerobically at 35 to 37^{o}C.
3. Observe a color change from green to blue along the slant.

Result Interpretation

Positive Reaction: Growth with color change from green to intense blue along the slant. Negative Reaction: No growth and No color change; Slant remains green.

Motility test

Principle

The isolated organisms are inoculated to semisolid media by straight wire through the center of the medium. Non-motile organisms grow only in the line of the inoculum, but motile bacteria 'swarm' and give a diffuse spreading growth that can be easily recognized by the naked eye**.**

Procedure

1. Prepare all the necessary materials
2. Take young colony from 24 hours culture growing on agar medium.
3. Inoculate the isolates through stab to a depth of only 1/3 to ½ inch in the middle of the tube once. Note: keep the needle in the same line to inoculate and remove also using the same line from the medium.
4. Incubate at 35°-37°C
5. Observe for a diffuse zone of growth out from the line of inoculation.

Result interpretation

- **Positive:** Diffuse spreading growth throughout the medium giving it slightly opaque.
- **Negative:** Growth occurred only in the line of the inoculum.

Kligler Iron Agar

Principle

Kligler Iron Agar contains peptone, HM peptone B and yeast extract, lactose, and glucose (dextrose). Phenol red is the pH indicator. Fermentation of dextrose results in the production of acid, which turns the indicator phenol from red to yellow. The detection of hydrogen sulfide production is evidenced by black color. Lactose fermenters produce yellow slants and butts. The

high amount of acids thus produced helps to maintain an acidic pH under aerobic conditions. Tubes showing the original color of the medium indicate the fermentation of neither glucose nor lactose. Gas production is detected by the formation of bubbles and cracks in the butt of the medium.

Procedure

1. Pick the center of well-isolated colonies obtained from solid culture media using an inoculating needle.
2. Stab the center of the medium into the deep of the tube to within 3-5mm from the bottom.
3. Withdraw the inoculating needle and streak the surface of the slant.
4. Loosen closure on the tube before incubating.
5. Incubate aerobically at 35ºC for 18-48 hours.
6. Read tubes for acid production of the slant/butt, gas, and hydrogen sulfide reactions

Result interpretation

- Red slant/ yellow butt – dextrose (+), lactose (-)
- Yellow slant/ yellow butt – dextrose (+), lactose (+)
- Red slant/ red butt – dextrose (-), lactose (-)

- Positive Test Hydrogen Sulfide Production – The black color of medium, a black ring at the juncture of the slant and butt, or a black precipitate in the butt.

- **Gas Production** – Bubbles, cracking, and displacement of the medium or separation of the medium from the side and bottom of the tube.

Catalase test

This test is used to differentiate those bacteria that produce the enzyme catalase such as Staphylococci from non-catalase producing bacteria such as Streptococci.

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.

Reagents and Supplies Used

H_2O_2 reagent (3%),

Glass slide

Swab

Sterile wooden or glass sticks or platinum loops or wires

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, remove several colonies of the test organism and immerse in the hydrogen peroxide solution.
3. Look for immediate bubbling.

Result interpretation

Active bubbling ----- Positive test-

No release of bubbles ----- Negative test

Coagulase test

This test is used to differentiate Staphylococcus aureus which produces the enzyme coagulase, from *S. epidermidis* and *S. saprophyticus* which do not produce coagulase.

Principle

Coagulase causes the plasma to clot by converting fibrinogen to fibrin. It can be detected by the clumping of bacterial cells in the rapid slide test. Before performing a coagulase test, examine a Gram-stained smear to confirm that the organism is a Gram-positive coccus.

Procedure

1. Place a drop of physiological saline on each end of a slide
2. With the loop, straight wire, or wooden stick, emulsify a portion of the isolated colony in each drop to make two thick suspensions.
3. Add a drop of human or rabbit plasma to one of the suspensions and mix gently.
4. Look for clumping of the organisms within 10 seconds.
5. No plasma is added to the second suspension to differentiate any granular appearance of the organism from true coagulase clumping.

Result interpretation

Clumping within …...S. aureus

No clumping …. No bound coagulase