ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES,

SCHOOL OF ALLIED HEALTH SCIENCES
DEPARTMENT OF MEDICAL LABORATORY SCIENCES

PREVALENCE AND DRUG SUSCEPTIBILITY PATTERN OF BACTERIA ASSOCIATED WITH URINARY TRACT INFECTION

AMONG HIV POSITIVE PATIENTS ATTENDING ALERT

CENTER, ADDIS ABEBA ETHIOPIA

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List of Abbreviation

AIDS	Acquired immune deficiency syndrome
ALERT	All Africa Leprosy & TB Rehabilitation Training Center
ARTC	Attending antiretroviral therapy clinic
ART	Antiretroviral therapy clinic
ASB	Asymptomatic bacteriuria
ATCC	American Type Culture collection
BA	Blood agar
CFU	Colony Forming Unit
CLED	Cysteine Lactose Electrolyte Deficient
Cons	Coagulase negative Staphylococci
HAART	Highly active antiretroviral therapy
HIV	Human Immunodeficiency Virus
MAC	MacConkey agar
MA	Micro albuminuria
MDR	Multi-Drug Resistant
MSU	Midstream urine
OIS	Opportunistic infections
UTI	Urinary Tract Infection
VCT	Voluntary counseling and Testing

Abstract

Background: Urinary tract infections (UTI) are one of the most common types of bacterial infections in humans occurring both in the community and the health care settings. UTI rank high amongst the most common causes that compel an individual to seek medical attention. HIV/AIDS is one of the greatest public health crisis faced by the global community without a complete cure. UTI represents a considerable health problem amongst HIV infected patients.

Objective; to determine the prevalence and drug susceptibility pattern of bacteria associated with UTI among HIV positive patients.

Methods: A cross sectional study was conducted in ALERT Center Addis Ababa Ethiopia from September to January 2015 among 165 adult HIV patients. Midstream urine (MSU) was collected from the study participants with sterile wide mouthed urine cups. Urine samples were inoculated in to Blood agar, MacConkey and Cysteine lactose electrolyte deficient and biochemical tests were performed to identify isolates. Drug susceptibility pattern of isolates was determined using the disc diffusion techniques.

Data were analyzed using SPSS version-20 software package. Chi – square (X^2) test was used to compare categorical data and to compare associations between proportions. Differences were considered significant when the p-values were < 0.05 at 95% confidences limit.

Result: Among the total 165 study participants 114 were females and the remaining 51 were males and the mean of age was 37.9. The overall prevalence of UTI was 15.7. High bacterial isolates were found in asymptomatic study participants than symptomatic HIV positive patients. Among Gram negative isolates *Escherichia coli* were the leading cause of UTI followed by Gram positive isolates *Staphylococci aureus*. Most bacterial isolates were resistant to Amp, TE, P and SXT and Gentamicin were susceptible for all isolates.

Conclusion: the prevalence of UTI was high, and both Gram-negative and Gram positive organisms were causes of UTIs. In this study the chance of acquiring UTI was higher among females than males. UTI prevalence was also high among study participants those have previous history of catheterization and UTI. *E. coli* were the most predominant organisms followed by *Staphylococci aureus* and most of the bacterial isolates were sensitive to Gentamicin, Ceftriaxone, Ciprofloxacin, Norfloxacine, Oxacilin and Nitrofurantion. Multi-drug resistance bacteria were common.

Key words: Asymptomatic UTI, Symptomatic UTI, urinary tract infection, HIV/AIDS, Ethiopia

1. INTRODUCTION

1.1 Background

Urinary tract infections are one of the most common types of bacterial infections in humans occurring both in the community and the health care settings. The urinary tract includes the organs that collect and store urine and release it from the body and these organs include the kidneys, ureters and bladder, urethra and accessory structures. UTI is the infection of any part of the urinary tract. Any part of these structures can become infected but bladder and urethra infections are the most common. The bladder infection is known as cystitis while that of the kidneys is known as pyelonephritis and it is more serious infection than the other structures. (1).

Urinary tract infection is one of the significant illnesses that increases disease burden on community. UTI is not only common nosocomial infection but an important source of morbidity in community as well. Main cause of UTI is obstruction of urinary tract including stone disease, pelvic-ureteric junction obstruction, benign prostate hyperplasia, urethral strictures and neuropathic bladder (2).

Urinary tract infection involves bacterial invasion and multiplication in the organs of the urinary tract system and it is manifested as at least 100,000 organisms per millimeter of urine in symptomatic and asymptomatic patient (3).

Asymptomatic UTI, also known as asymptomatic bacteriuria (ASB), is defined as the presence of significant bacteria ($\geq 10^5$cfu/ml) in an individual's urine without signs and symptoms of UTI (4). ASB is common with varying prevalence by age, sex, sexual activity and the presence of genito-urinary abnormalities. Women with ASB are more likely to experience symptomatic UTI than those without ASB (7).

Urinary tract infections are "uncomplicated" when they occur in a normal urinary tract with no structural, functional or underlying host illness to account for the infection, or "complicated" when an underlying abnormality is thought to have enabled the infection to occur (8).

The AIDS caused by HIV is the most important public health problem of 20th century. The infection is alarming due to the unique pathogenesis of the virus that decreases the CD4 cells, signaling the emergence of the opportunistic infections, in the host (11).

People living with HIV are likely to be more predisposed to UTI due to the suppression of their immunity and women in this category tend to get them more often due to the nature of their anatomy. Bacterial infections are a common cause of morbidity and mortality in HIV positive individuals (14).

The role of the virus in predisposition to infections of the urinary tract, naturally HIV predisposes to multi system/organ infection (18).

More than 90 % of UTIs are due to enteric Gram positive and Gram negative bacteria including *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis and Klebsiella pneumoniae*. These microbes have been implicated in some opportunistic infections caused by pathogenic bacteria in HIV/AIDS patients. (10)

1.2Statement of the problem

Worldwide, about 150 million people are diagnosed with UTI each year, costing the global economy in excess of 6 billion US dollar. UTIs are considered to be the commonest bacterial infections and account for a significant part of the workload in clinical microbiology laboratories. It also constitutes the most common infection in HIV patients in many hospitals

UTI is the most common health care associated group of bacterial infections affecting humans in world. UTI occur in both the community and hospital settings as well as in all age groups and in both genders. Hence it is considered as the major cause of morbidity in both the hospital and community settings and affecting both out and in patients. Worldwide, about 150 million people are diagnosed each year with UTIs, costing in excess of 6 billion dollars (1).

UTI represents a considerable health problem among HIV infected patients, where the incidence is between 5.0% and 20.0% (12).

There is evidence that bacteriuria is more common as HIV disease progresses. Studies have shown that the incidence of UTIs is greater among men and women infected with HIV than among non-infected. Some studies have indicated that the risk of bacteriuria and UTI may be increased in HIV-infected patients and is inversely related to CD4+ lymphocyte counts. UTI in HIV-positive patients tends to recur, requiring longer treatment and it is suggested that treatment should be culture-specific (12).

The management of other non HIV associated diseases in HIV patients has become increasingly important. In these regard UTI co infection with HIV is becoming a major challenge and confers high

costs. Less study has been done on UTI of HIV/AIDS patients, especially, in the African continent. There are very limited data available in Ethiopia on UTI in HIV-infected subjects. There were only two published information in Gonder and Jimma University in 2013. The Study conducted in Gonder Ethiopia in 2013 indicated that Patients with AIDS have UTI more frequently than HIV positive patients without AIDS. The reported incidence of bacteriuria in a patient with AIDS was 7%-50 %.(10). The study conducted in Jemma in 2013 also showed Of 467 examined urine samples, 56 (12%) had significant bacterial growth. Forty-six (12.5%) of the cases were ART users and 10 (10%) were nonusers. *E. coli* was the predominant isolate in both ART users 25 (54.3%) and nonusers 6 (6%).

So this cross sectional study was designed to determine the prevalence and antibiotic sensitivity pattern of bacteria associated with UTI among patients attending anti-retroviral ART clinic of ALERT Center from September 2014 to January 2015, Ethiopia.

1.3 Significance of the study

The research done by Alemu *etal* and Serkadis *etal* was done in 2013 and 2014 and these are the only studies that directly related to HIV with UTI and no study conducted in this study site as well as it can be a base line for further studies and it can Provide updated information. This study will determine the etiologic agent and antimicrobial susceptibility pattern of bacterial uropathogens among HIV positive patients in ALERT Center.

In view of the fact that individuals infected with HIV are immunocompromised, UTI in them can lead to other life threatening diseases, and as such, rapid and accurate detection of uropathogens from urine samples of HIV patients is practical to enhance their health status.

This study also alarming phenomenon might be inappropriate and incorrect administration of antimicrobial agents in empiric therapies and lack of appropriate infection control strategies, which can cause a shift to increase prevalence of resistant organism in the community and also it is informative for physicians about the most common resistant drugs.

2 LITERATURE REVIEW

2.1 Epidemiology of UTI in HIV positive individuals

Worldwide the disease burden of UTI is estimated to be 150 million cases annually, with different types of UTI posing serious health problems. The financial burden is also understandably enormous with an estimated annual cost of community-acquired UTI of approximately $1.6billion in United States of America (32).

Statistics in the United States estimate the incidence of HIV-associated UTI to be 3.5%–12.0%. If these were to be extrapolated to Sub-Saharan Africa, with an estimated 22.5 million people infected with HIV, 788,000–2.7 million people would be expected to have HIV-associated UTI (17).

In 1995 the study done in London on the incidence of symptomatic UTI in HIV seropositive patients and the use of co-trimoxazol as prophylaxis against *Pneumocystis carinii pneumoniae* showed the occurrence of UTI in 10 (5.7%) of 175 patients, with an incidence of 1.49 per hundred patients. UTI were significantly more common in patients with AIDS or a CD4 lymphocyte count below 200mm^3(or both) when compared to those without AIDS and a CD4 lymphocyte count above 200mm^3 (5.4 vs. 0.5) (29).

