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Bacterial profiles, antimicrobial susceptibility pattern and associated factors of urinary tract infections among pregnant women attending antenatal care at Tikur Anbesa Specialized Hospital and Lideta Dagim Hidasse Health Center, Addis Ababa, Ethiopia

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This is to certify that the thesis prepared by Sophiya Shemsu entitled:

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Abbreviations

AMR	Antimicrobial resistance
ATCC	American Type Culture Collection
CLSI	Clinical and Laboratory Standards Institute's guidelines
CoNS	Coagulase Negative Staphylococcus
DDST	Double-Disk Synergy Method
ESBL	Extended-spectrumbetalactamases
IRB	Institutional Review Board
LDHHC	Lideta Dagim Hidasse Health Center
MAC	McConkey agar
MDR	Multidrug Resistant
MHA	Mueller Hinton agar
MHT	Modified Hodge Test
UPEC	Uropathogenic*E. coli*
UTI	Urinary tract infection
PA	*Pseudomonas aeruginosa*
SB	Significant bacteriuria
SOP	Standard Operating Procedures
SXT	Sulfamethoxazole-Trimethoprim
TASH	TikurAnbesaspecializedhospital

ABSTRACT

BACKGROUND: Urinary tract infection is a common infection affecting any part of the urinary system,caused by the invasion and multiplication of microorganisms.Pregnant women are at high risk of developing UTIs due to physiological and anatomical changes.If left untreated,can lead to complications for both mother and fetus ,making early detection and management essential.

OBJECTIVE: This study was carried out to determine the bacterial profiles, antimicrobial susceptibility pattern and associated factors of urinary tract infection among pregnant women attending antenatal care in Tikur Anbesa Specialized Hospital and Lideta Dagim Hidasse Health Center, Addis Ababa, Ethiopia.

METHODS: A cross sectional study was conducted among 332 pregnant women from April to August 2024 at Tikur Anbesa Specialized Hospital and Lideta Dagim Hidasse Health Center. A clean-catch midstream urine sample was collected, delivered and inoculated on MacConkey agar and blood agar.Biochemical tests, antimicrobial susceptibility testing and ESBL and carbapenemase production tests were done.All demographic, clinical and laboratory data obtained from the study subjects were entered to SPSS version 20.Logistic regression ,descriptive statistics analyses and odds ratio at 95% confidence interval were carried out to conduct the output of the result.

RESULTS: From the total 332 participants in this study the overall prevalence of urinary tract infection among pregnant women was 30.1%.Among the isolated bacteria gram negative bacteria accounted for 58% while gram positive made up 42%.There was no significance association between urinary tract infection and socio demographic factors.In clinical diagnosis urine chemical examinationand microscopy was significantly associated with urinary tract infections Also no ESBL and carbapenem resistance gram negative bacteria were found.

CONCLUSION: From this finding we conclude that the prevalence of UTI is high. Any positive cases must be treated to prevent complication during pregnancy.As the study found a significant association between urine chemical examination and microscopy for diagnosing UTIs, regular screening is important for early detection.

KEYWORDS: Urine tract Infection, Bacterial uropathogens, pregnant women,antimicrobial resistancepattern

1. Introduction

1.1 Background

One of the key driving factors for maternal morbidity and mortality is bacterial infection. UTIs are more common during pregnancy,with endometritis posing a particularly high risk of post-vaginal-delivery infection(1).Urinary tract infections (UTIs)are becoming an increasin gly serious global health concern(2).In third-world countries, UTIs are one of the most sigificat health issues affecting women of reproductive age (3).

Urinary tract infection (UTI) is an infection that can occur in any part of the urinary system, including the kidneys, ureters, bladder, and urethra, caused by the invasion of microorganisms that then multiply and cause symptoms(4). Pathogenic bacteria are the primary cause of urinary tract infections; however, viruses and fungi can also cause infections on rare occasions. Bacteria can ascend the urethra,adhere,grow, and remain within the urinary system, resulting in a UTI(5).The presence of infectious pathogens in the genitourinary tract may potentially infiltrate the urinary system and surrounding tissues (6).

There are three subcategories of urinary tract infections first asymptomatic bacteriuria (ASB); second lower UTI (acute cystitis), which is defined by vaginal mucosa inflammation and irritative symptoms; and third acute pyelonephritis, also known as upper UTI which is a systemic illness also called symptomatic bacteriuria(7). In terms of clinical classification, UTIs are separated into two groups: simple and complex. A simple UTI, which includes cystitis and pyelonephritis(8). Urinary tract infections can cause the different symptoms like back pain, lower abdominal pain, fever, vomiting, nausea, chills, foul-smelling and cloudy urine, loin pain, dysuria, urine urgency and polyuria (9).

A pregnant woman is at an increased risk for urinary tract infection (UTI), which usually starts around the sixth week of pregnancy and peaks between the twenty-second and twenty-fourth weeks(1). The chances increase during pregnancy due to physiological changes,such as an infection-friendly environment of urine stasis, low peristalsis in ureter and urine retention induced by bacteria growth. As such, expectant mothers become more susceptible to UTIs(10). In addition, changes in the immune system and urinary tract during pregnancy increase the probability of asymptomatic bacteriuria or symptomatic infections, posing sigifica

nt threats to both mother and fetus(11).These infections are also facilitated by various physiological and hormonal changes occurring within the body during this time(12).

In addition, pregnancy-associated immunological changes facilitate this high susceptibility. Early labor pain associated with a urinary tract infection leads to maternal complications that including premature contractions (before 37 weeks' gestation), amnionitis,preeclampsia and pregnancy induced hypertension, and some degree of anemia (hematocrit level < 30 percent). The neonatal outcomes connected to UTIs include pneumonia and sepsis. A particularly high risk of post-vaginal-delivery infection is endometritis(13).

The rates of morbidity and mortality during pregnancy are greatly impacted by UTIs, which are most common in pregnant women(12). A number of uropathogens, including *Escherichia coli*, *Klebsiella pneumoniae, Enterococcus faecalis, Staphylococcus saprophyticus, Proteus minabilis,* and *Pseudomonas aeruginosa*, are responsible for UTIs. Both Gram-positive and Gram-negative bacteria can cause urinary tract infections; however, the majority of UTIs are caused by Gram-negative bacteria.*E. Coli* is the main cause of almost all UTIs (80%) (UPEC) (14).

Antimicrobial resistance occurs when microorganisms, including bacteria, viruses, fungi, and parasites, adapt to and flourish in the presence of medications, eluding the ways in which those medications work to eradicate them.Bacteria can become resistant to antimicrobial through mechanisms like gene transfer or mutation of antibiotic targets, or they can develop resistance intrinsically, due to bacterial characteristics(15).

Bacteria that cause UTIs produce extended-spectrum beta-lactamases which frequently develop resistance to third-generation cephalosporin's. The most recent generation of monobactams and cephalosporin's can be hydrolyzed, causing ESBL to develop resistance. In addition, resistance to carbapenems, a last-resort antimicroial is increasing due to the production of the enzyme by certain pathogens. Several previous studies in various contexts, particularly from sub-Saharan African countries, have also documented a significant incidence of carbapenemase-resistant, MDR, and ESBL-producing bacteria from patients with UTIs (16, 17).

1.2 Statement of the Problem

Urinary tract infections is one of the most prevalent bacterial infections in the world and becoming more and more common (18). Almost 150 million deaths worldwide are attributed to urinary tract infections and related conditions each year(19). Urinary tract infection impacting individuals of all age groups and resulting in treatment costs exceeding $6 billion for the global economy(20).

UTIs are currently the second most common infection in the body and affect people of all ages(21). Women experience UTIs at a rate of 8:1, which is significantly higher than that of men due to anatomy and physiology. In her lifetime, one in five adult women will get a UTI(22). In clinical settings, female urinary tract infections are commonly caused by bacteria and occur at a frequency of roughly 50–60%(23). Most affected are women between the ages of 16 and 35.10% of women receive a diagnosis of UTIs each year, and 60% will get an infection at some point in their lives(24).

Maternal urinary tract infections are the most prevalent non-intestinal infection in expectant mothers worldwide, impacting as many as 20% of these women(25).Most of the women never seek treatment for it because it is asymptomatic, but a small percentage go on to experience open symptoms and signs of urinary tract infection(26). Pregnancy-related UTIs are among the most extensively researched health issues; their prevalence ranges from 3% to 35% globally, with developing nations, particularly those in Sub-Saharan Africa, the Middle East, and Asia, showing the highest rates of infection(27). Asymptomatic bacteriuria affects 2–15% of expectant mothers and is a common risk factor for pyelonephritis if left untreated and linked to morbidity in both the mother and the fetus(28).

Pregnant women's incidence of UTIs varies greatly in Ethiopia. It falls between 9.8% and 26.6%. Ethiopia conducted a number of disjointed individual studies to gauge the prevalence of UTI and related factors among pregnant mothers(29). Increased age, the number of pregnancies, the frequency of weekly sex, diabetes, anemia, prior history of UTI, weakened immunity, literacy, residence, multigravida (women who have been pregnant more than once), multipara (viable pregnancies that last beyond 20 weeks), infection history, trimester status, inadequate individual sanitation, and urinary tract abnormalities are some of the factors that increase the risk of UTI during pregnancy(13, 30).

Antimicrobial resistance (AMR) is largely caused by the abuse of antibiotics in the treatment of urinary tract infections. AMR was predicted to be responsible for 700,000 deaths annually worldwide; if current trends continue, this number might rise to approximately 10 million fatalities annually by the year 2050. If something is not done, it is predicted that 4.1 million Africans will lose their lives to treatment failure by 2050(31). Western sub-Saharan Africa bears the greatest burden, with 27.3 deaths per 100,000 people attributable to antimicrobial resistance and 114.8 fatalities per 100,000 people linked with AMR in 2019(32).

Pregnant women and newborns are at risk for low birth weight, intrauterine growth restriction, fetal death, premature rupture of the membranes, and neonatal infections due to increased extended spectrum beta lactamases–Enterobacteriaceae associated UTI. A lot of the isolates that produce these enzymes are also resistant to aminoglycosides, trimethoprim, and quinolones. Other resistance mechanisms are frequently co-expressed on plasmids. Treatment options for bacterial infections that produce ESBL are incredibly rare(33). In hospital-based studies conducted in sub-Saharan African nations, the percentage of bacterial isolates that produced carbapenemase varied from 9% to 60%(34).

Therefore, many studies have been conducted in different parts of Ethiopia, both previously and recently; however, studies focusing on pregnancy-related UTIs and AMR, particularly concerning ESBL and carbapenem-resistant Gram-negative bacteria in health centers and Tikur Anbesa Specialized Hospital, do not fully represent the extent of this issue. Pregnant women should ideally receive their primary care from health centers, but these facilities are finding it challenging to effectively manage and treat UTIs because there aren't enough appropriate antimicrobial options available. Identifying the risk factors can help healthcare providers implement comprehensive antenatal care approaches that address UTIs, which can have negative effects on the health of both the mother and the fetus. This may lessen the frequency and consequences of UTIs in pregnant mothers. Also this study can fill in that knowledge void and add to the body of current medical literature.so this study aims in determination of common bacterial uropathogen, AMR and associated risk factors in hospital and health center.

1.3 Significance of the study

This study aimed to investigate the bacterial profiles, antimicrobial susceptibility pattern and associated factors of urinary tract infection among pregnant women attending antenatal care in Tikur Anbesa Specialized Hospital and Lideta Dagim Hidasse Health Center, Addis Ababa, Ethiopia. The antimicrobial resistance patterns, including multidrug resistance, such as the production of ESBL and carbapenemase, among pregnant women are increasing and becoming an emerging public health problem. Therefore, this study investigates the antimicrobial resistance of the identified pathogens

From the findings, policymakers and healthcare professionals can benefit by using the information to prevent the abuse and overuse of antibiotics, which in turn helps stop the spread of resistance. Additionally, it provides guidance for the creation of national treatment guidelines and the implementation of antimicrobial stewardship initiatives. Overall, this study is significant in highlighting the gap between health centers and hospitals and focusing on the necessary solutions to address these disparities.

2. Literature Review

From January 2020 to December 2022, Salmanov AG(33) conducted a cohort study in Ukraine on the prevalence rate of UTIs in pregnant women and the antimicrobial resistance of the pathogens causing them. The results showed that 29.5% of pregnant women had UTIs. The most frequently found pathogens were *P. mirabilis* (7%), *K. pneumoniae* (14.2%), *E. coli* (54.2%), and *P.aeruginosa* (5.5%). *S. aureus* and *CoNS* which are Gram-positive bacteria, exhibited high resistance to penicillin (73.5%) and erythromycin (65.7%). However, they demonstrated susceptibility to tetracycline, linezolid, vancomycin, and fusidic acid. The Enterobacteriaceae family accounted for 25.7% of the total production of extended-spectrum beta-lactamases (ESBL) (33).

A cross-sectional study conducted from March 2017 to May 2018 in Southwest Iran by Omidifar N. et al.(35) on antimicrobial susceptibility patterns in pregnant women with UTIs found *E. coli* (78.3%) as the most prevalent Gram-negative pathogen, followed by *Enterobacter spp.* (5.4%) and *Klebsiella spp.* (3.2%). Among Gram-positive bacteria, *S. aureus* (6.3%) and *CoNS* (4.07%) were most common. Age and gravidity were identified as risk factors. The highest susceptibility rates for Gram-negative bacteria were imipenem (96%) and amikacin (91.5%), while resistance was highest for amoxicillin (100%) and co-trimoxazole (71.2%). *S. aureus* and *CoNS* showed high resistance to ampicillin (75% and 66.6%, respectively), and oxacillin resistance was 21.4% for *S. aureus* and 55.5% for *CoNS*. The study concluded that bacteriuria screening and appropriate antibiotic use should be part of antenatal care guidelines(35).

A cross-sectional study conducted from March to May 2018 in Sana'a,Yemen, by Edrees WH et al.(36) on the prevalence and antibacterial susceptibility of uropathogens in pregnant women found a high prevalence of bacteriuria, with rates of 50% and 44.4%. The most commonly isolated bacteria were *E. coli* (50%), followed by *S. aureus* (27.7%), *Klebsiella spp.* (11.1%), *CoNS*, and *Pseudomonas aeruginosa* (5.6% each). Risk factors included education, gravidity, and age, although gravidity did not correlate with bacterial UTIs. *E. coli* showed susceptibility to nitrofurantoin (62.5%), vancomycin (75%), ampicillin, and cefepime (87.5%). *S. aureus* was sensitive to ampicillin, nitrofurantoin, and cefepime (80%, 60%, and 80%, respectively). *Klebsiella spp.* was 100% sensitive to cefepime and nitrofurantoin but resistant to eryth-

romycin and vancomycin. *P. aeruginosa* was resistant to erythromycin but 100% sensitive to cefepime, ampicillin, vancomycin, and nitrofurantoin (36).

A cross-sectional study conducted in West Africa, Nigeria, from July to October 2021 by Baba et al.(37) on antimicrobial susceptibility of urinary bacterial isolates in pregnant women attending antenatal clinics showed a 14.5% prevalence of UTIs. *E. coli* accounted for 5.7% of isolates, followed by *CoNS* (3.0%), *K. pneumoniae* (2.7%), *S. aureus* (2.3%), Enterococcus species (0.7%), and *Pseudomonas* species (0.3%). Sociodemographic factors like age, occupation, and education were associated with risk. Resistance was highest to ampicillin (73.1%), amoxicillin-clavulanic acid (65.4%), and cephalosporins (30.8%-46.2%). All Gram-negative isolates showed resistance to ceftazidime (26.7%) and nitrofurantoin (15.4%). Gram-positive isolates had 94.1% penicillin resistance, and resistance to ceftriaxone and amoxicillin-clavulanic acid ranged from 29.4% to 47.1%. The study emphasized the importance of early UTI detection during antenatal care(37).

A cross-sectional study in Kenya on urinary tract infections among pregnant women by Onyango HA et al.(38) from Jaunary to April 2018 found an overall UTI prevalence of 15.7%. The most common pathogens were *E. coli* (44.5%), *K. pneumoniae* (21.2%), *S. aureus* (15.1%), and *P. aeruginosa*, Acinetobacter species, and Enterococcus species (6.1%). Risk factors such as age, maternal age, parity, marital status, occupation, gestation, and education were identified, though no significant correlation with UTI was found. *S. aureus* showed resistance to ceftazidime, cefoxitin, imipenem, and ciprofloxacin but was sensitive to amoxicillin-clavulanic acid, nitrofurantoin, chloramphenicol, sulfamethoxazole/trimethoprim, and linezolid. *E. coli* isolates showed high resistance to ampicillin (88.9%), nalidixic acid (86.7%), and cephalosporins, but were susceptible to amoxicillin-clavulanic acid (86.6%), imipenem (100%), and nitrofurantoin (98.8%). 25.8% of Gram-positive strains and 70.2% of Gram-negative isolates exhibited multidrug resistance. ESBL production was found in 16% of *K. pneumoniae* and 22% of *E. coli* isolates (38).

A study conducted in South Africa from January 2015 to December 2019 by Zwane T.et al. (39) on the etiology and antimicrobial susceptibility of pathogens associated with urinary tract infections in women attending antenatal care found *E. coli* (56%) and *E. faecalis* (17%) as the most common Gram-negative pathogens, followed by *Klebsiella spp.* (3%), *Enterobacter spp.* (2%), and *Acinetobacter spp.* (0.4%). Gram-positive pathogens included *S. agalactiae* (5%), *Staphylococcus spp.* (2%), and *Enterococcus spp.* (0.3%). *E. coli* isolates were

susceptible to amoxicillin/clavulanic acid (82%), ciprofloxacin (88%), nitrofurantoin (95%), and gentamicin (93%). *K. pneumoniae* isolates were susceptible to ciprofloxacin (96%), gentamicin (95%), and trimethoprim/sulfamethoxazole (75%). The prevalence of ESBL-producing *E. coli* was 6–7%, and 8–9% in *K. pneumoniae*. The prevalence of multidrug-resistant uropathogens was low.**(39)**

A study by Derese et al.(40) conducted in Dire Dawa, Ethiopia, from February to March 2015 on the bacterial profile and antimicrobial susceptibility of urinary tract infections in pregnant women attending antenatal clinics found *E. coli* (34.6%) as the most common pathogen, followed by *CoNS* (19.2%), *P. aeruginosa* (15.4%), and *Klebsiella spp.* (11.5%). Sociodemographic factors such as age, education, family income, and marital status, as well as gestational age, were identified as risk factors, with gestational age significantly linked to UTI prevalence. Gram-negative isolates showed high resistance to ampicillin (89.5%), amoxicillin (73.7%), tetracycline (73.7%), nalidixic acid (52.6%), and nitrofurantoin (57.9%), but were sensitive to ceftriaxone, gentamicin, and ciprofloxacin. Gram-positive isolates were highly sensitive to gentamicin, erythromycin, ceftriaxone, and ciprofloxacin, but resistant to ampicillin, tetracycline, chloramphenicol, and trimethoprim-sulfamethoxazole. Multidrug resistance (MDR) was observed in 100% of the isolates.**(40).**

A study conducted by Belete MA (41) in North Eastern Ethiopia from February to May 2013 on the bacterial profile and ESBL screening of urinary tract infections among pregnant women found an overall UTI prevalence of 15.5%. The most common pathogens were *E. coli* (33.3%), *CoNS* (30%), *S. aureus* (27.5%), *K. pneumoniae* and *S. agalactiae* (3.9% each), and *Enterobacter* species (2%). Gram-positive bacteria showed high resistance to penicillin (93.5%) and trimethoprim-sulfamethoxazole (79.3%), but were fully sensitive to nitrofurantoin (100%), clindamycin (74.2%), ciprofloxacin (70%), and norfloxacin (75.9%). Gram-negative bacteria had a 65% resistance rate to ampicillin, with resistance to ciprofloxacin, tetracycline, trimethoprim-sulfamethoxazole, and ceftriaxone ranging from 30% to 50%. Both Gram-negative (80%) and Gram-positive bacteria (80.6%) exhibited multidrug resistance. Positive ESBL isolates were *E. coli* (66.7%) and *K. pneumoniae* (33.3%). (41)

A cross-sectional study conducted by Wabe YA et al. in Addis Ababa from July to September 2019 (42)on asymptomatic bacteriuria in pregnant women found a prevalence of 14.9%. *E. coli* was the most prevalent pathogen (43%), followed by *S. aureus* (20.4%) and *S. saprophyticus* (14.3%). Risk factors included being aged 26–34 years, being a housewife, living in

urban areas, having no formal education, and having anemia.Most Gram-negative bacteria were susceptible to nitrofurantoin (93.1%), gentamicin (86.2%), ceftriaxone (82.8%), and meropenem (75.9%), but resistant to amoxicillin (79.3%), cotrimoxazole (65.5%), and amoxicillin-clavulanic acid (37.9%). All Gram-positive bacteria were susceptible to nitrofurantoin (100%) and most were susceptible to azithromycin (90%), gentamicin, ciprofloxacin (85%), and clindamycin (80%). A significant proportion (57.1%) of the bacteria were MDR **(42).**

3. Objectives:

3.1. General objective

- To determine the bacterial profile, antimicrobial resistance pattern, and associated factors of urinary tract infections among pregnant women attending antenatal care at Tikur Anbesa Specialized Hospital and Lideta Dagim Hidasse Health Center Addis Ababa, Ethiopia.

3.2. Specific objectives:

I. To determine the prevalence of bacteriuria among pregnant women.
II. To determine the antimicrobial resistance pattern of isolated bacteria among pregnant women.
III. To determine extended-spectrum beta-lactamases and carbapenemase-producing Gram-negative bacteria among pregnant women.
IV. To assess the possible associated factors of urinary tract infection among pregnant women.

4. Hypothesis

Pregnant women attending antenatal care at Tikur Anbesa Specialized Hospital and Lideta Dagim Hidasse Health Center in Addis Ababa, Ethiopia,will have a significant prevalence of bacteriuria, with notable antimicrobial resistance patterns, including the presence of extended-spectrum beta-lactamases (ESBL) and carbapenemase-producing Gram-negative bacteria. Various sociodemographic and clinical factors are significantly associated with the occurrence of urinary tract infections among these women.

5. Materials and methods

5.1. Study area

The research was conducted at Tikur Anbessa Specialized Hospital and Lideta Dagim Hidasse Health Center, Addis Ababa, Ethiopia.

Tikur Anbessa Specialized Hospital, located in Lideta sub-city, was established in 1972 GC, and in 1998 was transferred to the school to become a university hospital. The hospital has 1000 beds, 450 physicians, 500 nurses, and 125 other medical professionals, with 200 specialists and 50 non-teaching doctors. ANC, a branch of the department of gynecology and obstetrics, provides care for over 1600 pregnant women per month and about 19,200 yearly(43).

Lideta Dagim Hidasse Health Center is one of the health centers from 8 health centers that are found in Lideta subcity, which is located around Torhailoch. Also, it is called Woreda 2. It was established in 2005, and it has 160 medical and other professionals. It provides services for over 600 patients monthly. ANC is one of the departments found under MCH that provides service for over 600 pregnant mothers yearly. The two sites were chosen for convenience purposes(44).

5.2. Study design and period

An institution-based cross-sectional study was conducted from April to August 2024. Data and clinical samples pertinent to the investigation were gathered from the study populations.

5.3. Population

5.3.1. Source population

All pregnant women who visited the antenatal care at Tikur Anbessa Specialized Hospital and Lideta Dagim Hidasse Health Center during the study period.

5.3.2. Study Population

All pregnant women who have visited the antenatal care and from whom urine is collected during the study period.

5.4. Inclusion and exclusion criteria

5.4.1. Inclusion criteria:

- Pregnant women who had follow-up in the antenatal care
- Participants who were willing to participate and gave informed consent

5.4.2. Exclusion criteria

- Pregnant women who took antibiotic treatment in the preceding 15 days.
- Pregnant women who were unable to provide urine at the time of collection.

5.5 Study variable

5.5.1. Dependent variables

- ✓ Prevalence of bacterial uropathogens in UTI
- ✓ Antimicrobial resistance pattern of isolated bacterial species
- ✓ Magnitude of extended-spectrum beta-lactamases and carbapenemase-producing gram-negative bacteria

5.5.2. Independent variables

- Socio-demographic characteristic
- Educational status
- Gestational age and parity

- Hygiene and sanitation of participant
- Underline chronic disease
- Prior history of UTI
- Visual and chemical, urine examination
- Microscopic urine examination

5.6. Sample size calculation and Sampling method

5.6.1. Sample size calculation

The sample size was calculated based on single sample size estimation. The value of p is taken as 14.7 %(0.147) from the study conducted by Getie M, et al (45).

Where n = sample size, Z = Z statistic for a level of confidence, P = expected prevalence or proportion (P = 0.5), and d = precision (d = 0.05), Z = Z statistic: For the level of confidence of 95%, which is conventional, Z value is 1.96.

$$\frac{(1.96)^2 \text{ X } 0.147\ (1\text{-}0.147)}{(0.05)^2} = 192.68$$

The 10% nonresponse rate added to the sample size was 211.

The 10% nonresponse rate added to the sample size was 211.So the sample size was calculated by referring to the yearly ANC flow in both TASH and LDHHC to make it proportional and calculating the ratio.so the total flow of LDHHC was 1000 and TASH was 18,500 so calculate the total and subdivided for each facilities.

Total=TASH+LDHHC=18,500+1000=19,500

TASH=18,500/19500*211=200 LDHHC=1000/19,500*211=11

The minimum sample sizes for Lideta Dagim Hidasse Health Center and Tikur Anbessa Hospital were 11 and 200 respectively. Nevertheless we collected 132 samples from Lideta Dagim Hidasse Health center. Therefore, the total number of samples collected was 332.