In Nigeria a study was carried out to detect the presence of HIV-1 & -2 co-infections with Multi-Drug Resistant (MDR) uropathogens in Port Harcourt, the result showed that urinary tract infection (UTI) (85.4%), and UTI/HIV (60.0%). Among HIV-negative subjects, 28(43.7%) had UTI while of the 50 HIV-positive subjects, 42(84.0%) had UTI. Females had the highest prevalence of HIV (78.0%) and UTI (70.0%) compared to males (2).

Another study done in Nigeria in 2013 among ART patients reported as *S. aureus* was the most prevalent isolated organism (n=34) followed by *K. pneumoniae* (n=17), *E. coli* (16) and *P. mirabilis* (n=8) (3).

A study done on Nigerian HIV positive children showed asymptomatic bacteriuria in 10.3% of the children. Prevalence of asymptomatic bacteriuria was found to be high in HIV infected children especially those in the school age group 6-17 years. The isolated bacteria were *Escherichia coli*, *Klebsiella* species and *Staphylococcus aureus,* accounting for 62%, 25% and 12.5% respectively (4).

A study in Ghana in 2012 also reported on gastrointestinal and urinary tract pathogenic infections among 500 HIV seropositive and 300 HIV sero-negative patients. Most, 60 (86%) out of 70, of the

urinary tract infection among the HIV seropositive patients was due to being the most predominant isolate, 28 (47%) (25).

A study was conducted in Ethiopia from 2012 in adult HIV patients. Overall prevalence of UTI in HIV patients was 10.7% with 75.6% occurrence in females. *Escherichia coli* was the most frequently isolated pathogen 23 (56.1%), followed by *Enterobacter* spp 6 (14.6%), *S. aureus* 6 (14.6%), Coagulase negative Staphylococci (CONS) 4 (9.8%) and *K. pneumoniae* 2 (4.9%) (10).

A study in Ethiopia in 2014 also reported on 367 ART users and 114 nonuser patients attending ART clinic. Results of 467 examined urine samples, 56 (12%) had significant bacterial growth. Forty-six (12.5%) of the cases were ART users and 10 (10%) were nonusers. *E. coli* was the predominant isolate in both ART users 25 (54.3%) and nonusers 6 (6%). Majority of the bacterial isolates were from females (58).

Study was performed in pokhara hospital in Nepal in 2012 on incidence of UTI among the patients visiting the hospital reports among 500 urine samples 116(23.2%) showed significant growth while 384(76.8%) samples showed no significant growth. Among 116 isolates *E.coli* was 50% accounted followed by *S.aureus* 13.79%, *Klebsiella* spp 10.34%, *P.aeruginosa*, 7.75%, *Enterobacter* spp (6.03%), *proteus* spp 5.17%, *citrobacter* spp 3.44%, *morganilla morganii* 1.72%, *S.saprophytics* 0.86% and *Entrococcus* spp 0.8% (63)

A study conducted in Saudi Arabia in 2013 showed *E.coli* as the major organism causing UTI 19.5% followed by *Staphylococci* 13.7%. *E.Coli* had been well known as the classical number one causative agent of UTI whereas *Staphylococci* were usually thought of as a rare cause of UTI (54).

A study conducted in India in 2013 showed UTI as one of the most important causes of morbidity in the general population, and is the second most common cause of infection, in spite of wide availability of antimicrobials. Resistance to commonly prescribed antibiotics for UTI is an expanding global problem both in developed as well as developing countries. Among 952 cases, 538 were positive. The maximum numbers of cases (66.9%) were isolated from females and Majority of the isolates was Gram negative bacteria (78.81%). Among this, *E.coli* accounted for 52.04% of the total cases followed by *Klebsiella species* (12.6%) and *Pseudomonas* species (7.62%). Out of 106 Gram positive organisms, *Enterococcus species* accounted for 8.17% of the cases, followed by *Staphylococcus aureus* (6.69%) and *Coagulase negative Staphylococcus* (4.83%), (5).

Another Nigerian study done on urogenital tract infection in asymptomatic male patients with infertility in 2012 showed that 164 (50.8%) infection rate was recorded. The dominant uropathogens detected or isolated were *Staphylococcus aureus* (14.0%), *Chlamydia trachomatis* (11.4%), *E.coli* (4.3%), *Micoplasma genitalium* (4.0%) and *K.aerogenes* (4.0%). Others were *Staphylococcus saprophyticus*, *Pseudomonas aeruginosa*, *Proteus mirabilis* with (2.7%) each respectively, *P. vulgaris, and Treponema pallidum* (2.1%), *Schistosoma haematobium* (0.9%) *Wulchereria bancrofti* (0.3%) (34).

The study conducted on 399 adult non-pregnant women attending Mulago hospital assessment Centre in Uganda showed, 40 pure significant bacterial growths ($\geq 10^5$ colony forming units (cfu)/ml of urine) and the isolates included *Escherichia coli*, 23 (57.5%), *Staphylococcus aureus,* 9 (22.5%), *Enterococci* spp, 6 (15%) and *Klebsiella pneumoniae*, 2 (5.0%) (8).

In 2013 Another study conducted in 2852 Iranian patients showed, 230(8.06) positive urine cultures of which 204 (88.69%) were females and the remaining 26 (11.3%) were males. 180 (83.17%) cases of isolated bacteria were Gram negative bacilli while 50 (21.73%) cases were Gram positive cocci (30).

The study conducted in Ethiopia in 2014 the prevalence and anti-biogram of bacterial isolates from UTI at Dessie Health Research Laboratory, showed that the male to female ratio of the patients was 1:1.96. Of the total 1404 samples, 319 (22.7%) were culture positive. *Escherichia coli* was the dominant isolate (63.6%) followed by *Klebsiella* spp 8.5%) and *Peroteus* spp. (8.2%) (39).

Across sectional study was conducted in Ethiopia in 2011 at Gandhi Hospital for ten months with 125 asymptomatic pregnant women in the age group of 15-35 years. Significant bacteriuria was seen in 21 (16%) of the women. The prevalence of bacteriuria showed a rise with increasing maternal age and increasing gestational period. The most common isolates were *Klebsiella pneumoniae* and *Staphylococcus aureus* (28%) each (40).

Study conducted in Hawassa Referral Hospital in 2013, among the Ninety urine specimens‘ analyzed *Escherichia coli* 35.5% was isolated from patients suspected for urinary tract infections (41).

Study carried out on urinary bacterial profile and antibiotic susceptibility pattern among pregnant women in North West Ethiopia in 2012 the result showed that out of 367 pregnant women, 37 were symptomatic and the rest 330 were asymptomatic. Bacteriological screening of urine samples revealed growth of bacteria in 8.5% (7/37) and 18.9% (28/330) for symptomatic and asymptomatic pregnant

women respectively with overall prevalence of 9.5%. The most common isolates detected were *E.coli* (45.7%) followed by coagulase negative *Staphylococcus* (17.1%) and *S.aureus* (8.6%) (48).

In Northwest Ethiopia, the University of Gondar Teaching Hospital conducted a study in pregnant women in 2012. The predominant bacterial pathogens were *Escherichia coli* (47.5 %) followed by coagulase-negative *staphylococci (*22.5 %), *Staphylococcus aureus* 10 %, and *Klebsiella pneumoniae* 10 % (47).

A cross sectional study was conducted in Tikur Anibessa specialized university Hospital Addis Ababa Ethiopia in 2012 among 413 diabetic patients, 181 (43.8%) were males and 232 (56.2%) were females. Nine (13.6%) of the symptomatic diabetic patients had bacteriuria compared with 36 (10.4%) of asymptomatic diabetes patients. The overall prevalence of UTI in the diabetic patients was 45 (10.9%). The predominant isolates were *Escherichia coli* and *Klebsiella pneumoniae* 6% and 28% followed by 2% and 6% in symptomatic and asymptomatic diabetic patients, respectively (28).

2.2 Risk factor for Urinary Tract Infection

Risk factors for UTIs include: sexual intercourse and past history of childhood UTIs, Prior antibiotic use may also increase the risk of UTI by altering the normal perianal flora. In postmenopausal women, mechanical and/ or physiologic factors that affect bladder emptying are strong risk factors for UTI. Other factors associated with UTI include urine incontinence; cystocele and large post void residual volumes, atrophic vaginitis, and a history of UTIs before menopause (43).

Tab le 1. Risk factors for complex UTI

Patient Factors	Male child <12 years, Pregnancy, Male >50 and immunosuppressed (diabetes, renal failure)
Structural/functional factors	Presence of indwelling catheter, chronic retention bladder outflow, obstruction, Polycystic kidneys upper tract calculi and Bladder stones
Bacterial factors	Nosocomial/ multi resistant organisms

Urinary tract infection affects all age groups, but women are more susceptible than men, due to short urethra, absence of prostatic secretion, pregnancy and easy contamination of the urinary tract with fecal flora (44).

Risk factors for UTI are sexual activity, chlamydia infection, lower socioeconomic status, history of recurrent UTI, diabetic mellitus, sickle cell disease (renal damage) and anatomic or functional genitourinary tract abnormalities. Pregnant women with untreated ASB in early pregnancy have a 20-40% risk of developing a symptomatic UTI, usually in the form of pyelonephritis, in later pregnancy (45).

2.3 Urinary Tract Infection and Human Immunodeficiency Virus Association

Human Immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) now ranks as one of the leading cause of morbidity and mortality in both children and adults, and increasingly more persons are either infected or dying from it (10).

Opportunistic infection (OIS) causes substantial morbidity and hospitalizations that necessitate toxic and expensive therapies and shorten the survival of people with HIV infection. Decrease in CD4+ count is at least partially responsible for the profounder immunodeficiency that leads to various OIS in HIV infected persons (13).

UTIs which accounts for a significant proportion of patients daily hospital visits is mainly caused by pathogenic bacteria, and their frequency are gradually increasing amongst HIV infected patients as an opportunistic infection. The emergence of HIV / AIDS has greatly increased the incidences of OIS especially UTIs amongst infected patients (3).

Some studies have shown varied prevalence rates of various urinary abnormalities in HIV infected adult population. The prevalence of hematuria in HIV infected adults was 3.3%, inability to concentrate urine was found in 16% of the subjects (15).