5.6.2. Sampling Method

A total of 332 study participants were selected using the convenient sampling method when they appeared for follow-up in antenatal care. A total of 132 pregnant women were included from Lideta Dagim Hidasse Health Center and 200 from Tikur Anbessa Specialized Hospital. Based on the inclusion criteria which are pregnant women who had follow-up in the antenatal care and who were willing to participate and gave informed consent were chosen until the target sample size was reached during the study period.

5.7 Measurement and Data collection

5.7.1. Data collection procedure

First, the patient follow-up process was done, including vital signs and other investigations by the nurses during the routine ANC follow-up. Then background information and the aim of the study were told to the study participant. Then an informed consent form was given to the study participants. For those who were willing to participate in the study and other investigations requested by the principal investigator,

Data on sociodemographic characteristics, possible associated risk factors, and all other required information was collected using structured, pretested questionnaires. The questioner was translated from English to Amharic. Pregnant women were asked to provide written, informed consent before sociodemographic, clinical, pregnancy-related, and health facility-related data were gathered.

5.7.2.1 Sample collection and Sample transportation

From each participant, 10 ml of clean catch mid-stream urine was collected using sterile leak-proof, wide-mouth, and sterile plastic universal containers after giving proper instructions to the women on how to collect midstream urine. A urine dipstick was used to examine approximately 5 ml of urine for leukocytes and nitrate using urinalysis (visual, chemical, and microscopic examination). The remaining 5 ml of urine was transported via an ice box from the LDHHC and TASH antenatal care laboratories to the TASH microbiology laboratory, where it was processed in less than two hours and transported to EPHI for antimicroial tests.

5.7.3. Bacteriological identification

*5.7.3.*1 Urine culture and identification

Urine samples were inoculated on MacConkey agar and blood agar using a sterile calibrated wire inoculating loop (0.001 ml) to determine colony-forming units. The plates were incubated at 37 ^{oC} aerobically for 24 hours. The presence of bacteria was then evaluated and to determine whether there was any significant bacteriuria, colonies were counted. The colony characteristics, biochemical tests, and gram stain were utilized to analyze all positive cultures obtained from urine samples. Both the total number of colonies and their different morphologies were tallied. [Bacteriuria with a count greater than 10^5 CFU/mL were considered significant(46).

All positive cultures of significant bacteriuria were subjected to Gram staining in order to classify the bacteria as either Gram-positive or Gram-negative based on their responses, cell structure, and arrangement. For gram-positive bacteria, catalase ,coagulase ible esculin biochemical tests were performed. For gram-negative bacteria, triple sugar iron, indole, citrate, urea, motility,oxidase tests were performed(47).

5.7.3.2 Antimicrobial resistance Patterns

Disk diffusion was used in the testing of antimicrobial resistance patterns. To test the antibiotic susceptibility of all the isolated Gram-negative bacteria, no>5 disks on 100-mm Muller-Hinton agar plates with a pH of 7.2-4 were inoculated using the Kirby-Bauer disc diffusion technique. Disc diffusion tests were conducted and interpreted in accordance with the Clinical and Laboratory Standards Institute's (CLSI) guidelines(48).

For antimicrobial resistance testing in this study, the following antibiotics were used: Ampicillin (10 µg, BD), Amikacin (30 µg, BD), Gentamicin (10 µg, BD), Nitrofurantoin (300 µg, BD), Trimethoprim-sulfamethoxazole (1.25/23.75 µg, BD), Tetracycline (30 µg, BD), Ceftriaxone (30 µg, BD), Cefotaxime (30 µg, BD), Ceftazidime (30 µg, BD), Amoxicillin-clavulanic acid (20/10 µg, BD), Meropenem (30 µg, BD), Cefazolin (30 µg, BD), Ciprofloxacin (5 µg, BD), Cefoxitin (30 µg, BD), Nalidixic acid (30 µg, BD), Norfloxacin (10 µg, BD), and Ertapenem (10 µg, BD) for Gram-negative bacteria. For Gram-positive bacteria, Penicillin (10 µg, BD), Cefoxitin (30 µg, BD), Trimethoprim-sulfamethoxazole (1.25/23.75 µg, BD), Tetracycline (30 µg, BD), Gentamicin (10 µg, BD), Nitrofurantoin (300 µg, BD), Ciprofloxacin (5 µg, BD), Norfloxacin (10 µg, BD), and Novobiocin (5 µg, BD). The results were categorized as sensitive (S), intermediate (I), or resistant (R) according to the interpretive standards set by the Clinical and Laboratory Standards Institute. (48).

5.7.3.3ESBL Detection

Using a disk diffusion method, we looked for resistance to any of the three third-generation cephalosporin antibiotics: Cefotaxime, ceftazidime, and ceftriaxone. The organisms were screened for ESBL using the CLSI recommendation. It was suggested that ESBL production might be taking place based on these CLSI (M100, 2021) recommended breakpoints for ceftriaxone (⩽ 25 mm), Cefotaxime (⩽ 27 mm), and ceftazidime (30 µg)(49).

The ESBL disk screening method underwent an ESBL disk confirmation test by using the double-disk synergy method (DDST). Third-generation test disks containing amoxicillin/clavulanate (Augmentin) and cephalosporin (Cefotaxime, Ceftazidime, and Ceftriaxone) were spaced 20 mm apart, center to center, on inoculated Mueller-Hinton agar. A clear extension of the edge of the inhibition zone of cephalosporin toward the Augmentin disc was interpreted as positive for ESBL(49).

All Gram negative bacteria's like *E.coli, P.vulgaris, P.stuarti, Citrobacte*r, *K.ozeae, K.oxytoca, and Enterobacter* were screened for ESBL production.

5.7.3.4 Carbapenemase Detection

The bacteria were grown on Muller-Hinton agar using a disk containing 10 μg of imipenem, Ertapenem, or meropenem. Diffusion testing on Muller-Hinton agar-grown bacteria revealed resistance to carbapenemase discs such as imipenem, meropenem, or Ertapenem of 10 μg, in accordance with CLSI (M100, 2021) recommended breakpoints. Then For 16–18 hours, incubate the plate at 35–37 °C. Measured the diameters of the zones of inhibition surrounding each carbapenem disc after it was incubated. The zones surrounding the carbapenem disks were measured after incubation. Depending on the kind of carbapenem disk that was utilized, the CLSI established distinct interpretation guidelines for the zone sizes. As per CLSI 2021 guidelines, the production of an inhibition zone size of ≤19 mm suggested resistance to carbapenemase when a meropenem disk was used(50).

Gram negative bacteria's were like *E.coli, P.vulgaris, P.stuarti, Citrobacter, K.ozeae, K.oxytoca, Enteroboacter* were screened for Carbapenemase production.

5.7.3.5Quality control

Strictly adhering to the Standard Operating Procedures (SOP). Media, reagents, and antibiotic disks used were checked to be in date before use. After the sterility check, visual inspection of media was made for cracking and/or thickness/cell debris/contamination, freeze-dried medium/agglomerates/bubble(s). New lots were tested for quality control before being used by the *E. coli* ATCC 25922 and/or Staphylococcus aureus ATCC 25923 standard strains. In addition, ESBL-positive *K. pneumoniae* ATCC 700603 and ESBL-negative *E. coli* Enterobacteriaceae ATCC 25922 control strains were tested for confirmation of the presence or absence of an ESBL mechanism by disk diffusion test(48).

A phenotypic assay to detect the existence of carbapenemase enzymes in *E. coli* ATCC 25922 reference strain is modified Hodge test. At 10 μg, carbapenem disks were positioned as interpreted in the CLSI guidelines. The heat map analysis was overlaid on an image of a corresponding strain with or without the introduced plasmid to show that *E. coli* ATCC

25922 had a feature, such as enhanced growth surrounding its streak arcs. This implied that this organism is carrying carbapenemase.

The *E. coli* ATCC 25922 streaks appeared normal and did not exhibit increased growth or impressions, the results were interpreted as clearly negative / no MHT was applied for this strain in our laboratory. It shall be considered as the test organism is non-carbapenemase producer(51).

Completeness and representativeness of the data were verified during input. Pre-tested questioners: The uncertainty on data collection that was not clear in the pre-test and mother tongue explanation is reasonably presented.

5.8 Data analysis and interpretation

Analysis was performed using the SPSS software package, version 20. Frequencies, means, and standard deviations were used as descriptive statistics. Both bivariate and multivariate logistic regression analyses were applied to examine the relationship between the dependent and independent variables. The results of the study were finally interpreted using words and tables. Additionally, data cleaning and double data entry were performed to ensure the integrity of the data.P value <0.05 considered as stastically significant.

5.9 Data Quality Assurance

Before the data input was carried out, the correctness and range of information extracted from my site in a regular manner were checked. These were added and analyzed using Statistical Packages for the Social Sciences (SPSS) after results on culture and antibiotic susceptibility tests were recorded. To improve data quality, further steps such as controlling or double entry of some variables were performed.

5.10. Operational definitions:

I. Significant Bacteriuria-the presence of 100,000 CFU of bacteria per mL of urine.
II. MDR(multi drug resistance)-bacteria resistance to at least three different classes of antibiotic(betalactamase,cephalosporin,aminoglycosideand fluoroquioloes)(52).

5.11. Ethical considerations

Ethical approval was obtained from the "Department of Research and Ethical Review Committee" (DRERC), Department of Medical Laboratory Science, College of Health Sciences, Addis Ababa, Ethiopia with reference number MLS/164/24.

The research sites were contacted for approval, and informed consent was obtained from all participants during the study period. All data collected were kept confidential to ensure privacy. Patients with positive bacteriuria results were informed by the attending physician and provided with further clinical management based on laboratory findings at subsequent visits during the study period.

5.12. Dissemination of the result

The result of the study submitted to the Department of Medical Laboratory Sciences, School of Allied Health Sciences, College of Health Sciences,Addis Ababa University, TikurAnbesa Specialized Hospital and Lideta Dagim Hidasse Health Center. The result of the thesis was submitted to the international national peer reviewed journal for publication.

6. Result

6.1 Socio-demographic characteristics

A total of 332 pregnant women were included in the study, with ages ranging from 15 to 38 years. The majority of participants (148, 44.6%) were in the age group 21-26, followed by 114 women (34.3%) in the age group 15-20. Regarding educational background, most participants (134, 40.4%) had completed elementary school, while 91 women (27.4%) had completed high school and preparatory school (Table 1).

Table 1.Socio-demographic characteristics of study participants attending TASH and LDHHC from April to August2024 (n=332)

Variables		Frequency(N)	Percentage (%)
Age in groups	15-20	114	34.3%
	21-26	148	44.6%
	27-32	66	19.9%
	33-38	4	1.2%
Education level	Unable to read & write	25	7.5%
	Read & write only	6	1.8%
	Complete elementary	134	40.4%
	Complete high school & preparatory	91	27.4%
	Certificate & diploma	47	14.2%
	Degree & above	29	8.7%
Sample collected area	Lideta Dagim Hidasse Health center	132	39.8%
	Tikur Anbesa hospital	200	60.2%

6.2 Clinical characteristics of study participants attending TASH and LDHHC

Significant bacteriuria were identified in 26% (51/194) of multigravida women and 28.4% (62/218) in the third trimester. Among those with chronic diseases, bacterial growth was found in diabetes (23.07%, 3/13), hypertension (35.3%, 6/17), HIV (33.3%, 3/9), anemia (54.54%, 6/11), and other conditions such as hepatitis, thyroid disorders, heart disease, and hereditary diseases (32.26%, 10/31). In the absence of chronic conditions, significant growth was detected in 28.68% (72/251).Culture positivity was 34.09% (30/88) in women with one parity. Among those with no previous UTI history, 20.98% (68/324) had significant bacteriuria, while 30.59% (97/317) of those without STI history were culture positive. In previously diagnosed UTI cases, those who received treatment had a positivity rate of 33.33% (28/84), while untreated cases showed 32% (8/25).Asymptomatic bacteriuria was found in 25.47% (67/263). Among culture-positive cases, 28.46% (76/267) washed the genital area after urination, 34.4% (32/93) had weekly sexual activity, and 30.09% (99/329) cleaned the genital area from back to front (Table 2).

Table 2 Clinical characteristics of study participants attending TASH and LDHHC

Characteristics		Positive	Negative	Total
	Prime- Gravid	29(29)	45(19.4)	74
Gravidity	Multi-gravid	51(51)	143(61.6)	194
	Grand-multigravida	20(20)	44(19)	64
Stage of pregnancy	First trimester	11(11)	17(7.4)	28
	Second trimester	27(27)	59(25.4)	86
	Third trimester	62(62)	156(67.2)	218
Chronic conditions present	Diabetes	3(3)	10(4.3)	13
	Hypertension	6(6)	11(4.7)	17
	HIV/AIDS	3(3)	6(2.6)	9
	Anemia	6(6)	5(2.2)	11
	Others	10(10)	21(9.1)	31
	None	72(72)	179(77.2)	251
number of parity	0	30(30)	58(25)	88
	1	39(39)	98(42.2)	137
	2	19(19)	43(18.5)	62

	3	7(7)	20(8.6)	27
	4& above	5(5)	13(5.6)	18
Have diagnosed with UTI in previous pregnancy	Yes	32(32)	66(28.4)	98
	No	68(68)	166(71.5)	234
History of STIS	Yes	3(3)	12(5.2)	15
	No	97(97)	220(94.8)	317
Got treatment for UTI	Yes	28(28)	56(24.1)	84
	No	8(8)	17(7.3)	25
	None	64(64)	159(68.5)	223
Symptomatology of UTI	Lower abdominal pain	12(12)	20(8.6)	32
	Urgency of passing urine	12(12)	12(5.2)	24
	Pain or burning sensation	9(9)	4(1.7)	13
	No symptoms	67(67)	196(84.5)	263
How many times do you wash genital area	Once	0(0)	1(0.4)	1
	Twice	2(2)	11(4.7)	13
	Three	22(22)	29(12.5)	51
	Every time u urinate	76(76)	191(82.3)	267
Frequency of sex during pregnancy	Daily	2(2)	0(0)	2
	Twice per week	10(10)	30(13)	40
	Weekly	32(32)	61(26..3)	93
	Twice per month	25(25)	51(22)	76
	Monthly	13(13)	52(22.4)	65
	None	18(18)	38(16.4)	56
Cleaning of genitalia	Back to front	99(99)	230(99.1)	329
	front to back	1(1)	2(0.9)	3

6.3 The laboratory results for study participants who visited TASH and LDHHC

78.9% of the urine samples had a clear appearance. Urine dipstick tests revealed leukocytes and blood in 158 samples (47.6%), while 45.5% showed negative results. Microscopic examination showed pus cells and RBCs in 47.6% of samples, while 42.8% had normal findings. Gram-positive results were observed in 42 samples (12.7%), and gram-negative results in 58 samples (17.5%) (Table 3).

Table 3. The laboratory results for study participants who visited TASH and LDHHC

Variables		Frequency	Percentage
Urine appearance	Clear	262	78.9
	Turbid	70	21.1%
Urine dipstick	Negative	151	45.5%
	Have leukocyte & blood	158	47.6%
	Positive for nitrite	11	3.3%
	Have glucose	12	3.6%
Microscopy	Have pus cell & RBC	158	47.6%
	Have bacteria	32	9.6%
	Have normal finding	142	42.8%
Gram stain result	Positive	42	12.7%
	Negative	58	17.5%
	None	232	69.9%
Culture result	significant growth	100	30.1%
	Insignificant growth	63	19.0%
	Fungi	5	1.5%
	No growth	164	49.4%

6.4 Prevalence of isolated bacteria from study participants attending TASH ad LDHHC

According to culture results, 5 samples (1.5%) contained fungi, 63 (19.0%) showed insignificant growth, and 100 (30.1%) had significant growth. Among the identified pathogens, the most frequently detected microorganism was *E. coli* (27% of positive cultures), followed by *coagulase-negative staphylococci* (26%), *Citrobacter spp.* (11%), *Enterobacter aerogenes* (6%), *Klebsiella oxytoca* (2%), *Klebsiella ozaenae* (4%), *Providencia stuartii* (5%), *Staphylococcus aureus* (4%), *Proteus vulgaris* (3%), *Staphylococcus saprophyticus* (2%), *Streptococcus agalactiae* (2%), and *Enterococcus* species (8%) (figure 1).

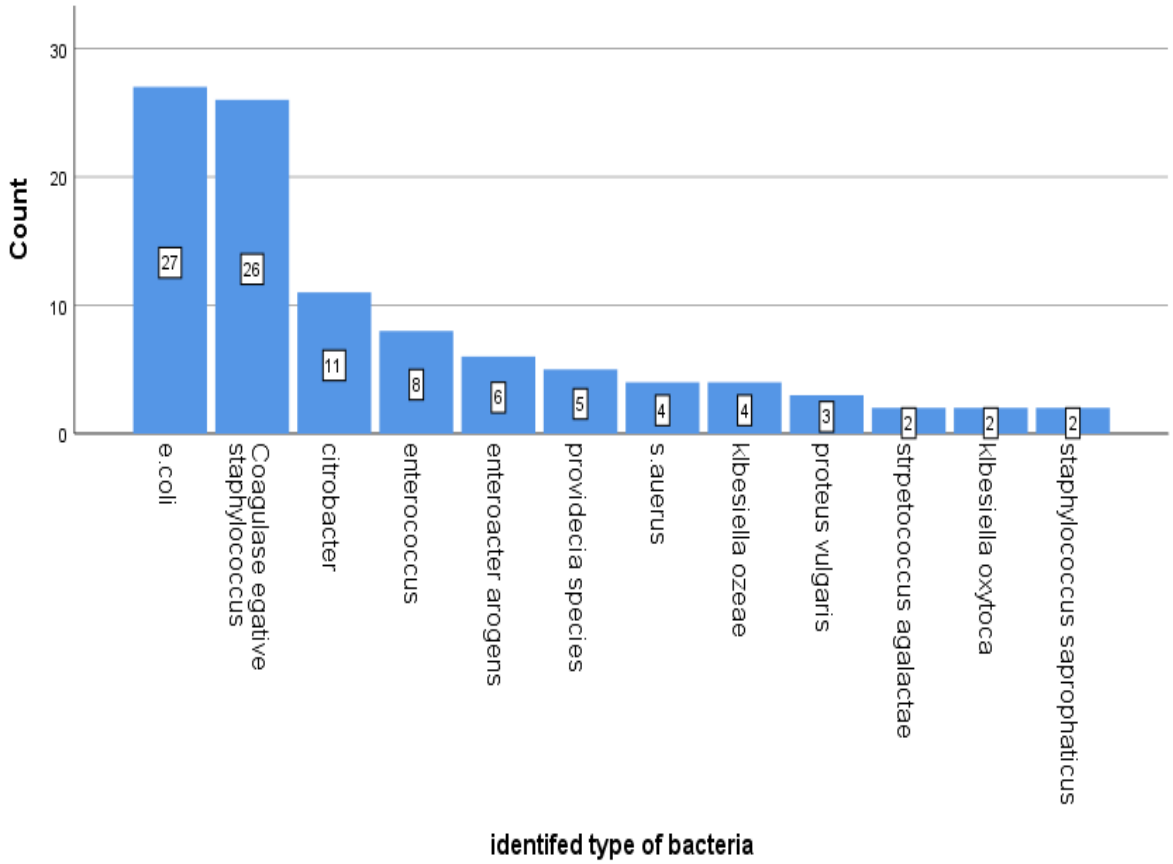

Figure 1 Prevalence of isolated bacteria from study participants attending TASH ad LDHHC

6.4 Antibiotic susceptibility pattern of identified gram positive bacteria

The most dominant gram-positive bacteria identified were *coagulase-negative staphylococci* (26%), followed by *Enterococcus* species (8%). Most gram-positive isolates in this study were susceptible to gentamicin (40%), nitrofurantoin (26%), tetracycline (24%), and trimethoprim/sulfamethoxazole (22%), but showed resistance to penicillin (81%), cefoxitin (27%), and ciprofloxacin (21%). The dominant gram-positive *Enterococcus* species (8%) were resistant to penicillin (19%), nitrofurantoin (3%), and tetracycline (3%), while being susceptible to ampicillin (12.1%), gentamicin (8%), trimethoprim/sulfamethoxazole (5%), and ciprofloxacin (5%)(Table 4).

6.5 Antibiotic susceptibility pattern of identified gram negative bacteria

The most dominant gram-negative bacteria identified were *E. coli* (27%), followed by *Citrobacter spp.* (11%) and *Enterobacter aerogenes* (6%). Among the isolated gram-negative bacteria, most were resistant to ampicillin (59.1%), cefotaxime (44.1%), and cefazolin (36.2%), while being susceptible to ertapenem (100%), meropenem (100%), amikacin (89.7%), and cefotaxime (75.9%). The most dominant gram-negative bacteria, *E. coli*, were resistant to ampicillin (28.8%), nalidixic acid (17.2%), and cefazolin (13.8%), while being susceptible to meropenem (100%), ceftriaxone (46.6%), cefotaxime (41.4%), amikacin (41.4%), and amoxicillin-clavulanate (41.4%)(Table 5).

Table 4 Antimicrobial susceptibility pattern for identified gram positive bacteria

Bacterial isolates		Antimicrobial susceptibility pattern for identified gram positive bacteria																						
		AM		GM			CIP			FM			TS		SXT			FOX			PG			NOV	
		R	S	R	S	I	R	S	I	R	S	I	R	S	R	S	I	R	S	I	R	S	I	R	S
CoNs (n=26)		NA	NA	1 1	25 25	0	11 11	15 15	0 0	10 10	16 16	0 0	14 14	12 12	14 14	12 12	0 0	17 16	9 10	0 0	19 45.2	7 16.7	0 0	0 0	25 92.6
S.agalactae (n=2)		N	NA	1 1	1(1)	1 1	1 1	1 1	0 0	0 0	2 2	0 0	0 0	2 2	0 0	2 2	0 0	2 2	0 0	0 0	2 4.8	0 0	0 0	NA	NA
S.saprophaticus(n=2)		N A	NA	0 0	1 1	1 1	2 2	0 0	0 0	1 1	1 1	0 0	1 1	1 1	0 0	2 2	0 0	1 1	1 1	0 0	1 2.4	1 2.4	0 0	2 7.4	0 0
Enterococcus(n=8)		0 0	8 12.1	0 0	8 8	0 0	2 2	4 4	2 2	3 3	3 3	2 2	3 3	5 5	2 2	5 5	1 1	3 3	5 5	0 0	8 19.0	0 0	0 0	NA	NA
S.auerus (n=4)		N A	NA	0 0	4 4	0 0	0 0	4 4	0 0	1 1	3 3	0 0	0 0	4 4	4 4	0 0	0 0	4 4	0 0	0 0	4 9.5	0 0	0 0	NA	NA
Total	N	0	8	2	40	0	16	24	0	15	25	0	18	24	20	21	1	27	15	0	34	0	0	2	25
	%	0	12.1 %	2%	**40%**	**0**	16%	24 %	2	15%	**25%**	**2 %**	18%	**24%**	20%	**21%**	**1%**	27%	15%	0	81%	0	0	7.4%	**92.6%**

Abbreviations: AM- Ampicillin, GM= Gentamicin, CIP= Ciprofloxacin, FM = Nitrofurantoin, TS=Tetracycline, SXT=Trimethoprim/ Sulfamethoxazole, FOX=cefoxitin, PG = penicillin, NOV=Novobiocin, S= Susceptible, R= Resistant I= Intermidate NA=Not AppliedTable 5Antimicrobial susceptibility pattern for identified gram negative bacteria

bacterial isolates	**Table 5 Antimicrobial susceptibility patter for identified gram negative bacteria**																														
	AM		GM		AUG		CAX		MEM		CRO		CIP		FM			TS		SXT			AMK		*NA*		KZ		FOX		
	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	I	R	S	R	S	I	R	S	R	S	R	S	R	S	I
p.stuarti (n=*5)*	1 1.5	4 6.1	0 0	5 5	0 0	5 8.6	0 0	5 8.6	0	5 8.6	0 0	5 8.6	0 0	5 5	3 3	0 0	2 2	4 4	1 1	4 4	0 0	1 1	0 0	5 8.6	0 0	5 8.6	0 0	5 8.6	0 0	4 4	1 1
p.vulgaris(n=*3)*	3 7.7	0 0	1 1	2 2	1 1.7	2 3.4	0 0	3 5.2	0	3 5.2	0 0	3 5.2	1 1	2 2	2 2	1 1	0 0	2 2	1 1	2 2	1 1	0 0	2 3.4	1 1.7	0 0	3 5.2	2 3.4	1 1.7	0 0	3 3	0 0
E.coli (n=*27)*	19 28.8	8 12.1	4 4	23 23	3 5.2	24 41.4	3 5.2	24 41.4	0	27 46.6	0 0	27 46.6	6 6	21 21	1 1	26 26	0 0	12 12	15 15	10 10	17 17	0 0	3 5.2	24 41.4	10 17.2	17 29.3	8 13.8	19 32.8	2 2	25 25	0 0
Citrobacter (n=*11)*	6 9.1	5 7.6	0 0	11 11	6 10.3	5 8.6	6 10.3	5 8.6	0	11 19	3 5.2	8 13.8	0 0	11 11	0 0	10 10	1 1	0 0	11 11	0 0	9 9	2 2	1 1.7	10 17.2	3 5.2	8 13.8	7 12.1	4 6.9	0 0	10 10	1 1
k.ozeae (n=*4)*	4 6.1	0 0	1 1	3 3	1 1.7	3 5.2	1 1.7	3 5.2	0	4 6.9	0 0	4 6.9	1 1	3 3	2 2	2 2	0 0	0 0	2 2	1 1	3 3	0 0	0 0	4 6.9	1 1.7	3 5.2	2 3.4	2 3.4	0 0	4 4	0 0
k.oxytoca (n=*2)*	2 3	0 0	0 0	2 2	2 3.4	0 0	1 1.7	1 1.7	0	2 3.4	0 0	2 3.4	1 1	1 1	0 0	2 2	0 0	3 3	1 1	1 1	1 1	0 0	0 0	2 3.4	0 0	2 3.4	2 3.4	0 0	0 0	2 2	0 0
Enteroboacter (n=*6)*	4 6.1	2 3	0 0	6 6	0 0	6 10.3	3 5.2	3 5.2	0	6 10.3	6 10.3	0 0	3 3	3 3	0 0	5 5	1 1	5 5	1 1	0 0	6 6	0 0	0 0	6 10.3	0 0	6 10.3	0 0	6 10.3	1 1	5 5	0 0
Total *N*	39	19	6	52	13	45	14	44	*0*	*58*	*9*	*49*	*12*	*46*	8	46	4	26	32	19	36	3	6	52	3	3	21	37	3	53	2
%	59.1	28.8	6	52	22.4	**77.6**	44.1	**75.9**	0	***100***	*15.5*	***84.5***	12	46	8	46	4	26	32	19	36	3	10.3	**89.7**	3	3	36.2	**63.8**	3	53	2