Opportunistic pathogens of bacterial, fungal, parasitic and viral origin immensely contribute to mortality and morbidity in AIDS patients. Microbiologic analysis of clinical specimens from this group such as cerebrospinal fluid, sputum, stool, blood have shown an expanded spectrum of pathogens however, less work has been done on urine samples of HIV/AIDS patients, especially, in the African continent (16).

2.4. Antibiotic Susceptibility Pattern

The extensive and inappropriate use of antimicrobial agents has invariably resulted in the development of antibiotic resistance which, in recent years, has become a major problem worldwide. In patients with suspected UTI, antibiotic treatment is usually started empirically, before urine culture results are

available. To ensure appropriate treatment, knowledge of the organisms that cause UTI and their antibiotic susceptibility is mandatory (21).

The prevalent pathogens of UTIs have been found to be resistant to most chemotherapeutic agents; the antimicrobial susceptibilities of these pathogens are highly predictable. Development of resistance to these antimicrobial agents in UTI cases will therefore affect future treatment and management of the infection with these drugs (27).

Adequate treatment and control of these conditions need a good knowledge of the causative bacterial species their susceptibility and resistance pattern to antimicrobial agents. Majority of the treatments are started empirically, so the knowledge of the organisms, their epidemiological characteristics and their antibacterial susceptibility is therefore mandatory. Data obtained are essential to optimize the treatment and avoid the emergence of bacterial resistance, which is responsible for the increasing number of therapeutic failure. (27).

A study done in Nigeria in 2010 of 500 HIV positive patients and 500 control groups found the most common organisms isolated were *E.coli* in both groups. Most of the urinary bacterial isolates from both groups were highly sensitive to Ceftazidime (95%) and ciprofloxacin (88%). There was generally low sensitivity of bacterial isolates to most antibiotics in common use such as co-trimoxazole (40%), tetracycline (25%), chloramphenicol (33%) and ampicillin (40%) (16).

Another study in Nigeria in 2006 of 257 sample size showed that *Escherichia coli* and *Klebsiella* species were 100% sensitive to ofloxacin, and 100% and 66.7% to ciprofloxacin respectively but completely resistant to Cotrimoxazole, amoxicillin and Clavulanic-acid potentiated amoxicillin (18).

A study in Nigeria in 2010 showed that *E.coli* and *S.aureus* were the common isolates Ciprofloxacin, Ceftriaxone and Augmentin were found to be the most effective antibiotics against the urinary isolates (31). A study in Nigeria in 2013 of 315 asymptomatic bacteriurias among secondary school students *S.aureus* and *E.coli* the most predominant bacteria were for Nitrofurantion and pefloxacin the most active antibacterial agent (37).

Another study in Brazil in 2012 showed that *E. coli* was responsible for 98 (76.56%) cases of significant bacteriuria; 34 (34.69%) were resistant to trimethoprim-sulphsmethsoxazole, and 21 (21.42%) to fluoroquinolones (36)

A study in India in 2009 from 75.46% of HIV reactive and 24.54% of HIV non-reactive patients were culture positive. About (36.48%) were *E. coli*, (31.10%) were *Pseudomonas aeruginosa* and (24%) were *Klebsiella* spp. The antibiogram of selected urinary isolates in HIV reactive as well as HIV non-reactive patients indicates that urinary isolates in HIV reactive patients were more drug resistant than HIV non-reactive patients (11).

3 OBJECTIVES OF THE STUDY

3.1 General objective

To determine the prevalence and drug susceptibility pattern of bacteria associated with UTI among HIV positive patients attending ALERT Center, Addis Ababa Ethiopia.

3.2 Specific objectives

- To determine bacteria associated with symptomatic and asymptomatic UTI among HIV patients
- To determine drug susceptibility pattern of isolates

3.3 Hypothesis

Bacterial isolates and its AST in UTI among HIV patients are similar with previous studies done in Ethiopia.

4 MATERIALS AND METHODS

4.1. Study area

The study was conducted at ALERT center, Addis Ababa, Ethiopia. ALERT center is one of the largest referral and training center that provides services mainly in Dermatology and ART treatment for patients throughout the country. The hospital is located at the City in *Kolfe kernaio* Sub city, Addis Ababa, Ethiopia

4.2. Study Design and Period

A Hospital based cross-sectional study in ALERT Center was conducted from September 2014 to January 2015

4.3. Source population

All HIV positive individual who are attending ALERT Center during the study period were considered as source population for this study.

4.4. Study population

All HIV patients that were examined in ALERT Center ART Clinic either UTI symptomatic or asymptomatic patients during the study period were included in this study.

4.5. Inclusion and exclusion criteria of study participants

The study subjects were recruited prospectively based on clinical examination by clinicians.

4.5.1 Inclusion Criteria

- Males and females that are ≥18 years old and are HIV positive.
- UTI symptomatic and asymptomatic HIV infected patients.
- Patients who were giving consent to participate in the study.

4.5.2 Exclusion Criteria

- Patients who are on antibiotics (either oral or parenteral) for more than two weeks prior to the time of enrolment.

4.6 . Description of variables

4.6.1 Dependent Variables

- Bacterial isolates from urine culture
- Antimicrobial susceptibility pattern

4.6.2 Independent Variables

- Age, Sex, Marital status, educational level, HAART naive or started
- Clinical Sign and Symptoms of UTI
- CD4+cell count
- Cotrimoxazole usage

4.7. Sample size determination

The required sample size was determined by using single population formula considering the following assumptions:

Prevalence = 11% (prevalence of culture proven HIV UTI patients that was previously done in Ethiopia (10)

Sample size, $\frac{(Z\alpha/2)^2 * p(1-p)}{d^2}$ Where:

n= the required sample size Z = Z score for 95 % confidence interval = 1.96,

p = prevalence; 11 (0.11) d = tolerable error =5 %.

1-p=Q= negative proportion There by n = ((1.96)2 x 0.11x (1-0.11))/ (0.05)2=150

10% non-response rate=15

By considering 10% non-response rate; the overall sample size was found to be:

Total study participants=165

4.8. Sampling technique

All consecutive HIV patients were included in the study by convenient technique. Relevant information including; age, sex, source of infection, were gathered on a prepared questionnaires. Laboratory data including urine microscopy, culture and sensitivity results were recorded.

4.9. Methods of data collection

The informed consent was obtained from the study participants by Nurses. The socio demographic data, history of exposure for the possible associated factors, and years of drug taking and other relevant information was collected using structured questionnaire. The participants‘ current CD4 value was taken from their medical records upon which urine sample and other relevant data were collected.

4.9.1 Sample collection

The urine sample collections were performed after adequate explanation by the clinical nurses to the patients and obtaining there consents, a clean catch MSU was collected in sterile, wide mouthed, screw capped container were immediately processed in ALERT microbiology laboratory. The collected urine specimens were divided in to two sterile test tubes; one container for microscope investigation and the other for culture inoculation.

4.10 Laboratory investigation

4.10.1. Microscopy

The samples were then mixed properly in a container and Ten ml of each well–mixed, urine samples were centrifuged at 2000g for 5 minutes. After centrifugation the supernatant were discarded and a drop or two of the sediment placed on the grease free slide, coverslip applied and examined under the microscope using the high power field. Reporting system for microscopic identification was at high magnification for pus cells, red blood cells, epithelial cells, casts, crystals, yeast cells (Annex X).

4.10.2 Culture

Calibrated loop designed to deliver 0.001 milliliter of well mixed un-centrifuged urine was inoculated on to the surface of blood agar, Cysteine Lactose Electrolyte Deficient (CLED) medium and MacConkey agar (MAC). The media were prepared as the manufacturer instruction and incubated at 35^0C-37^0C. After 24-48 hours of incubation, the plates were examined for the presence of colonies (8).

4.10.3 Bacterial Identification

A number of more than 100 colonies per 0.001ml of urine were considered significant. The number of colony forming units (CFUs) was multiplied by 1000 to determine the number of microorganisms per milliliter in the original specimen (8).

Gram negative bacterial identification was done following standard procedures, with use of biochemical tests which included kligler iron agar, Simmon‘s citrate agar, lysine iron agar, urea, motility tests and indol. (38) Were used. Cultures from MacConkey and blood agar were sub-cultured onto nutrient agar for the appropriate biochemical tests to be carried out (Annex III).

Species identification for Gram positive bacteria was carried out using Gram staining reaction, catalase, coagulase, Novobiocin tests and manitol salt agar.

4.10.4 Antimicrobial susceptibility testing (AST)

The antimicrobial susceptibility testing of the isolate were performed by the disk diffusion technique as modified by the Clinical and Laboratory Standard Institute (49). From a pure culture 3-5 selected colonies of bacteria was taken and transferred to a tube containing 5 ml sterile normal saline and mixed gently to make homogenous suspension and the turbidity of the suspension was adjusted to a McFarland 0.5 standard (49). A sterile cotton swab was used to streak the plates and the excess suspension was removed by gentle pressing and rotation of the swab against the inside wall surface of the tube. The swab was then used to distribute the bacteria evenly over the entire surface of Mueller Hinton agar (MHA). The inoculated plates were left at room temperature to dry for 3-5 minutes and a set of 13 antibiotic discs were impregnated on the MHA (49).

The twelve antibiotic disks (Oxoid Ltd, Basingstoke, and Hampshire, UK) include: Ampicillin (AMP) 10µg, Amoxicillin-Clavulanic acid (AMC) 20µg, Ceftazidime (CAZ) 30µg, Ceftriaxone (CRO) 30µg, Ciprofloxacin (CIP) 5µg, Trimethoprim-sulphamethazole (SXT) 25µg, Erythromycin (E) 15µg, Gentamicin (CN) 10µg, Nitrofurantion (F) 300µg, Norfloxacine (NOR) 10 µg, and Tetracycline (TE) 30µg, penicillin (P) 10µg (49).