Abbreviations: AM- Ampicillin, GM= Gentamicin, AUG= Amoxicillin-Clavulanate, CAX=Cefotaxime, MEM= Meropenem, CRO= Ceftriaxone CIP= Ciprofloxacin, FM = Nitrofurantoin, TS=Tetracycline, SXT=Trimethoprim/ Sulfamethoxazole, AMK =Amikacin, NA=Nalidixic acid,FOX=cefoxitin,KZ=Cefazolin,S=Susceptible,R=Resistant,I=Intermidate

6.5 Bivariate logistic regression analysis for associated factors with significant bacteriuria

A p-value < 0.05 in the bivariate logistic regression analysis showed that grand-multigravida status, frequency of genital washing (every time after urination), symptomatology of UTI (lower abdominal pain, urgency of passing urine, pain or burning sensation), urine dipstick results, and microscopy findings were significantly associated with bacteriuria. Morover,in the multivariate analysis, pain or burning sensation [AOR: 0.281, 95% CI: 0.082–0.965], urine dipstick showing leukocytes and blood [AOR: 8.042, 95% CI: 1.159–55.813], and microscopy showing pus cells and RBCs [AOR: 0.075, 95% CI: 0.024–0.236] remained statistically significant(Table 6)

Table 6 Bivariate logistic regression analysis for associated factors with significant bacteriuria

		Bi-variable		Multi-variable	
Variables	Positive	COR(95% CI)	P value	AOR((95% CI)	P value
Age in groups					
15-20(114)	34(10.2%)	Ref	0.856	Ref	.754
21-26(148)	45(13.6%)	2.353 (0.318-17.397)	0.402	3.057	.304
27-32(66)	19(5.7%)	2.289 (0.313-16.761)	0.415	2.649	.368
33-38(4)	2(0.6%)	2.474 (0.325-18.856)	0.382	2.880	.334
Education level					
Complete elementary(134)	46(13.8%)	3.529 (0.364-34.185)	0.276	2.515(0.238-26.596)	0.443
Complete HIS& prep (91)	24(7.2%)	1.265 (0.558-2.869)	0.574	1.056(0.432-2.582)	0.905
Certificate & diploma(47)	13(3.9%)	1.971 (0.822-4.721)	0.128	1.868(0.741-4.707)	0.185
Degree & above(29)	12(1.2%)	2.310 (0.849-6.286)	0.101	2.144(0.748-6.146)	0.156
Gravidity					
Prime- Gravid(74)	29(29)	Ref*	0.244	Ref*	0.091
Multi-gravid(194)	51(51)	0.655(0.322-1.334)	0.654	.426 (0.148-1.227)	0.114
Grand-multigravida(64)	20(20)	1.153(0.618-2.150)	**0.002***	1.100(0.534-2.266)	0.795
Stage of pregnancy					
First(28)	11(11)	Ref*	0.482	Ref*	0.459
Second(68)	27(27)	0.614(0.272-1.386)	0.240	0.572(0.572-0.230)	0.230
Third(218)	62(62)	0.868(0.505-1.494)	0.610	0.838(0.838-0.467)	0.552

Chronic conditions present					
Yes(251)	28(28)	1.104(0.644-1.892)	0.718	0.999(0.564-1.769)	0.996
No(81)	72(72)	0.906(0.528-1.552)	0.718	1.036(0.562-1.911)	0.910
number of parity					
0(88)	30(30)	Ref*	0.922	Ref*	0.784
1(137)	39(39)	0.782(0.255-2.406)	0.669	1.601(0.363-7.073)	0.534
2(62)	19(19)	0.966(0.323-2.892)	0.951	0.965(0.267-3.486)	0.957
3(27)	7(7)	0.808(0.253-2.578)	0.718	0.900(0.236-3.429)	0.877
4& above(18)	5(5)	1.099(0.287-4.211)	0.891	1.163(0.279-4.842)	0.836
How many times do you genital area					
Three(51)	22(22)	2.188(0.474-10.106)	0.316	2.526(0.511-12.479)	0.255
Every time u urinate(23)	76(76)	0.525(0.284-0.970)	**0.040***	0.528(0.274-1.017)	0.05
Have diagnosed with UTI in previous pregnancy					
Yes(98)	32(32)	1.265(0.763-2.099)	0.362	0.750(0.4360-1.290)	0.299
No(234)	68(68)	1.265(0.763-2.099)	0.362	1.334 (0.775-2.295)	0.299
History of STIS					
No(317)	97(97)	0.567 (0.156-2.055)	0.388	1.578(0.400-6.218)	0.514
Symptomatology of UTI					
Lower abdominal pain	12(12)	Ref*	**0.001**	Ref*	0.075
Urgency of passing urine	12(12)	0.149(0.044-0.499)	**0.002**	0 .205(0.039-1.075)	0.061
Pain or burning sensation	9(9)	0.283(0.121-0.663)	**0.004**	0.281(0.082-0.965)	**0.044**
No symptoms	67(67)	0.558(0.259-1.204)	0.137	0.825(0.302-2.251)	0.707
Fluid you in take					
Water	80(80)	Ref*	0.996	Ref*	0.998
Hot drink	20(20)	1.013(0.309-3.323)	0.983	0.762(.0145-4.003)	0.748
Average daily water in take					
0-1L	52(52)	Ref*	0.516	Ref*	0.708
2-3L	44(44)	0.978(0.250-3.832)	0.974	1.062(0.138-8.149)	0.954
Engage physical exercise					
Yes	87(87)	1.182(0.566-2.468)	0.657	1.136(0.417-3.094)	0.803

No	13(13)	1.182(0.566-2.468)	0.657	1.136(0.417-3.094)	0.803
Frequency of sex during pregnancy					
Twice per week	10(10)	0.000	0.999	0.000	0.999
Weekly	32(32)	1.149(0.468-2.820)	0.761	0.750(0.210-2.678)	0.658
Twice per month	25(25)	0.831(0.408-1.694)	0.610	0.678(0.241-1.904)	0.461
Monthly	13(13)	0.838(0.4-1.759)	0.641	0.566(0.189-1.697)	0.210
None	18(18)	1.925(0.826-4.490)	0.129	0.868(0.258-2.917)	0.819
Cleaning of genitalia					
Back to front	99(99)	0.000	0.999	0.000	0.999
Urine dipstick					
Have leukocyte & blood	81(81)	9.733(2.001-47.337)	0.05	8.042(1.159-55.813)	**0.035**
Positive for nitrite	11(11)	0.317(0.083-1.214)	0.094	0.759(0.130-4.431)	0.760
Microscopy					
Have pus cell & RBC	86(86)	Ref*	**0.000**	0.075(0.024-0.236)	**0.000**
Have bacteria	10(10)	0.024(0.009-0.069)	**0.000**	0.338(0.058-1.978)	0.229

Ref *Reference COR = Cruds odds ratio, AOR=Adjusted odds ratio, CI=Confidence interval

6.6 prevalence of multi drug resistance of isolated bacteria's

Multiple drug resistance was described in this study as a bacterial isolate that possesses resistance to three or more antibiotics in three or more antimicrobial agent classes. Among the isolates, about 34 (34.7%) of the study participants showed MDR. From gram-negative bacteria *E. coli* shows 10(29.4%),*Enterobacter.aerogens* (11.8) *K. ozaena* 2(5.9%) and *P.vulgaris*1 (2.9%).whereas from gram-positive bacteria *CoNs* 11(32.4%), *Enterococcus* 2(5.9%) ad *S.auerus* 4(11.8%). ESBL and carbapenemase production were not detected in all isolated bacteria(Table 7).

Table 8 Multi drug resistance of isolated bacteria's

Bacterial isolate	R1	R2	R3	R4	R5	R6	MDR	
							N	**%**
Gram-positive bacteria								
CoNs(26)	1	12	6	3	1	1	11	32.4
Enterococcus(8)	0	2	0	2	0	0	2	5.9
S.agalactae(2)	1	2	0	0	0	0	0	0
S.saphrophaticus(2)	1	1	0	0	0	0	0	0
S.auerus(4)	1	4	4	0	0	0	4	11.8
Total	4	21	10	3	1	1	17	
Gram negative bacteria								
E.coli(27)	12	13	3	3	2	2	10	29.4
Citrobacter(11)	0	0	0	0	0	0	**0**	**0**
K.ozaena(4)	0	3	1	1	0	0	2	5.9
K.oxytoca(2)	0	2	0	0	0	0	0	0
E.aerogens (6)	0	3	4	0	0	0	4	11.8
P.stuartin(5)	4	0	0	0	0	0	0	0
P.vulgaris(3)	0	2	1	0	0	0	1	2.9
	16	23	9	6	2	2	17	
Total	**20**	**44**	**19**	**9**	**3**	**3**	34	34.7(98)

For gram-positive bacteria R1 refers to resistance to one class of antibiotics, specifically beta-lactam antibiotics**.** R2 indicates resistance to two classes, which include both beta-lactam antibiotics and second-generation cephalosporins**.** R3 denotes resistance to three classes of antibiotics, including beta-lactam antibiotics**,** second-generation cephalosporins, and sulfonamides**.** R4 represents resistance to four classes: beta-lactam antibiotics, second-generation cephalosporins**,** sulfonamides**,** and tetracyclines**.** R5 indicates resistance to five classes, which includes beta-lactam antibiotics**,** second-generation cephalosporins**,** sulfonamides**,** tetracy-

clines, and fluoroquinolones. Finally, R6 refers to resistance to six classes, covering beta-lactam antibiotics, second-generation cephalosporins, sulfonamides, tetracyclines, fluoroquinolones, and nitrofurans.

For gram- negative bacteria R1 refers to resistance to one class of antibiotics, beta-lactam antibiotics. R2 indicates resistance to two classes, which include both beta-lactam antibiotics and tetracyclines. R3 denotes resistance to three classes of antibiotics, including beta-lactam antibiotics ,tetracyclines and fluoroquinolones. R4 represents resistance to four classes beta-lactam antibiotics ,tetracyclines ,fluoroquinolones and first-generation quinolone. R5 indicates resistance to five classes, which includes beta-lactam antibiotics ,tetracyclines ,fluoroquinolones, first-generation quinolone and aminoglycosides. R6 refers to resistance to six classes, covering beta-lactam antibiotics, includes beta-lactam antibiotics ,tetracyclines ,fluoroquinolones,first-generation quinolone ,aminoglycosides and second-generation cephalosporins.

7.Discussion

The overall prevalence of significant bacteriuria in this investigation was found to be 30.1% (100/332, 95%CI:1.65,1.75)Which was greater than some studies conducted in AddisAbaba14.7%(45),Mekelle21.1%(41),Hawasssa 7.8%(53),Harar15.5 %(54) , Southeast Ethiopia 23.9%(55),Nigeria 24.2%(56),Libya 22%(57),India 7.4%(58) and Iraq 12.40%(59)and lower than the prevalence of some studies in Saudarebia 57.01% (60) ,Ghana 39.8%(61), Uganda 35 %(62),Somali 38.3% (63) Egypt 49.3%(64),central Ethiopia 40.%(65). Possible reasons for the observed variation include differences in study populations, such as demographic disparities, sample size, study design, and timeframe, as well as cultural and behavioral factors like hygiene practices, sexual activity, and healthcare-seeking behavior.

The demographic category of people in the average age range of 15–26 years had the highest incidence of UTI recorded in this study . This aligns with findings from other research studies, like in southern Ethiopia(53) it contradicts the findings from other studies conducted in Nigeria, where the more prevalent age group was 34-49 (66) and age group from 25-34 prevalent in Dire Dawa (40). This might be as a result of the higher prevalence of sexual activity among women in this age group, which may increase their vulnerability to UTI.From the positive cultures obtained, 51% belonged to multigravida, and 62% were in the third trimester. This corresponds with studies conduct in central India(67),Kenya,(68)south Ethiopia (Wachemo) (69) and contradicts with studies in Ghana(25)Yemen(4). The disparities across studies may be due to factors such as age and sexual activity, regional differences in healthcare access, prevention programs, cultural practices and pregnancy management.

Our investigation examined the potential correlation between culture-positive urine UTI and various socio-demographic factors like age, educational background, residence, and clinical characteristics like gravidity, parity, previous UTI and STI history, and presence of chronic disease.However, there was no statistically significant association between these factors and UTI.This finding agrees with studies conducted in Southern Ethiopia(53)Somali(70) ,Lebanon(71),Nigeria(72),Cameroon(73) and contradicts studies done in uganda (74),Ethiopia ,Dessie (75) The differences observed across studies may be due to variations in sample size, study design, and methodologies. Additionally, differences in healthcare access, regional disease burden, cultural practices, and the prevalence of risk factors such as

sexual behavior, hygiene, and antibiotic use may influence the presence of significant correlations in some regions but not others.

Both gram-positive and gram-negative bacteria can cause urinary tract infections. Nonetheless, gram-negative bacteria are the most frequently found, with *E. coli* accounting for the majority of bacterial uropathogens globally(76).From the significant bacteriuria, the most frequently detected microorganism from the identified pathogens in this study was *E.coli* (27% of positive cultures), followed by *Citrobacter spp* (11%). The fact that frequently detected microorganism was *E.coli* consistent with findings in Ethiopia [(77),(78, 79),(80, 81)]and from other countries Nigeria (56) Pakistan (82) Iran (83) Libya (84) Ghana (61). *E.coli* followed by *CoNS* (*coagulase-negative staphylococci*) (26%) and *Citrobacter species* (11%) which is comparable with other findings in AddisAbeba[(42, 85)Diredawa, Ethiopia *E.coli*(34.6%)& *CoNS*(19.2%) (40),central Ethiopia *E.coli*(34.4%)& *CoNs*(15.6%) (65) Somali land *E.coli*(43.5%)& *CoNs*(16%)(86)Jigjiga *E.coli* (40%) & *CoNs* (12%) Ghana *E.coli*(27.8%)& *CoNs*(13.5%) (61). Geographic factors, including climate, topography, and water quality, can significantly influence the types of microorganisms present in different regions. Additionally, variations in healthcare systems, the availability of diagnostic tools, and laboratory capabilities can impact the detection and frequency of uropathogens such as *E. coli* and *CoNS.*

According to our finding the majority of the identified bacteria were resistant to penicillin (81%), ampicillin (59.1%), and tetracycline (44%), cefotaxime (44.1%) trimethoprim/ sulfamethoxazole (39%), cefazolin (36.2%). On the other hand, most bacterial isolates were sensitive for ertapenem (100%), meropenem (100%), Gentamicin (92%), amikacin (89.7%), ceftriaxone (84.5%), amoxicillin-clavulanate (77.6%) and cefotaxime(75.9%). These findings align with studies conducted in Ethiopia Hosanna (65)Uganda(87) but contradict findings from Ghana(61). Gram-positive bacteria showed high resistance to penicillin, while gram-negative bacteria were more resistant to ampicillin,consistent with earlier studies in Ethiopia [Bahirdar(41)Adigrat(80)]. However, these findings contradict studies conducted in AddisAbeba (81), Libya (72) Yemen (82)Pakistan (79). Variations in antibiotic resistance patterns across studies may result from differences in antibiotic prescription practices**,** healthcare infrastructure**,** and diagnostic capabilities**.** Regions with higher antibiotic misuse tend to have increased resistance. Additionally, genetic variations in bacterial strains**,** environmental factors**,** and public health policies influence resistance development. Poor sanitation, self-medication, and weak infection control can contribute to higher resistance in some areas.

In this study, the most dominant gram-negative bacteria identified were *E.coli* and *Citrobacter species* which were resistant to ampicillin (59.1%), cefotaxime (44.1%) and cefazolin (36.2%).Similar finding have been reported in previous studies in Hargeisa, Somaliland(86),Jimma(88)Diredawa (40)Kenya(76) Iran(89). However, these bacteria were highly susceptible to ertapenem and meropenem (100%), Cefotaxime (96.6%), Gentamicin and Amikacin (89.7%), Ceftriaxone(84.5%), Amoxicillin-Clavulanate (77.6%) and Cefotaxime (75.9%). Among gram-positive bacteria, the most dominant isolates were CoNS (*coagulase-negative staphylococci*) (26%) and *Enterococcus* (8%) . These bacteria showed high resistance to penicillin (81%), cefoxitin (27%) less resistance to Ciprofloxacin (21%), Trimethoprim/ Sulfamethoxazole (20%), and susceptible for Gentamicin (40%), Nitrofurantoin(26%) and Tetracycline (24%). susceptible to gentamicin correlates with other studies in Ethiopia[(40) (53),(77),(79)]Saudi Arabia(60) Pakistan(90) and it contradicts findings from earlier studies in Libya(84). Differences in antibiotic resistance patterns across regions may stem from variations in antibiotic use, healthcare infrastructure, bacterial genetics, diagnostic capabilities, and public health policies. Areas with higher antibiotic misuse and weaker infection control measures tend to experience increased resistance. Genetic mutations and plasmid-mediated resistance play a role in bacterial adaptation, while variations in diagnostic methods and study designs can impact reported findings. The discrepancy in gentamicin resistance across studies may be attributed to regional prescribing practices, bacterial evolution, and differences in laboratory testing methodologies.

Our study's observed drug resistance pattern (34.7%) was comparable with other studies in Ethiopia[Bale zone](80) but lower than studies conducted in Dire Dawa (40), Jigjiga (96.0%)(81),Kenya(96%)(76),Jimma (79.2%) (88) North east(80.4%) (41) AddisAbeba(80%)(45). Local healthcare practices, such as antibiotic over-prescription, self-medication, and weak infection control, contribute to higher MDR rates. Environmental factors, including poor sanitation, contaminated water, and climate conditions, also promote the growth of resistant bacteria. Additionally, genetic diversity in bacterial strains and the spread of resistant clones impact MDR prevalence. Finally, variations in antibiotic regulations and international travel further influence resistance patterns, with regions with stricter antibiotic control measures typically seeing lower MDR rates, which is more favorable compared to other regions.

In this study, there were no ESBL producers or carbapenemase-resistant gram-negative bacteria. This may be due to the fact that the majority of identified bacteria were sensitive to broad-spectrum antibiotics, including carbapenems. This finding corresponds with studies in Nigeria (91)and but contradicts studies in Saudi Arabia (92). The absence of ESBL producers and carbapenemase-resistant gram-negative bacteria, despite broad-spectrum antibiotic sensitivity, may be attributed to factors such as more strict antibiotic use regulations, differences in healthcare infrastructure, local epidemiology, and lower levels of antibiotic misuse, compared to regions like Saudi Arabia, where higher resistance rates are often observed due to more widespread antibiotic overuse, weaker infection control, and different bacterial strains circulating in the population.

In the current study clinical diagnosis done using urine chemical examination and microscopy was significantly associated with urinary tract infections. Pregnant women showed blood and leukocyte more likely to have urinary tract infections compared .Urine microscopy showed pus cell & RBC were likely to have urinary tract infections. The correlation between urine chemical examination and UTIs may stem from the body's immune response to infection.Which correlates with the study has been done in Cameroon (73) The consistency of these findings across different studies, including the one in Cameroon, may be due to similar diagnostic methods, regional prevalence of UTIs, and shared physiological mechanisms involved in the immune response to urinary infections.

8. Strength and Limitation

8.1 strength of the study

- It provides insights about resistance patterns like (MDR).

8.2 Limitation of the study

- A cross-sectional design is used so for resistance patterns it may not capture trends over time.
- Diagnostic limitation like (molecular testing to detect resistance genes that are not phenotypically expressed.
- No molecular test for conformation of identified bacteria.

9. Conclusion and recommendation

9.1 conclusions

The overall prevalence of UTI in this study was relatively high. Antimicrobial susceptibility testing revealed that carbapenems (ertapenem, meropenem) were effective treatment options, whereas ampicillin and tetracycline were unsuitable due to high resistance rates. Routine screening for UTIs at least once during pregnancy is essential to prevent adverse pregnancy outcomes. Additionally, this study identified a significant association between UTIs and findings from urine chemical examination and microscopy.

9.2 Recommendation

- Strength antimicrobial stewardship program to minimize the misuse ad overuse of antibiotics.
- Enhance laboratory capacity including phenotypic and molecular testing to accurately detect resistance mechanism.
- Educate health care professional and pregnant women about the risk of anti-microbial resistance and importance of completing prescribed antibiotics courses.

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11. Annexes

Annex I- Laboratory procedures (CLIS guidelines)

Urine sample collection

Midstream specimen of Urine (MSU) for microbiological examination will be collected as follows:

Give the patient a sterile, dry, wide-necked, leak proof container with participant code and request a 10 ml specimen. Clean, dry paper for cleaning the vulva and alcohol wipe or antiseptic solution for disinfecting the area before collecting the specimen will be given by the principal investigator

Important: Explain to the patient the need to collect the urine with as little contamination as possible, i.e. a 'clean-catch' specimen.

Female patients: Wash the hands. Cleanse the area around the urethral opening with clean water from back to front or with the wipes dry the area with a sterile gauze pad, and collect the urine with the labia held apart.

Label the container with the date, the name and number of the patient, and the time of collection.

As soon as possible, deliver the specimen with a request form to the laboratory. When immediate delivery to the laboratory is not possible, refrigerate the urine at 4–6 °C. When a delay in delivery of more than 2 hours is anticipated, add boric acid preservative to the urine.

2. Culture and identification for bacteria

i .Suspected growth of bacteria from the urine on MacConkey

a. Positive/present □

b. Negative/absent □

ii. Identification steps for suspected colonies

a) Gram stain result
    a. Gram positive
    b. Gram negative

b) Lactose fermentation from MacConkey agar

a.Lactose Fermenter

b. Late Lactose fermenter

c.Non lactose fermenter

iii. Biochemical reactions

Identification of gram positive bacterial isolates involves the use of biochemical screening includes catalase test and coagulase test.

Biochemical reactions		Catalase	Coagulase test
Result	Positive		
	Negative		
Gram positive			

a. Catalase test

Pour 2-3 ml of the hydrogen peroxide solution into a test tube.Using a sterile wooden stick or a glass rod remove several colonies of the test organism and immerse in the hydrogen peroxide solution. If active bubbling seen positive and if no release of bubbles it is negative.

b. Coagulase Test

Place a drop of distilled water on each end of a slide or on two separate slides.Emulsify a colony of the test organism (previously checked by Gram staining) in each of the drops to make two thick suspensions.Add a loopful (not more) of plasma to one of the suspensions, and mix gently. Look for clumping of the organisms within 10 seconds. If Clumping seen within 10 seconds positive if no clumping negative.

Identification of bacterial isolates involves the use of biochemical screening Medias. Indole, Urease, Triple sugar iron (TSI), Citrate, Motility, Lysine Decarboxylase, and Oxidase tests.

Biochemical reactions		Indo	Urea	TSI	Cit	Mot	OX	H2S
Result	Positive							
	Negative							
Gram negative rods								

Key: Triple sugar iron (TSI),Ox = Oxidase test, Cit = Citrate test, Mot = Motility, Ind = Indole test, Urea = Urease, H2S = Hydrogen sulphide (blackening)

A. Indole test: Few colonies of the culture will be inoculated into peptone water and incubated at 37oC for 24 hours. Few drops of indicator (Kovac's reagent) will be added and gently shake to mix well. Color change will be then observed. If the layer of indicator reagent turns to red within 1 minute, it is Indole positive (positive result). If the layer of indicator reagent remains yellow within 1 minute, it is indole negative (negative result).

B. Urease test (Christensen's (modified) urea broth): Urea agars will be inoculated heavily over the entire surfaces of the slants in bijou bottles. The cap will be loosened and then incubated at 37oC for 3-12 hours. A urease-positive culture produces an alkaline reaction in the medium, evidenced by pinkish red color of the Medium. Urease-negative organisms do not change the color of the medium, which is pale yellow-pink.

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

D. Citrate utilization test using Simmon's citrate agar: Simmon's citrate slopes will be prepared in bijou bottles as recommended by the manufacturer (stored at 2-8oC). And the slopes will be then stabbed and incubated at 37oC aerobically for 48 hours.Bluecolour indicates a positive reaction and if Simmon's citrate agar slopes remained as green in colour indicate negative reaction.