The plates were incubated in aerobic atmosphere at 37^{o}C for 16-18 hours. Diameters of the zone of inhibition around the disc was measured to the nearest millimeter using a graduated caliper in millimeters, and the isolates were classified as sensitive, intermediate, and resistant according to the standardized table supplied by the CLSI (49)

4.11 Data Quality Assurance

The quality of the data collection process was checked by giving adequate training for data collectors & for improvement of the data collection tool corrective measures was taken accordingly for any gap.

The quality of culture media was tested for sterility and performance, sterility of culture media was checked by incubating overnight at 35-37 ºC without specimen inoculation. Any physical change like cracks, excess moisture, color, hemolysis, dehydration, & contamination was assessed & expiration date was also checked. Standard strains of *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) was used for quality control QC throughout the study for culture and antimicrobial susceptibility test. For Gram staining reagents *S.aureus* (Gram positive) & *E.coli* (Gram negative) was used as QC. Temperature of incubator & refrigerator was monitored daily.

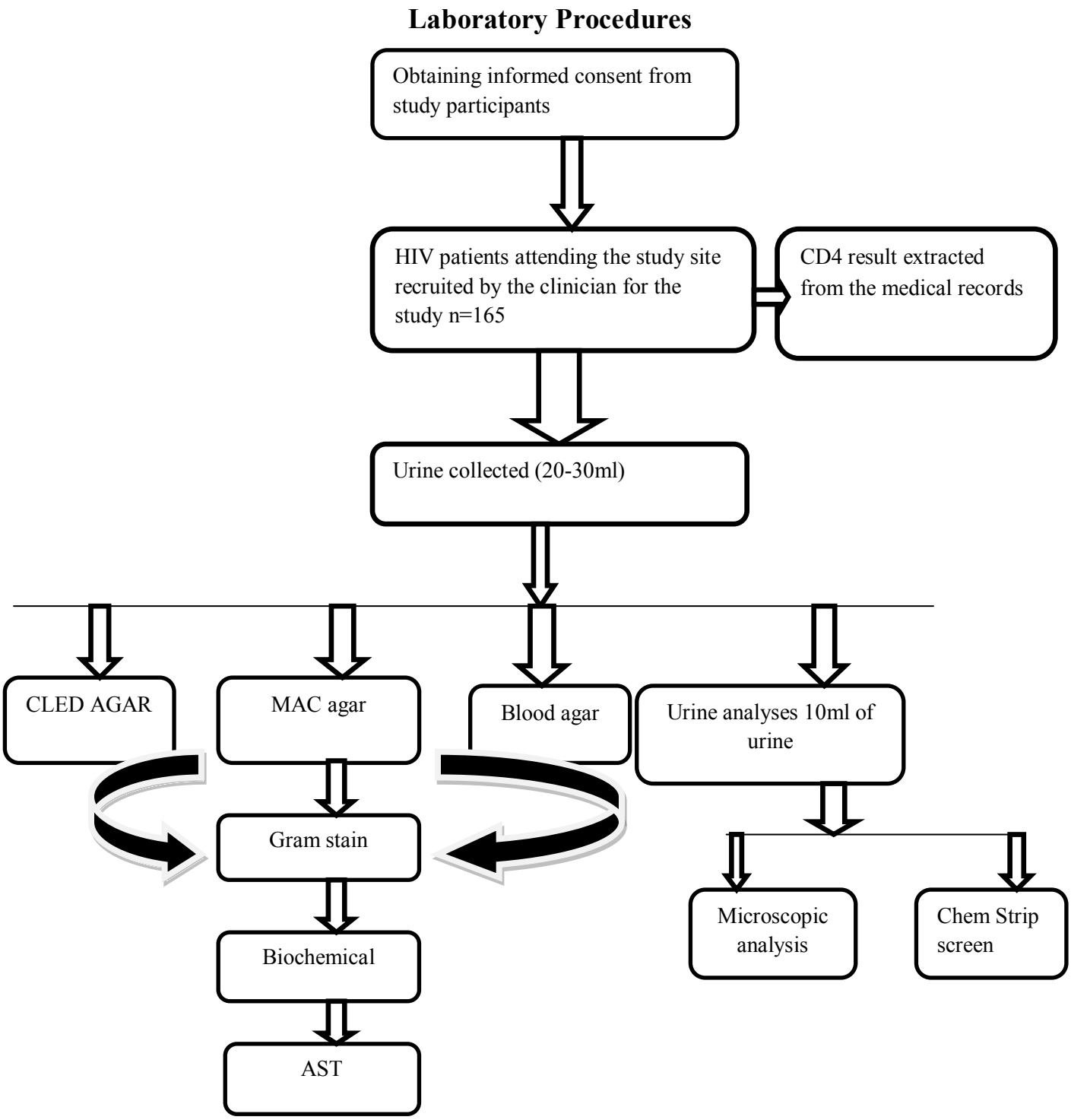

Figure 1. Study Flow Chart for HIV patients attending ALERT Center Addis Ababa, Ethiopia (September 2014 to January 2015)

4.12 Data analyses

The clinical and laboratory data collected from each study subject was recorded on a standard registration format. After the data was checked for its completeness it was analyzed using SPSS version-20 software package. Chi–square (X^2) test was used to compare categorical data and to compare associations between proportions and was used to determine the presence of association between culture results and HIV positive participants. Differences were deemed to be significant where p-values < 0.05 at 95% confidences limit.

4.13 Operational Definitions

UTI: A urinary tract infections are one the most common types of bacterial in humans that can happen anywhere along the urinary tract.

Urinary tract includes the Bladder, Kidneys Ureters -the tubes that take urine from each kidney to the bladder, Urethra - the tube that empties urine from the bladder to the outside (1).

Asymptomatic UTI: It is the presence of significant bacteria ($\geq 10^5$ cfu/ml) in an individual‘s urine (7).

Symptomatic UTI: defined when a patient has one or more of the following signs or symptoms with other recognized cause: fever (temperature, $>38^0$C), urgency, frequency, dysuria, or suprapubic tenderness and a urine culture positive for 10^5 or more microorganisms per milliliter (4).

MSU: a specimen obtained from the middle part of urine flow

Sensitive (S): bacterial isolates are inhibited by the usual achievable concentration of antimicrobial agent. (38).

Intermediate (I): bacterial isolates with antimicrobial agent minimum inhibitory concentrations (MICs) that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates(38, 49).

Resistant (R): bacterial isolates are not inhibited by the usually achievable concentrations of the agent with normal dosage schedule (38, 49).

4.14 Ethical considerations

Ethical clearance and permissions were obtained from the Department of ethics committee of medical laboratory science in Addis Ababa University; and a letter of support was written to ALERT Center from the department. Official permission also was obtained from AHRI/ALERT Ethics Review Committee. All study records that identify subjects were kept confidential. All information collected in this study was given code numbers and no names were recorded. The key to this code numbers and paper files were kept in a locked file and computerized files were password-protected and all were only accessible to authorized staff.

All the investigations done for participants of this study was free of charge but hospital care and treatments were paid according to the rule of the hospital. Study participants were not compensated for their participation in this study.

5 RESULTS

5.1 Socio-demographic Characteristics

A total of 165 urine samples were collected from HIV patients who were attending ART clinic in ALERT Center during September 2014 to January 2015. Demographic features of patients included in this study (i.e. persons living with HIV/AIDS) indicated that of the 165 study participants most of the study participants were female, and majority of them had either primary or no education than those with some level of secondary and tertiary education. Most of the study participants were married. Their ages ranged from 20 to 70 years with a mean of 37.9 (Table 2).

Table 2Sociodemographic characteristics of HIV positive patients attending ALERT Center ART referral clinic, (September 2014 - January 2015).

Scio-demographic variables	Total	Bacterial culture		P-value
		Negative=139	Positive=26	
Age group				
18-27	14	11(78.6%)	3(21.4%)	0.87
28-37	71	60(84.5%)	11(15.5%)	
38-47	58	48(82.8%)	10(17.2%)	
48-57	13	12(92.3%)	1(7.7%)	
≥58	9	8(88.9%)	1(11.1%)	
Sex				
Male	51	47(92.2%)	4(7.8%)	0.06
Female	114	92(80.7%)	22(19.3%)	
Residence				
Urban	150	125(83.3%)	25(16.7%)	0.31
Out of AA	15	14(93.3%)	1(6.7%)	
Marital status				
Married	68	57(83.8%)	11(16.2%)	0.99
Single	38	32(84.2%)	6(15.8%)	
Divorced	30	25(83.3%)	5(16.7%)	
Widowed	29	25(86.2%)	4(13.8%)	
Occupation				
Civil servant	28	24(85.7%)	4(14.3%)	0.26
Private employed	60	52(86.7%)	8(13.3%)	
Daily laborer	18	16(88.9%)	2(11.1)	
Driver	10	10(100%)	0(0%)	
House Wife	49	37(75.5%)	12(24.5%)	
Educational background				
Illiterate	25	21(84%)	4(16%)	0.91
Elementary	93	79(84.9%)	14(15.1%)	
High school	37	30(81.1%)	7(18.9%)	
University	10	9(90%)	1(10%)	

The age group was based on WHO age group classification where the different age groups comprise 18-27 14(8.5%), 28-37, 71(43%), 38-47, 58(35.2%), 48-57, 13(7.9%) and ≥58, 9(5.5%) respectively as it is depicted in. Majority of the UTI infected patients were found in urban 150 (90.9%) Majority of the participants 159(96.4%) were currently on HAART and the rest 6(3.6%) were HAART naive (Table 2).

Among 165 study participants seventy of them were symptomatic and the different symptoms of UTIs frequency was observed, the most common symptom which accounted 62(88.5%), having high frequency of urine, followed by burning during micturition and fever accounted 47(67.1%). (Table 3).

Table 3 Frequency of Signs and Symptoms for UTIs among HIV positive patients attending ART clinics, ALERT Center , Addis Ababa, Ethiopia (September 2014 -- January 2015).

Symptoms	Frequency	%
Having frequency of urine	62	88.5
Dysuria	47	67.1
Having fever at time of visit of participants	47	67.1
Urgency	41	58.5
Flank pain	40	57.1
Renal stone	4	5.7

5.2. Microscopic findings

Microscopic identification of urine samples had been also done for all the study participants; almost one third 48(29.1%) of the study had pus cells, followed by 29(17.6%) RBC, 4(2.4%) Granular casts and 2(1.2%) of them were yeast cells, amorphous phosphates, epithelial cells account for 16(9.7%).

5.3. Culture isolates

Among 165 study participants 13(13.7%) were UTI symptomatic and 13(18.6%) were UTI asymptomatic HIV patients, symptomatic individuals‘ showed high percentages of bacteriuria

(18.6%) as is shown in the table below. The overall prevalence of UTIs in both groups was 26(15.8%) as shown in (Table 4)

Out of the 165 samples, bacteria were isolated from 26 samples giving an overall prevalence of 15.75% with 4(7.8%) occurring in males and 22(19.2%) in females and the remaining 139 had no significant growth. UTI is high in females than males and based on their age frequency of UTI is higher in younger age group than older one (Table 2).

Regarding their current address among 26 isolated organisms 25(16.7%) UTI were found in urban dwellers and 1(6.7%) was out of Addis Ababa the rest 125(83.3%) and 14(93.3%) do not show growth. UTI also have high frequency isolated organisms among the divorced 5(16.7%) and married women 11(16.2%) and then single and widowed 6(15.8%), 4(13.8%) respectively.

Out of 165 study participants ten known diabetic patients were found and among these UTI was found in 1(10%) and the rest 9(90%) do not show any significant growth of bacteria.

Based on CD4+cell count of the patients, the highest proportion of bacteria were isolated from patients who had CD4+cell count of $<200mm^3$ 5(33.3%). Among 165 respondents 7(15.6%) were using co-trimoxazole and 19(15.8%) did not use, but similar UTI outcome was observed in both groups (Table 4).

Table 4. Prevalence of UTI and clinical characteristic of study population (n=165) at ALERT Center ART department September 2014 - January 2015.

Clinical factors	Total	Bacterial Culture		P. value
		Culture -ve=139	culture +ve =26	
History of diabetic				
Yes	10	9(90%)	1(10%)	0.61
No	155	130(83.9%)	25(16.1%)	
Status HAART				
HARRT naïve	6	6(100%)		
On HAART	159	133(83.7)	26(16.3%)	
Using Co-trimoxazole				
Yes	45	38(84.4%)	7(15.7%)	0.965
No	120	101(84.2%)	19(15.8%)	
History of catheterization				
Yes	8	6(75%)	2(25%)	0.46
No	157	133(84.7%)	24(15.3%)	
History of UTI				
yes	21	15(71.4%)	6(28.6)	0.08
No	144	124(86.1%)	20(13.89%)	
Current CD4 value				
<200	15	10(66.7%)	5(33.3)	0.09
201-350	31	27(87.1%)	4(12.9%)	
351-500	48	38(79.2%)	10(20.8%)	
≥501	71	64(90.1%)	7(9.9%)	
Symptoms of UTI				
Symptomatic	70	57(81.4%)	13(18.6%)	0.75
Asymptomatic	95	82(86.3%)	13(13.7%)	

Of all the bacteria isolated (n=26), equal proportion of both Gram-negative and Gram-positive bacteria were found 13(50%). As shown in(table 5) among Gram negative bacteria the most commonly isolated bacteria were *E. coli* 10 (38.5%), followed by *P.mirabiles* 3(11.5%) and among Gram positive *S. aureus* 8 (30.8%), *S.saprophytics* 3(11.5%) and *S. epidermidis* 2 (4.9%). Among the 10 isolates of *E. coli* 8(80%) were from females and 2(20%) from males. And in both symptomatic and asymptomatic patients *E.coli* and *S.epidermides* found equally (50%) and *P.mirabiles* found 2(66.6%) in symptomatic and 1(33.3%) in asymptomatic, whereas 5(62.5%) *S.aureus* was found in symptomatic and 3(37.5%) *S.aureus* was found in asymptomatic and *S.saprophytics* was found only in asymptomatic patients 3(100%) (Table 5).

Table 5Frequency of bacterial isolated associated with symptomatic and asymptomatic UTI among HIV positive individuals (n=26) at ALERT Center ART Clinic, Addis Ababa Ethiopia September 2014 - January 2015.

Bacterial etiologic agents	Symptomatic HIV No (%)	Asymptomatic HIV No (%)	Total No (%)
E.coli	5(50%)	5 (50%)	10(38.5%)
P.mirabiles	2 (66.7%)	1 (33.3%)	3 (11.5%)
S.aureus	5 (62.5%)	3 (37.5%)	8 (30.5%)
S.saprophyticus	0 (0.00%)	3 (100%)	3 (11.5%)
S.epidermidies	1(50%)	1(50%)	2(7.7%)
Total	13(100%)	13(100%)	26(100%)

5.4. Antimicrobial susceptibility pattern of bacterial uropathogens

The susceptibility testing results of the isolates showed that *Escherichia coli* strains were 100% sensitive to Ciprofloxacin and Gentamicin whereas 90% sensitivities was observed for Ceftriaxone and Norfloxacine and 80% to Ceftazidime. There was a higher rate of resistance to ampicillin (90%), tetracycline (100%) and Trimethoprim-sulphamethoxazole (80%) and Amoxicillin-Clavulanic acid (80%). *Proteus mirabilis* were 100% sensitive to ciprofloxacin, Ceftazidime, ceftriaxone, and gentamicin, and over 66% sensitive to nitrofurantion, Amoxicillin-Clavulanicacid and Norfloxacine

whereas Trimethoprim Cotrimoxazole showed a high rate of resistant (100%), to ampicillin and tetracycline (66.7%).

S.aureus, which predominant Gram-positives isolates (30.8%), were 100% sensitive to nitrofurantion followed by (75%) sensitive to gentamicin, whereas, they showed a high rate of resistance to tetracycline and Norfloxacine, (87.5%), Trimethoprim-sulphamethoxazole and penicillin (75%), ciprofloxaciline (62.5%) and erythromycin (50%).

Coagulase negative staphylococci (Cons) were resistance to most of the antibiotics tested. *S.saprophyticus* showed high resistance for most drugs. Hundred percent resistances were observed for penicillin, tetracycline, Norfloxacine, Trimethoprim-sulphsmethsoxazole and ciprofloxaciline but it showed 66.7% sensitivity for nitrofurantion and gentamicin.

S.epidermides showed 100% sensitive for erythromycin, nitrofurantion, ciprofloxaciline and norfloxaciline, whereas 50% resistance for gentamicin and penicillin and 100% resistance for Cotrimoxazole.

Table 6. Antimicrobial Susceptibility Patterns of Bacteria Isolated from Urinary Tract Infections from HIV positive patients who were attending ALERT Center ART clinics, Addis Ababa Ethiopia (September 2014 - January 2015).

Etiologic agents		Antimicrobial agents											
		AMP	AMC	CN	CAZ	E	p	F	Te	SXT	CRO	NOR	CIP
Escherichia coli (n=10)	S*	1	3	10	8	0	0	9	0	1	9	9	10
	I*	0	5	0	1	0	0	0	0	1	0	0	0
	R*	9	2	0	1	0	0	1	10	8	1	1	0
Proteus mirabilus (n=3)	S	0	2	3	3	0	0	2	0	0	3	2	3
	I	1	1	0	0	0	0	0	1	0	0	0	0
	R	2	0	0	0	0	0	1	2	3	0	1	0
S.epidermides (n=2)	S	0	0	1	0	2	0	2	0	0	0	2	2
	I	0	0	0	0	0	1	0	0	0	0	0	0
	R	0	0	1	0	0	1	00	2	2	0	0	0
S.auras (n=8)	S	0	0	6	0	2	2	8	0	2	0	1	2
	I	0	0	0	0	2	0	0	1	0	0	0	1
	R	0	0	2	0	4	6	0	7	6	0	7	5
S.saprophyticus (n=3)	S	0	0	2	0	1	0	2	0	0	0	0	0
	I	0	0	1	0	1	0	1	0	0	0	0	0
	R	0	0	0	0	1	3	0	3	3	0	3	3
Total (n=26) **	S	7.6%	38.5 %	84. 6%	75 %	41.6 %	15%	88.4 %	0	11. 5 %	87.5 %	53.4 %	65.4 %
	I	7.6%	46.2 %	3.8 %	6.3 %	25%	7.6 %	3.8 %	7.6 %	3.8 %	0	0	3.8%
	R	84.6 %	15.4 %	11. 5%	18. 8%	41.6	69%	7.6%	92%	84. 6%	12.5 %	46.5 %	30.8 %

*S= Sensitive *I=Intermediate *R=Resistant ** Expressed in percent.

AMP: Ampicillin; AMC: Amoxicillin-Clavulanic acid; CN: Gentamicin; CAZ: Ceftazidime E: Erythromycin: F: Nitrofurantion; TE: Tetracycline; SXT: Trimethoprim-sulphsmethsoxazole; CRO: Ceftriaxone; NOR: Norfloxacine; CIP: Ciprofloxacin

5.4.1 Multiple drug resistance patterns of the isolates

The frequency of multiple drug resistances to two or more drugs of the urinary pathogens was found in all isolates (100%). Nineteen% of bacteria were resistant to at least two antibiotics, there were no isolates that were sensitive to all antibiotics tested (Table 7).

Table 7 Multiple drug resistance patterns of bacteriuria from HIV positive patients attending at ART clinics, of ALERT Center Addis Ababa Ethiopia (September 2014 - January 2015)

Isolated organisms	N0 of isolates	R2	R3	R4	R5	R6	R7
E.coli	10	2	5	2	1	0	0
		20%	50%	20%	10%	0%	0%
P.mirabiles	3	1	0	0	2	0	0
		33.3%	0%	0%	66.7%	0%	0%
S.aureus	8	1	1	1	2	3	0
		12.5%	12.5%	12.5%	25%	37.5%	0%
S.saprophytics	3	0	0	0	1	0	2
		0%	0%	0%	33.3%	0%	66.7%
S.epidermides	2	1	0	1	0	0	0
		50%	0%	50%	0%	0%	0%
Total	26	5	6	4	5	4	2
		19%	23.1%	15.4%	19%	15.4%	2.7%

R2=Resistance to two antibiotics

R3=Resistance to three antibiotics

R4=Resistance to four antibiotics

R5=Resistance to five antibiotics

R6=Resistance to six antibiotics

R7=Resistance to seven antibiotics

6 Discussion

This study describes a study undertaken to evaluate the prevalence and susceptibility patterns of bacterial strains isolated from both UTI symptomatic and asymptomatic HIV positive patients in ALERT Center.