E. Motility Test (using motility agars): Motility agar will be prepared and inoculated with a straight inoculating needle making a single stab about 1-2cm down into the medium. The motility will be examined after 35-37oC for 24 hour. Motility will be indicated by the presence of diffuse growth (appearing as coloring of the medium) away from the line of inoculation.

G. Oxidase test: A piece of filter paper is soaked with a few drops of oxidase reagent. A colony of the test organism is then smeared on the filter paper. Alternatively an oxidase reagent strip can be used. When the organism is oxidase-producing, the phenylenediamine in the reagent will be oxidized to a deep purple colour.

3. Antibiotics susceptibility result for Bacteria isolates

Isolated bacteria	Antibiotics		GM	F	CRO	STX	AMP	CFN	AMI	FOX	NOR	C	MEM
	Susceptibility pattern	S											
		I											

		R											

Note;Gentamicin(GM), Amoxicilin-Clavulanic acid(AmC), Cefoxitine(FOX) , Mere-penem(MEM) Ampicilin(AMP), Amikacin **(**AMI**)**,Nitrofurantoin(f),Trimethoprim–sulphamethoxazole (SXT) ,Cefixime (CFX) ,Ceftriaxone (CFN), Ceftazidime (CFD) ,Chloramphenicol(c) and Amoxicillin-clavulanic acid (AMX-C)

Procedure for Performing the Disc Diffusion Test Inoculum Preparation

At least three to five well-isolated pure colonies of the same morphological type will be selected from Blood or MacConkey agar plate. The top of each colony is touched with a loop, and the growth is transferred into a tube containing 4 to 5 ml of tryptone soy broth.

The turbidity of the broth culture will be adjusted with that of the 0.5 McFarland standards.

Inoculation of Test Plates

After adjusting the turbidity of the inoculum suspension within 15 minutes Using a sterile inoculation loop or swab, pick up bacterial culture to be tested from the adjusted suspension. The swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level. Gently streak the inoculum onto the surface of the Mueller-Hinton agar plate in a zig-zag pattern or in a lawn streaking method. Be careful not to press too hard to avoid damaging the agar surface.The dried surface of a Mueller-Hinton agar plate is inoculated by streaking the swab over the entire sterile agar surface.

NOTE: Extremes in inoculum density must be avoided. Never use undiluted overnight broth cultures or other unstandardized inocula for streaking plates.

Application of Discs to Inoculated Agar Plates

The predetermined battery of antimicrobial discs is dispensed onto the surface of the inoculated agar plate. Each disc must be pressed down to ensure complete contact with the agar surface.

The plates are inverted and placed in an incubator set to 37□C within 15 minutes after the discs are applied.

Reading Plates and Interpreting Results

After 16 to 18 hours of incubation, each plate is examined. The diameters of the zones of complete inhibition (as judged by the unaided eye) are measured, including the diameter of the disc. Zones are measured to the nearest whole millimeter, using sliding calipers which is held on the back of the inverted plate.

4.Data collection form for enterobacteriaceaesolates

Isolate identification laboratory:_____

Patient referring Hospital:_____

Age _____ Sex _____

Identification number of the isolate at site:_____

Identification number of the isolate for the study:_____

Isolated Enterobacteriaceaespps:

Escherichia coli

Klebsiella species

Citrobacter species

Enterobacter species

Klebsiella species

Proteus vulgaris

Staphylococcus saprophyticus

Pseudomonas aeruginosa

Staphylococcus aureus

Other:_____

A.Methods used for isolation:_____

b.Type specimen/Disease type:_____

C. Prior Antibiotics used

D. Name of antibiotics used:_____

E. Others_____

Can the laboratory perform ESBL test?

A.Yes___ b.No_____

If Yes which method they use?

A. DDST (*Double-disk* synergy test)

B. DDT (Double-disk diffusion test)

C. If automated, name of machine_____

Result of AST pattern of the isolate at peripheral laboratory:

S.No	Antimicrobial Agent	Disk content	Zone diameter near-est whole mm(48)			Result of AST (S/I/R)/(Zone diameter in mm)
			R	I	S	
	Ampicilin	10 µg	≤ 13	14-16	≥ 17	
	Gentamicin	10 µg	≤ 14	15-17	≥ 18	
	Amikacin	30 µg	≤ 14	15-16	≥ 17	
	Amoxicillin+clavulanic acid	20/10 µg	≤ 13	14-17	≥ 18	
	Ceftriaxone	30 µg	≤ 19	20-22	≥ 23	
	Cefotaxime	30 µg	≤ 22	23-25	≥ 26	
	Cefazolin	30 µg	≤ 19	20-22	≥ 23	
	Cefoxitin	30 µg	≤ 14	15-17	≥ 18	

	Meropenem	10 µg	≤ 19	20-22	≥ 23	
	Trime-thoprim+Sulfamethoxazole	1.25/23.75	≤ 10	11-15	≥ 16	
	Ciprofloaxacin	5 µg	≤ 20	22-25	≥ 26	
	Nitrofurantoin	300 µg	≤ 14	15-16	≥ 17	
	Nalidic acid	30 µg	≤ 13	14-18	≥ 19	
	Tetracycline	30 µg	≤ 14	15-16	≥ 17	
	penicillin	30 µg	≤ 28	-	≥ 29	
	Imipenem, or Ertapenem	10 µg	≤19 mm	20-22	≥23 mm	

If the lab perform ESBL test, what is the result? ESBL POS____ Neg ____

If the lab perform Carbapenemase what is the result? Carbapenemase POS____ Neg ____

Sources

CLSI performance standards for antimicrobial susceptibility testig 33rd ed.CLSI supplement M100

Annex II: English version of participant information sheet, consent form and questioner

2.1. Participant information sheet

Department of Medical Laboratory Science, School of Allied Health Sciences, Collage of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia

Title: Bacterial uropathogen, antimicrobial susceptibility pattern and associated factors of urinary tract infection among pregnant women attending antenatal care in TikurAnbesa Specialized Hospital and Lideta Dagim Hidasse Health Center Addis Ababa, Ethiopia,2016

First of all I would like to thank you in advance for your cooperation and consent in participation in this study. Please read or listen when it is read for you about the general information of the study. If you have any question regarding the study please ask freely.

Background information

When bacteria enter the urinary tract and grow, they can lead to an infection and a urinary tract infection. Due to hormonal changes that impact the urinary system and physical changes in the urinary tract brought on by the uterus's expansion, pregnancy raises the risk of urinary tract infections. About 10% of pregnant mothers experience UTIs during their pregnancy. During pregnancy, it's critical to recognize and treat UTIs as soon as possible to avoid complications. To find out which antibiotics work best at eliminating the particular bacteria causing the infection, susceptibility testing is done. This information is essential for making informed decisions about the best course of action, as it guarantees that the antibiotic of choice will effectively combat the bacteria causing the UTI.

Aim of the study

The purpose of the study is to determine the Bacterial uropathogen, antimicrobial susceptibility pattern and associated factors of urinary tract infection among pregnant women attending antenatal care in Tikur Anbesa Specialized Hospital and Lideta Dagim Hidasse Health Center Addis Ababa, Ethiopia.

Benefits for participants

Study participants will not have any financial incentives or other inducements from participating on this study. However, the results will be given to your physician for treatment or to get counseling. Most importantly, this study will contribute to provide information or data for future and further nationwide study and to develop health programs for health policy makers.

Risks and complications

There is no risk to the participants in participating in this study other than collecting 5-10ml of clean catch mid stream urine sample in a sterile comfortable wide mouth urine cup.

Confidentiality

In order to maintain the confidentiality of participants' information, your name will not be given and the samples will be coded. Participants will not be prohibited to stop or withdraw at any time from the study. No personal information will be disclosed to third party or will not appear in any report from this study.

Assurance of Principal Investigator

I put my signature below to confirm you that I take over the responsibility for the scientific ethical and technical conduct of the research project and for provision of progress reports for all stakeholders of the research project.

Sophiya Shemsu(PI):signature:_____ Date:_____

Note: If you have any questions about this study, feel free to ask now or anytime throughout the study by contacting:

PI Address: Sophiya Shemsu Department of Medical Laboratory Sciences, Collage of health sciences, Addis Ababa University, Addis Ababa, Ethiopia

E-mail: sophiyashemsu11@gmail.com; Tel.: +251913151036

2.2 Informed consent

Patient ID-

I have been informed about the study which plans to determine the Bacterial uropathogen, antimicrobial susceptibility pattern and associated factors of urinary tract infection among pregnant women attending antenatal care in TikurAnbesa Specialized Hospital and Lideta Dagim Hidasse health center Addis Ababa, Ethiopia.

The objective and the application of the study were briefly explained to me. Moreover, I have been well informed of my right to refuse information, decline to cooperate and drop out of the study if I want and none of my actions will have any bearing at all on my overall health care.

It is therefore with full understanding of the situation that I agreed to give the informed consent voluntarily to the researcher to give my urine for the mentioned study. I agreed that the specimen would be tested for uropathogens. I have had the opportunity to ask questions about the project and received clarification to my satisfaction in a language I understand. I was also informed that results for the analysis of urine isolates of uropathogens will be given to the health facility and that I may ask the information if I want.

I _____ hereby give my consent for giving of the requested information and specimen for this study.

Participant code:_____ Signature: _____ Date: _____

2.3 Questionnaire for to determine the Bacterial uropathogen, antimicrobial susceptibility pattern and associated factors of urinary tract infection among pregnant women attending antenatal care in TikurAnbesa Specialized Hospital and Lideta Dagim Hidasse Health Center Addis Ababa, Ethiopia,2016

A. Patient Identification

1. Patient name----------
2.MRN----------
3.Laboratory ID----------
4. Clinical diagnosis ----------

B. Socio-demographic factors (filled by the patient)

1. Age

A.15-20 B.21-26 C.27-32 D.33-38 e.39-44 f.45-50

2. What is your educational level presently?

A. Unable to read & write

B. Read & write only

C. complete elementary

D. complete high school

E.Certeficate &Diploma

F.Degree & above

C. medical related and pregnancy details

1. Any pre-existing medical conditions,

A.Yes B. No

1.1 If yes

A.D.M B.Hypertension C.Anemia D.others

2. Is there history of sexually transmitted infections (STIs)?

A.Yes B. No

3. Stage of pregnancy

A.1st trimester B.2nd trimester C.3rd trimester

4. Number of Gravidity?

A. 0 B. 1 C.2 D.3 E. 4& above

5. Number of Parity?

A. 0 B. 1 C.2 D.3 E. 4& above

6.Is there previous history of UTI?

A. Yes B. No

6.1 .If yes 1.How many times it happened

A. In the last one year

B.In the last two years

C.In the last three years

6.2.Duration of UTI existed

A. 1-5 days B. 6-10 days C.11-15 days D. 16-20 days E.more than 20 days

6.3. Any previous treatment received?

A.yes B.no

If Yes What was the treatment?

A.Antibiotics

D. Hygiene practices and Sexual activity

1.How many times per day u do you wash your genetial area?

A.Once

B.Twice

C.Three

D. Every time u urinate

E.No at all

2. what wiping techniques do you use after using the toilet?

A.Washing with water

B.Using dry cotton

C. Soft

D. Using nothing

E. . Using wipes

3.How frequent do you do sexual intercourse?