From our study we found that the incidence of UTI was high among females (19.3%) than males (7.8%). Evidence from various epidemiological studies showed that UTIs were more common in females than in their male counterparts, which is in agreement with many reports from Ethiopia, India and Nigeria (1, 3, 9, 14, 16, 50 and 56).

Factors such as short urethra and its closeness to the anus as well as sexual activity, decrease of normal vaginal flora, less acidic pH of vaginal surface, poor hygienic conditions, in general physiological and anatomical differences are accounted for the differences in males and females (1, 26). But studies in different counters and including our study disagree with the study done in Nigeria that show high prevalence of UTI in males than females (22).

The prevalence of UTI in relation to age groups indicated that age group18-27 years had the highest prevalence (21.4%) followed by 38-47 years (17.2%) while the least was recorded within the age group 48-57 years with a prevalence of (7.7%) as shown in (Table 2). This disagree with Nigerian‘s study who reported higher prevalence of UTI in age group 35-45 (31.6%) and ≥ 46 (38.2%) (12), but our study agrees with other finding in Nigeria in age group 18-23 (3). This indicates that UTI is distributed in all the age groups but it has no significant association (P>0.05). The occurrences of the infection in all the age groups may be attributed to their exposure to HIV/AIDS being the major predisposing risk factor and probably other risk factors such as diabetes, pregnancy, increased sexual activity or reproductive age group and contamination from anus after defecation. These findings disagree with that of other studies conducted in Nigeria which accounts (44.6%) (20), in Iran (41.7%) (59), but it is in line with the study in Tikur Anbessa hospital Ethiopia (27.1%) (53), In other words, more cases of UTIs were recorded in our study among young and middle age patients.

The prevalence of UTI in relation to occupation indicated that housewives had the highest prevalence of UTI (24.5%) followed by civil servants (14.3%). Other groups such as private employed, daily laborers and drivers were found to have prevalence of (13.3%), (11.1%) and (0.00%) respectively. However a statistically significant relationship was not observed ($p > 0.05$). The higher frequencies of occurrence among housewives and private employed may be attributed due to different risk factors

prevalence of UTI in relation to marital status reveals that the divorced had the highest prevalence of UTI with (16.7%), followed by married with (16.2%) and the singles and widowed showed prevalence of (15.8%) and (13.8%) respectively. This study's findings was disagree with that Hawassa study which showed high prevalence found in married (41) though statistically there was no significant association among the different groups ($p > 0.05$) this study disagree with the study done in Nigeria that showed more UTI among widowed (41.7%) followed by married and single with the number of married (22.7%) and single (22.2%) (14), the differences in the prevalence in the groups may be linked to the immune status of the individuals and other possible risk factors.

Regarding their residence the prevalence of UTI in relation to those who live in urban accounts (16.7%) followed by those live out of Addis (6.7%) this may be due to less number of participants who came out of Addis and their geographical different as well . There is no significant association between their residence and bacterial isolates (P>0.05).

Based on their educational level the prevalence of UTI was higher in high school students (18.9%), but it is not significantly associated P>0.05, our finding is almost similar with the study done in Hawassa that showed high UTI among educated groups (41, 52).

Regarding diabetics 10 study participants were known diabetic mellitus patients that accounts (10.0%) this result is in line with other studies done in Ethiopia (Tikur Anbessa (10.9%) (28). But higher number of diabetics patients with UTI were reported from the study done in Gonder 17.8% (35, 51) whereas it is not significantly associated P>0.05.

In this study, there was no statistically significant difference between the magnitude of UTI among HAART users (16.4%) compared to HAART naive (0.00%), P>0.05. Our finding was almost similar with a study conducted in Jimma which had (12%) prevalence of UTI on ART users (58).

The prevalence of UTI among those using co-trimoxazole and those do not use co-trimoxazole were almost similar 15.7% and 15.8% respectively. This might be because all HIV infected participants were taking co-trimoxazole as prophylaxis so they were equally protected.

Regarding the CD4+ value of the HIV infected participants, high prevalence of UTI (33.3%) was reported for CD4+<200 /mm^3. This could be attributed to the decreased level of immunity but it is not significantly associated (P>0.05), this finding was not in agreement with the study done in Nigeria

(92.3%) (22) and South Africa (18%) (62), but it is in line with another study in Nigeria (30%) (23, 61).

The prevalence of UTI among the study participants with previous history of UTI was higher than those without previous history of UTI but it is not significantly associated ($p > 0.05$). This result disagrees with the research conducted in Gonder (1, 51), and Pakistan (44), but it is similar to the study done in Jimma (58) and Hawassa (41) The reasons for these findings might be due to the presence of resistance strains from those who had previous history of UTI as well as because of recurrent (relapsing) infections and failures of drugs in destroying the bacteria (41). Our study also showed high prevalence of UTI in HIV positive individuals with previous history of catheterization than those without but it was not significant ($p>0.05$). It was different from previous reports in Ethiopia and Saudi Arabia (1, 10, 54, and 47). This could be due to long duration of catheterization, repeated catheterization or contamination during inserting catheters.

In this study, the presence of bacteriuria in symptomatic and asymptomatic respondents UTI was high in symptomatic one (18.6 %). Our result was almost similar with previous study conducted in Ethiopia (13.6%) in symptomatic and (10.4%) asymptomatic and in Uganda (17.9%) for symptomatic and13% for asymptomatic (8, 24 and 28).

The overall prevalence of UTI in this study was 15.75%. This is almost similar with the reports of other workers 10.7% (10), 12 % (58), 10% (8), 17.8 % (51). Wheras our finding is lower than similar UTI studies reported, 19.2%, 25 %, respectively (29, 16). Much higher prevalence rates have been reported by some other authors (19, 20, 9,) stating prevalence rates of 26%, 39.6% and 63.5% respectively. These might be accounted for HAART treatment as well as use of SXT as a prophylaxis which might have inhibited the growth of the bacteria or it might have protected them from the bacterial infection. Geographical distribution in sample size, socio-economic status and sexual behavior and disease stage differences among the study participants might have also contributed to the difference prevalence (8, 58).

Worldwide, *E.coli* has been demonstrated as the most common uropathogens in women. Of five uropathogens species isolated in this study, *Escherichia coli* were the most frequent isolate accounting for 38.5%, followed by *Staphylococcus aureus* (30.8%). This finding is similar with two Ethiopian studies from Gonder (1, 42), Nigeria (60, 61, 14, 22 and 33) Saudi Arabia (54) Khartoum (55). The Gram positive isolates were coagulase negative staphylococci (11.5%) *S.saprophyticus* and (4.9%)

S.epidermidies respectively. According to the study done in Nigeria, Benin and Calabar City the predominant bacteria among HAART users were *S. aureus* (87.2%) followed by *E. coli* (84%)while *E. coli* was common among non-HAART users. (58) *E. coli* is considered the most prominent uro-pathogenic due to a number of virulence factors specific for colonization and invasion of the urinary epithelium, such as the P-fimbriae and S fimbriae adhesions or it could be due to the presence of unique structure in Gram negative bacteria which help for attachment to the uro-epithelial cells and prevent bacteria from urinary lavage, allowing for multiplication and tissue invasion (46, 47 and 52). Urinary tract infections due to *E. coli* are a common finding in women and it is associated with microorganisms ascending from the peri-urethral areas contaminated by fecal flora due to the close proximity to the anus and warm, moist environment.

Staphylococcus aureus is the second highest isolate in our study. This finding is in agreement with commonly isolated bacteria in other studies of general population elsewhere in the world and in Ethiopia (1, 2, 60 and 61).

There is no use of isolating pathogens from diseased human body site alone without a corresponding prescription of appropriate antibiotic therapy. Besides, the need for constant monitoring of susceptibility of pathogens in different populations to commonly used antimicrobial agents has been suggested (1).

Antimicrobial resistance among uropathogens to the commonly used antibiotics is increasing that left clinicians with very few choices of drugs for the treatment of UTI (1). In this study, *E.coli* were sensitive to ceftriaxone (90%), Gentamycin and ciprofloxacin (100%), Norfloxacine, and nitrofurantion (90%) and Ceftazidime (80%) whereas, it showed a high rate of resistance to ampicillin, (90%), and tetracycline (100%) and co-trimoxazole (80%). *P.mirabilis* isolates were sensitive for Gentamycin, Ceftazidime, Ciprofloxacin and Ceftriaxone 100% but resistance to Ampicillin (66.7%) trimethoprim sulphsmethsoxazole (66.7%) and Tetracycline (100%). This finding is supported by a study done in Ethiopia Tikur Anbessa on antibiotics susceptibility patterns of Gram negative bacteria (*E. coli, Klebsiella* spp, and *Proteus* spp) isolated from urine samples were highly susceptible to Ciprofloxacin; however, they showed a high rate of resistance to Ampicillin (81.2%), Amoxicillin (85.12%) and Tetracycline (80.6%) (53), another study in Gonder also had similar findings that showed high sensitivity to ceftriaxone (96.3 %), ciprofloxacin (96.3 %), gentamicin (92.6%), Norfloxacine (92.6%) and amoxicillin-Clavulanic acid (59.3 %) (47), another study in Gonder also had almost similar result with this study Chloramphenicol (100%), Ceftriaxone (100%), Ciprofloxacin

(96.8%), and Norfloxacine (90.3%), however, they showed a high rate of resistance to ampicillin and amoxicillin 29 (93.5%), co-trimoxazole 26 (83.9%), and tetracycline 25(80.6%) (10 and 16). This may be due to easy access and availability uncontrolled and indiscriminate use of commonly used drugs such as Ampicillin, Amoxicillin, Tetracycline and Co-trimoxazole that led to an increased resistance (57 and 8). Most studies; however, found the isolates resistant to co/trimoxazol and penicillin. The cotrimoxazole resistance could be explained due to its use as prophylaxis in the HIV clinic for the treatment of several opportunistic bacterial infections (58, 59, 40, 42, 19 and 20). Our findings were disagrees with the study done in Nigeria (32) and in Ethiopia (47).

In this study *S.aureus* was sensitive to Gentamycin (75%), Oxacilin (62.5%) and Nitrofurantion (100%) where as Norfloxacine and Tetracycline (87.5%), Cotrimoxazole and Penicillin (75%), Ciprofloxacin (62.5%) and Erythromycin (50%) were resistant. This finding supported the study conducted in Gonder that was shown little or no susceptibility to tetracycline, amoxicillin, ampicillin and Cotrimoxazole (1, 31 and 16). On the other hand Coagulase negative staphylococci (Cons) such as *S.saprophyticus* was found to be sensitive for Gentamycin and Nitrofurantion (66.7%) whereas were highly resistance for Tetracycline, Penicillin, Cotrimoxazole, Norfloxacine and ciprofloxacine (100%) and *S.epidermides* isolates were sensitive for Erythromycin, Oxacilin, Nitrofurantion, Norfloxacin and ciprofloxacine, but resistance for tetracycline and co-trimoxazole (100%). Our study is in agreement with the study conducted in Iran (30)

Multi drug resistance (MDR= resistance in ≥2 drugs) was seen in (96 %) of the isolated bacterial uropathogens. This is comparable with reports from Gonder (87.2%) (1), these findings were very high compared with former study done in Gondar that showed (68%), MDR and (74%) from Tikur Anbessa Hospital (53). These might be due to an irrational and unnecessary use of antibacterial agents. The consequence of this could lead to the emergence of multidrug resistance bacterial strains. In the present study most of the isolated pathogens showed MDR of two and more antibacterial agents tested (46). This reflected the fact that ampicillin, tetracycline, and co-trimoxazole were the most easily available in the market without prescription beside their cheap price. The widespread use and more often the misuse of antimicrobial drugs has led to a general rise in the emergence of resistant bacteria. Globally there are many reports of MDR for instance higher resistant strains were reported in USA to ampicillin and Cotrimoxazole. Whereas ciprofloxacin was considered as an option for therapy to UTIs, since its multiple mechanisms of action seem to have enabled it to retain potent activity against *E. coli*

compared with other commonly used agents, such as Ampicillin and SXT. In this study also Ciprofloxacin had shown 100% sensitivity (57).

In this study over (90%) of all isolates were resistances to ampicillin, tetracycline and Trimethoprim-sulphamethaxozole, which is comparable to other findings, reported in Ethiopia (10). This might be due to the fact that many antibiotics are easily available for self-medication and they are being used indiscriminately.

Resistance of UTI pathogens to commonly used antibiotics may be connected to their frequent prescription in hospitals, their easy availability in the community without prescription and their low cost which make them subject to abuse (1).

7 Limitation of the study

The design of the study did not include control group due to budget constraints.

8 Conclusion and Recommendation

In our study both Gram positive and Gram negative isolates are obtained equally. In the current study, the overall prevalence of asymptomatic and symptomatic UTI was 15.75 %. *E. coli* isolates were the most predominant bacterial isolates followed by *Staphylococci aureus* and most of the bacterial isolates were sensitive to gentamicin, ceftriaxone, ciprofloxacin, Norfloxacine and nitrofurantion. A large number of the isolates were resistant to the commonly used antibiotics ampicillin, tetracycline, penicillin and co-trimoxazol. Multi-drug resistance bacteria were common. Periodic and continuous follow up are mandatory to reduce the consequence of symptomatic and asymptomatic bacteriuria and multi-drug resistance bacteria in HIV positive patients. In this study the chance of acquiring or being exposed to UTI was higher among females HIV positive patients than males in the presence of associated risk factors such as lowered immunity and their anatomical structure.

The following recommendations are made based on the findings of the present study

- ✓ According to our study Ampicillin, Tetracycline, penicillin and co-trimoxazole, were found quite ineffective to treat UTI infections hence in an area with no culture facility, these antimicrobials should not be prescribed as empirical treatment.
- ✓ In the absence of laboratory facility for isolation of microorganisms and performing antimicrobial testing, it is recommended to use empirically broad spectrum antimicrobials like Gentamicin.
- ✓ There is a need for a continuous surveillance for resistant bacteria to provide the basis of alternative treatment.

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Annex I: GENERAL INFORMATION FOR THE STUDY PARTICIPANTS

Date.....

Introduction

My name is …… and I am MSc student of Addis Ababa University, School of Medical laboratory Sciences. I am doing a research entitled **Prevalence of UTI in HIV infected people**. Currently UTI have a great burden on HIV positive patients as different studies indicate so this study will indicate in ALERT hospital HIV patients the commonly isolated bacteria and there drug susuptability tests and it will help the physicians to treat based on culture results.

What is the reason of this study?

The objective of this research is to study the prevalence of UTI in HIV patients and to know their drug susuptability. If you agree to participate in the study, about 20-30 ml (a cup of coffee) of midstream urine will be collected from you. Additionally we will take your CD4 value from your card.

Will my information be kept confidential?

All the data obtained will be kept strictly confidential and locking the data, only study personnel will have access to the files. Anonymous testing will be undertaken, that means samples will be coded and positive results will not be identified by names. There will not be any payment or direct benefit for participating and you are not asked to pay for the laboratory examination. Your result will be reported back to the physicians if it is found significant for further diagnosis and treatment.

What are the costs? All the investigations done for participants of this study will be free of charge.

What about compensation? You will not be compensated for your participation in this study directly but it may help indirectly for other patients those who may have the same problem as you.

What about my rights to decline participation or withdraw from the study?

Your participation in this study is purely voluntary, and you may stop the participation at any time or you may refuse to answer some of the questions if you feel uncomfortable. You are free to refuse to participate in the study or you can withdraw your consent at any time, without giving reasons and this will not involve any penalty or loss of benefits to which you are entitled such as proper care and

treatment. Your access to treatment will not be dependent on your participation in the study. If you are not comfortable please feel free to stop it at any level of the study.

I appreciate your cooperation greatly. If you have questions regarding this study or would like to be informed of the results after its completion, please contact me through the following address.

Principal investigator: Yemisrach Getu

TEL. 0935-025301/09-11-160930 E-mail: birutgetu@gmail.com

ALERT /AHRI Ethical Review Committee 0118-962183

Annex II: **GENERAL INFORMATION FOR THE STUDY PARTICIPANTS IN AMHARIC**

አጠቃላይ መረጃ

አዲስ አበባ ዩንቨርሲቲ የድህረ ምረቃ ት/ት ቤት

የላብራቶሪ ሳይንስ ትምህርት ክፍል

በጥናቱ የሚሳተፉ ግለሰቦች የፈቃድ መጠየቂያ እና መቀበያ ፎርም

<u>መግቢያ</u>

ስሜ----------እባላለሁ::የአ.አ.ዩ. የላበራቶሪ ሳይንስ የትምህርት ክፍል የማስተርስ ድግሪ ተማሪ ነኝ በአሁኑ ሰአት የኩላሊት እና የሽንት ቱቦ ህመም በኤች አይቪ ቫይረስ ታካሚዎች ላይ የሚያመጣውን ህመም እና ያለዉን የስርጭት መጠን ለማወቅ ጥናት እያካሄድኩ ነው ፡የኩላሊት እና የሽንት ቱቦ ኢንፌክሽን አምጭ ባክቲሪያ የኤች አይ ቪ ህመማን ላይ የተለያዩ ችግሩች ሲያመጡ ይታያል፡፡ ይህ ጥናት በአለርት ሆስፒታል የኤች አይ ቪ ህመማን የኩላሊት እና የሽንት ትቦ ኢንፌክሽን ምልክት የሚሳዩትን እና የማያሳዩትን በየትኛዉ ባክቲሪያ እንደተጠቁ መለየት እና ባክቲሪያዉ በየትኛዉ መድሀኒት ሊጠፉ እንደሚችል ለማመልከት ሲሆን ይህም ለሃኪሙ ህመማንን ለማከም የሚያግዝ ሲሆን በተጨማሪም ተያያዥነት ያላቸዉን ችግሮች ለማዉቅ እና የመፍትሔ ዕርምጃ እንዲወሰድ ለማመልከት ነዉ፡፡

<u>የጥናቱ አላማ</u>

የጥናቱ አላማ የኩላሊተ እና የሽንት ቱቦ ኢንፌክሽን ህመም በኤች አይቪ ቫይረስ ህመማን ለይ ምን ያህል እንደሆነ ለማውቅ ነው፡፡ እርስዎ በጥናቱ ለመሳተፍ ፈቃደኛ ከሆኑ 20-30 **ሚ.ሊ. ወይም ግማሽ የቡና ስኒ የሚሆን የሽንት ናሙና ይሰጣሉ፡፡ በተጨማሪም የ**CD4 **ዉጤተወን ከህክምና ካርድዎት ላይ እንወስዳለን፡፡**

<u>ስለ እኔ የሚያዙ መረጃዎች በሚስጥር ይጠበቃሉ?</u> የሚሰጡት መረጃ ሚስጥራዊነቱ የተጠበቀ ነው በስም አይጻፉም የዚህ ኮድ መፍቻ በፋይል ተቆልፎ የሚቀመጥ ሲሆን የተፈቀደለት ሰው ብቻ ፋይሉን ማየት ይችላል፡፡ ከዚህ ጥናት በሚወጡ ዘገባዎች ወይንም የህትመት ውጤቶች ላይ ስምም ወይም ሌላ የእርስዎን ማንነት የሚገልጽ መረጃ አይኖርም፡፡ ከምርመራ የሚገኘውም ውጤት ወይም ሌላ መረጃ ለሚመለከታቸው አካላት ለምሳሌ፤ እርስዎን የሚንከባከቡ የህክምና ባለሙዎች እና ጥናቱን ለሚያካሄዱት ባለሙያዎች እንዲሁም ጥናቱ ስነምግባርን ጠብቆ መከናወኑን