A.Daily B.Twice per week C.Weekly D. Twice per month E.Monthly

E.Fluid intake:

1.The fluid you take more

A.Water

B.Coffee

C. Alcohol

D. Soft drinks

E.Tea

2. The average daily water intake

A.0-1L B .2-3L C.3-4L D.4. E. >5L

F.Lifestyle factors

A.Do u have a habit of smoking

A.yes B.No

b.Do u engage in any physical activity

A.yes B.No

AnnexIII: የአማርኛ እትም የተሣታፊ መረጃ ወረቀት፣ የስምምነት ቅጽ እና ጠያቂ

3.1 የተሳታፊ መረጃ ወረቀት

የሕክምና የላቦራቶሪ ሳይንስ ትምህርት ክፍል፣ የሕብረት ጤና ሳይንስ ትምህርት ቤት፣ የጤና ሳይንስ ኮሌጅ፣ አዲስ አበባ ዩኒቨርሲቲ፣ አዲስ አበባ፣ ኢትዮጵያ

ርዕስ፡- በጥቁር አንበሳ ስፔሻላይዝድሆስፒታል እና በልደታ ዳግም ህዳሴ ጤና ጣቢያ አዲስ አበባ፣ ኢትዮጵያ፣ 2016 የባክቴሪያ uropathogen፣ ፀረ-ተህዋስያን ተጋላጭነት እና ተያያዥነት ያላቸው የሽንት ቧንቧ ኢንፌክሽን ምክንያት ነፍሰ ጡር እናቶች የቅድመ ወሊድ ህክምና

በመጀመሪያ በዚህ ጥናት ውስጥ በመሳተፍ ለምታደርጉት ትብብር እና ፈቃድ በቅድሚያ ላመሰግናችሁ እወዳለሁ። ስለ ጥናቱ አጠቃላይ መረጃ ሲነበብ እባክዎን ያንብቡ ወይም ያዳምጡ። ጥናቱን በተመለከተ ማንኛውም ጥያቄ ካለዎት እባክዎን በነጻነት ይጠይቁ።

ዳራ መረጃ

ባክቴሪያዎች የሽንት ቱቦ ውስጥ ገብተው ሲያድጉ ወደ ኢንፌክሽን እና የሽንት ቱቦ ኢንፌክሽን ሊያስከትሉ ይችላሉ. በሆርሞን ለውጦች ምክንያት የሽንት ስርዓት እና በሽንት ቱቦ እርግዝና ላይ አካላዊ ለውጦች በሽንት ቧንቧ ኢንፌክሽን የመያዝ እድልን ይጨምራሉ. 10% የሚሆኑት ነፍሰ ጡር እናቶች በእርግዝና ወቅት የሽንት ቧንቧ ኢንፌክሽን ያጋጥማቸዋል. በእርግዝና ወቅት፣ ውስብስቦችን ለማስወገድ በተቻለ ፍጥነት የሽንት ቧንቧ ኢንፌክሽን ን ማወቅ እና ማከም በጣም አስፈላጊ ነው። የትኞቹ አንቲባዮቲኮች ኢንፌክሽኑን የሚያስከትሉ ተህዋሲያንን ለማስወገድ በተሻለ ሁኔታ እንደሚሠሩ ለማወቅ የተጋላጭነት ምርመራ ይደረጋል። ይህ መረጃ የተመረጠው አንቲባዮቲክ የ ሽንት ቧንቧ ኢንፌክሽን ን የሚያመጣውን ተህዋሲያን ውጤታማ በሆነ መንገድ እንደሚዋጋ ዋስትና ስለሚሰጥ ስለ የተሻለው እርምጃ በመረጃ ላይ የተመሰረተ ውሳኔ ለማድረግ አስፈላጊ ነው።

የጥናቱ ዓላማ

የጥናቱ ዓላማ በጥቁር አንበሳ ስፔሻላይዝድ ሆስፒታል እና በልደታ ዳግም ህዳሴ ጤና ጣቢያ አዲስ አበባ፣ ኢትዮጵያ፣ በቅድመ ወሊድ ክትትል ላይ በሚገኙ ነፍሰ ጡር እናቶች ላይ ያለውን የባክቴሪያ uropathogen፣ ፀረ ተህዋሲያን ተጋላጭነት እና ተያያዥ ምክንያቶችን ለማወቅ ነው።

የተሳታፊዎች ጥቅሞች

የጥናት ተሳታፊዎች በዚህ ጥናት ላይ ለመሳተፍ የገንዘብ ማበረታቻዎች ወይም ሌሎች ማበረታቻዎች አይኖራቸውም። ይሁን እንጂ ውጤቱ ለሐኪማቸው ሕክምና ወይም ምክር ለማግኘት ይሰጣል። ከሁሉም በላይ ይህ ጥናት ለወደፊት እና

ለቀጣይ ሀገር አቀፍ ጥናት መረጃ ወይም መረጃ ለማቅረብ እና ለጤና ፖሊሲ አውጪዎች የጤና ፕሮግራሞችን ለማዘጋጀት አስተዋፅኦ ያደርጋል.

አደጋዎች እና ውስብስቦች

በጥናቱ ላይ ለመሳተፍ ተሳታፊዎች 10ml ንጹህ የተያዙ መካከለኛ ጅረት የሽንት ናሙና ከመሰብሰብ በቀር ምቹ ምቹ በሆነ ሰፊ የአፍ ሽንት ኩባያ ውስጥ ምንም አይነት ስጋት የለም።

ሚስጥራዊነት

የተሳታፊዎችን መረጃ ምስጢራዊነት ለመጠበቅ, ስሙ አይሰጠውም እና ናሙናዎቹ ኮድ ይደረጋሉ. ተሳታፊዎች በማናቸውም ጊዜ ከጥናቱ ለማቆም ወይም ለማቆም አይከለከሉም. ምንም የግል መረጃ ለሶስተኛ ወገን አይገለጽም ወይም በዚህ ጥናት ውስጥ በማንኛውም ዘገባ ላይ አይታይም።

የዋና ተመራማሪ ማረጋገጫ

ለምርምር ፕሮጀክቱ ሳይንሳዊ ስነ-ምግባር እና ቴክኒካል ስነምግባር እና ለምርምር ፕሮጀክቱ ባለድርሻ አካላት የሂደት ሪፖርቶችን የማቅረብ ሀላፊነት እንደምወስድ ለማረጋገጥ ፊርማዬን ከዚህ በታች አስቀምጬላችኋለሁ።

ሶፊያ ሸምሱ(ዋና ተመራማሪ):ፊርማ:_____ ቀን:_____

ማሳሰቢያ፡ ስለዚህ ጥናት ማንኛውም አይነት ጥያቄ ካሎት አሁኑኑ ወይም በማንኛውም ጊዜ በጥናቱ ወቅት በመደወል ለመጠየቅ ነፃነት ይሰማዎ፡-

የ ዋና ተመራማሪ አድራሻ፡- ሶፊያ ሸምሱ የህክምና ላቦራቶሪ ሳይንስ ትምህርት ክፍል፣ የጤና ሳይንስ ኮሌጅ፣ አዲስ አበባ ዩኒቨርሲቲ፣ አዲስ አበባ፣ ኢትዮጵያ

ኢሜል፡ sophiyashemsu11@gmail.com; ስልክ፡ +251913151036

3.2 በመረጃ የተደገፈ ስምምነት

የታካሚ መታወቂያ-

በጥቁር አንበሳ ስፔሻላይዝድ ሆስፒታል እና በልደታ ዳግም ህዳሴ ጤና ጣቢያ አዲስ አበባ፣ ኢትዮጵያ፣ 2016 በቅድመ ወሊድ ክትትል ላይ በሚገኙ ነፍሰ ጡር እናቶች ላይ ያለውን የባክቴሪያ የዩሮፓቶጅን፣ ፀረ-ተህዋሲያን ተጋላጭነት ሁኔታ እና የሽንት ቧንቧ ኢንፌክሽንን ተያያዥ ምክንያቶች ለማወቅ ስለታቀደው ጥናት ተነግሮኛል።

የጥናቱ ዓላማ እና አተገባበር በአጭሩ ተብራርቶልኛል። በተጨማሪም፣ መረጃን የመከልከል፣ የመተባበር እና የማቋረጥ መብቴን መጠቀም እንደምችል በደንብ ተነግሮኛል፣ እና የትኛውም ድርጊቶቼ በአጠቃላይ የጤና አጠባበቅ ላይ ምንም ተጽእኖ አይኖራቸውም።

ስለዚህ ለተጠቀሰው ጥናት ሽንቴን ለመስጠት ለተመራማሪው በፈቃደኝነት በመረጃ ላይ የተመሰረተ ስምምነት ለመስጠት የተስማማሁት ሁኔታውን በሚገባ በመረዳት ነው። ናሙናው ለ ዩሮፓቶጅን እንደሚሞከር ተስማምቻለሁ. ስለ ፕሮጀክቱ ጥያቄዎችን ለመጠየቅ እድል አግኝቻለሁ እናም በምረዳው ቋንቋ እርካታዬን አግኝቻለሁ። እንዲሁም የሽንት ተውሳክ የዩሮፓቶጅንን ትንተና ውጤት ለጤና ተቋሙ እንደሚሰጥ እና ከፈለግኩ መረጃውን እንደምጠይቅ ተነግሮኛል።

እኔ _____ የተጠየቀውን መረጃ እና የዚህ ጥናት ናሙና ለመስጠት በዚህ ፈቃድ ሰጥቻለሁ።

የተሳታፊ ኮድ፡_____ ፊርማ፡ _____ ቀን፡ _____

3.3 በጥቁር አንበሳ ስፔሻላይዝድ ሆስፒታል እና በልደታ ዳግም ህዳሴ ጤና ጣቢያ አዲስ አበባ፣ ኢትዮጵያ፣ 2016 በቅድመ ወሊድ እንክብካቤ ላይ በሚገኙ ነፍሰ ጡር እናቶች ላይ የባክቴሪያ ዩሮፓቶጅንን፣ ፀረ ተሕዋስያን ተጋላጭነት እና ተያያዥ ምክንያቶችን ለማወቅ መጠይቅ

ሀ. የታካሚ መታወቂያ

1. የታካሚ ስም ----------

2. የላቦራቶሪ መታወቂያ----------

3ታካሚ መታወቂያ----------

4. ክሊኒካዊ ምርመራ ----------

ለ. ሶሺዮ-ስነ-ሕዝብ ምክንያቶች (በታካሚው የተሞላ)

1.እድሜ -

ሀ.10-14 ለ.15-19 ሐ.20-30 መ.30-40 ሠ.40-50 ረ.>50

2.በአሁኑ ጊዜ የትምህርት ደረጃዎ ስንት ነው?

ሀ. ማንበብ እና መጻፍ አልተቻለም

ለ. ማንበብ እና መፃፍ ብቻ

ሐ. የመጀመሪያ ደረጃ ሙሉ

መ. የሁለተኛ ደረጃ ትምህርት ቤት

ሠ ዲፕሎማ እና ከዚያ በላይ

ሐ. የሕክምና እና የእርግዝና ዝርዝሮች

1.ማንኛውም ቅድመ-ነባር የሕክምና ሁኔታዎች,አለ?

አ.አዎ ለ.አይ

1.1.እሺ ካለ

ሀ.የስኳር በሽታ ለ.የ የደም ግፊት ሐ. የደም ማነስ መ. ሌሎች

2.በግብረ ሥጋ ግንኙነት የሚተላለፉ ኢንፌክሽኖች ,አለ?

አ.አዎ ለ.አይ

3.የእርግዝና ደረጃ

ሀ.1ኛ አጋማሽ ለ .2 ኛ አጋማሽ ሐ .3 ኛ አጋማሽ

4የእርግዝና ብዛት

ሀ.የለም ለ.1 ሐ.2 መ.3 ሠ.4 ናከአራት በላይ

5. የወሊድብዛት

ሀ.የለም ለ.1 ሐ.2 መ.3 ሠ.4 ናከአራት በላይ

6. የሽንት ቱቦ እርግዝና የቀድሞ ታሪክ አለ?

ሀ .አለ ለ.የለም

6.1እሺ ካለ ስንት ጊዜ ተከሰተ

ሀ. 1-5ቀናት ለ. 6-10 ቀናት ሐ.11-15ቀናት መ. 16-20 ቀናት ሠ.>20ቀናት

6.1. የሽንት ቧንቧ ኢንፌክሽን ቆይታ ነበረ

ሀ. ባለፈው አንድ ዓመት ውስጥ

ለ. ባለፉት ሁለት ዓመታት

ሐ.ባለፉት ሶስት አመታት

6.2. ከዚህ በፊት የታከም የሽንት ቧንቧ ኢንፌክሽን አለ

ሀ .አለ ለ.የለም

መ.የንፅህና አጠባበቅ ልምዶች

1.የብልት አካባቢዎን በቀን ስንት ጊዜ ይታጠቡ?

ሀ.አንድ ጊዜ ለ. ሁለት ጊዜ ሐ . ሶስትጊዜ መ. በሽኑ ጊዜ ሁሉ ሠ.በጭራሽ

2. ሽንት ቤት ከተጠቀሙ በኋላ ምን ዓይነት የማጽዳት ዘዴዎች ጥቅም ላይ ይውላሉ?

ሀ. በውሃ መታጠብ

ለ..ደረቅ ጥጥ መጠቀም

ሐ. ለስላሳ

መ. ምንም መጠቀም

3.የግብረ ሥጋ ግንኙነት ድግግሞሽ?

a.በየቀኑ ለ ሁለት ጊዜ በሳምንት ሐ.ሳምንት መ.. በወር ሁለት ጊዜ ሠ በወርአንዴ

ሠ.የፈሳሽ መጠን፡

1.የሚወስዱት ፈሳሽ አይነት

ሀ.ውሃ

ለ. ቡና

ሐ. አልኮል መጠጣት

መ. ማንኛውም ልዩ መጠጦች

2. የነዚህ አማካይ ዕለታዊ ፈሳሽ መጠን

ሀ.0-1ሊ ለ..2-3ሊ ሐ .3-4ሊ መ.>5L

ረ.የእናናር ሁኔታዎች

1.ማጨስ ልማድ አለህ?

ሀ .አለ ለየለም

2. አካላዊ እንቅስቃሴ ታደርጋለህ ?

ሀ .አለ ለ.የለም

12. Declaration

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been duly acknowledged.

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Date of submission:_____

This thesis has been submitted with our approval as advisors.

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Signature: _____

Date: _____

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Advisor: Mr.DessieAbera(MSc, PhD candidate)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Dr.Eskinder Kebede(MD Associate professor of Obstetrics and Gynecology Reproductive Endocrinology and Infertility subspecialist)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Mr.zeleke Ayenew

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.