ለሚከተሉት የኮሚቴ አባላት ብቻ ይገለፃል፡፡ ኮምፒውተር ላይ ያሉ መርጃዎች ምስጢራዊነታቸው የተጠብቀ ሲሆን በወረቀት ያሉ መረጃዎችም ደህንነቱ በሚጠበቅ ቦታ የሚቆለፉና የተፈቀደለት ሰው ብቻ ሊያያቸው እንዲችል ተደርጎ ይጠበቃሉ፡፡ውጤቱ ተጨማሪ ምርመራ የሚያስፈልገዉ ከሆነ እና ህክምና ካሰፈለገዉ ለሀኪሙ ውጤቱ ይሰጠዋል፡፡

<u>በጥናቱ መሳተፍ ምን ጥቅም ይኖረዋል?</u> በጥናቱ በመሳተፈዎ ምንም አይነት ክፍያ አይጠየቁም ወይም የሚያገኙት ገንዘብ አይኖርም ነገር ግን የኩላሊተ እና የሽንት ቱቦ ኢንፊክሽን ህመም ካለዉ ወይም የምርመራ ውጤቱ ህክምና የሚያስፈልገው ከሆነ ተጨማሪ ምርመራ እና ህክምና እንዲያገኙ የረደወታል፡፡ነገር ግን ከጥናቱ በሚገኘው እውቀት በኩላሊተ እና በሽንት ቱቦ ኢንፊክሽን ባክቴሪያ አማካኝነት የሚመጣውን በሽታ በተሻለ ደረጃ ለመቆጣጠርና ለበሽታው ትክክለኛውን ፀረ ባክቴሪያ ለመምረጥ ሀኪሞችንይረዳል፡፡

<u>በጥናቱ መሳተፍ እሚያስከፍለዉ ክፍያ ይኖራልን?</u> ሁሉንም ዓይነት ለጥናቱ የሚያስፈልጉ ምርመራዎች በነፃ የሚሰሩ ሲሆን የህክምናና/የሆስፒታል ወጪዎች በሆስፒታሉ አሰራር መሰረት ምንም ክፍያ አይኖረዉም፡፡

<u>ስለማበረታቻ (ማካካሻ)፡</u> በዚህ የዳሰሳ ጥናት ውስጥ ስለተሳተፉ ወይም እንዲሳተፉ ለማድረግ ምንም ዓይነት ማካካሻ ወይም ማበረታቻ አይሰጥም፡፡

<u>በጥናቱ ለመሳተፍ ፈቃደኛ አለመሆን ወይም መሳተፍ ከጀመሩ በኋላ ራስን የማግለል መብት</u>

በጥናቱ የሚሳተፉት ፈቃደኛ ከሆኑ ብቻ ነው፡፡ስለዚህ መሳተፍ አለመሳተፍ ከጀመሩ በኃላ ማቋረጥ ወይም መመለስ የማይፈልጉት ጥያቄ ከሆነ ይለፈኝ ማለት ሙሉ መብትዎ ነው፡፡ በጥናቱ መሳተፍ ወይም አለመሳተፍ አገልግሎት ላይ ምንም አይነት ጥቅምም ሆነ ጉዳት አይኖረውም፡፡ ጊዜወትን መሰዋት አድርገው ሰለተባበሩኝ ከልብ አመሰግናለሁ፡፡

ይህንን ጥናት አስመልክቶ ጥያቄ ካላዎት፡፡ ወይም የጥናቱ የመጨረሻ ውጤት ምን እንደሆነ ለማዉቅ ከፈለጉ በሚከተለዉ አድራሻ ሊያገኙን ይችላሉ፡፡

የምስራች ጌጡ የስልክ ቁጥር 09-35-025301 ኢሚይል birutgetu@gmail.com እና አለርት/አህሪ የምርምር ስነምግባር ኮሜቴ 0118-962183

Annex III CONSENT FORM

For adult patients who are able to respond:

I _____, after being fully informed about the detail of this study like

- I understand that my urine sample will be used for the study Yes…….No…….
- I agreed that my CD4 result to be extracted from my medical card Yes…….No……
- I have understood that there is no any compensation for my participation Yes……No……

Hereby give my consent to participate in this study being voluntary.

Signature_____ Date_____

For families or attendants of patients unable to read and write:

I_____ parent/guardian/attendant, after being fully informed about the purpose of this study, here by give my consent on the patient‘s participation in this study by knowing the following points.

- I understand that his or her urine sample will be used for the study Yes…….No…….
- I agreed that his or her CD4 result to be extracted from his or her medical card Yes…….No……
- I have understood that there is no any compensation for participation Yes……No……

Signature: _____ Date_____

Annex IV CONSENT FORM IN AMHARIC
የስስምምነት መጠየቂያ ቅጽ

በዚህ ጥናት ለሚዳሰሱ ጥናቶች ማንበብ እና መጻፍ ለሚችሉ

እኔ----------የዚህ ጥናት ዐላማ በግልጽ ስለተረዳሁ በጥናቱ ለመሳተፍ

- **ግማሽ የቡና ስኒ የሚሆን የሽንት ናሙና እንደምሰጥ ተረድቻለሁ አዎ…….አይደለም….**
- **የሲዲ4 ውጤቴ ከመዝገብ ላይ እንደሚወሰድ ተረድቻለሁ አዎ……..አይደለም…….**
- **በጥናቱ ላይ በመሳተፌ ምንም አይነት ጥቅም እንደማላገኝ ገብቶኛል አዎ…..አይደለም…..**

ከላይ የተጠከሱትን ለመስጠት መሰስማማቴን በፊርማየ አረጋግጠለሁ፡፡

ፊርማ----------ቀን----------

ማንበብ እና መጻፍ ለማይችሉ

እኔ----------የበሽተኛዉ አስታማሚ ስሆን የዚህን ጥናት አላማ በዉል ተረድቻለሁ፡፡ ስለሆነም በሽተኛዉ

- **ግማሽ የቡና ስኒ የሚሆን የሽንት ናሙና እንደሚሰጥ ተረድቻለሁ አዎ…….አይደለም….**
- **የሲዲ4 ዉጤት ከመዝገብ ላይ እንደሚወሰድ ተረድቻለሁ አዎ……..አይደለም…….**
- **በጥናቱ ላይ በመሳተፌ ምንም አይነት ጥቅም እንደማላገኝ ገብቶኛል አዎ…..አይደለም…..**

ከላይ የተጠከሱትን ህመምተኛዉ ቢሰጥ መሰስማማቴን በፊርማየ አረጋግጠለሁ፡፡

ፊርማ----------ቀን----------

Annex V QUESTIONNAIRE:

A DATA COLLECTION FORM

ADDIS ABABA UNIVERSITY SCHOOL OF MEDICAL

LABORATORY SINCE

QUESTIONNAIRES: Administered for investigation to determine prevalence of urinary tract infection in HIV positive patients and drug susceptibility pattern.

I. Patient identification

Date of data collection.....

Study‘s code no .....Age..... Sex.....Address…Urban ------- out of AA----------

Card No. ……...Code No ……OPD……Ward……..Bed No……Marital status; married…....single……..divorced……widowed……

Date of Hospitalization……

Educational Level: Illiterate-------Elementary----------High school----------University----- Occupational work----------family size…….?

Duration of HAART started …… (wks./months/years)

II. Clinical data

Do you have the following symptoms of UTI?

	YES	NO
Fever	☐	☐
Dysuria	☐	☐
Frequency of urine	☐	☐
Urgency	☐	☐

Diabetics ☐ ☐

Flank pain ☐ ☐

Renal stone ☐ ☐

Duration of present complaints or symptoms of UTI……..? (Days/months)

	YES	NO
Antibiotic use less than 72 hours	☐	☐

If yes specify……..……..

Are you taking antibiotics currently?	☐	☐

If yes specify……

Reason of taking antibiotics (Diagnosis)…….

Are you taking co-trimoxazole?	☐	☐

Do you have catheterization previously? Yes……No……

Do you have previous history of UTI? Yes……No…….

Are you pregnant? Yes……No…….

III. Laboratory data

Current CD4value----------

Annex VI QUESTIONNAIRE IN AMHARIC

የበሽተኛ መለያ መጠይቅ

ቀን_____ የጥናቱ መለያ ቁጥር_____ እድሜ _____ጾታ _____

አድራሻ፡-ከተማ _____ከ አለ ዉጭ _____

ካርድ ቁጥር_____ ኦፒዲ _____ዋርድ _____የአልጋ ቁጥር _____

የጋብቻ ሁኔታ፡- ያገባ/ች _____ ያላገባ/ች_____ የተፋታ/ች _____ የሞተበት/ባት _____

ወደ ሆስፒታል የመጡበት ቀን_____

የትምህርት ደረጃ፡-ማንበብና መፃፍ የማይችል _____ የመጀመሪያ ደረጃ ____ ሁለተኛ ደረጃ ____
ዩንቨርሲቲ _____የስራ አይነት_____የቤተሰብ አባላት ብዛት ____

የጸረ ኤ.ች.አይ.ቪ መድሃኒት መውሰድ ጀምረዋል? ____ አዎ ____ አይደለም ____

አዎ ከሆነ መልሱ፣ የጸረ ኤች አይቪ መዳኒት መዉሰድ የጀመሩበት ጊዜ፡-

በሳምንት ____ በወር ____ በአመት _____

1. የጤንነት ሆኒታ መጠየቂያ

የሚከተሉት የኩላሊት እና የሽንት ቱቦ የህመም ምልክቶች ታይቶብዎት ያዉቃል?

	አዎ	አይደለም
ትኩሳት	☐	☐
ሲሸኑ ማቃጠል	☐	☐
ቶሎ ቶሎ ማሸናት	☐	☐
ሽንትን መቆጣጠር አለመቻል	☐	☐
የስኳር ህመም	☐	☐

የጎን እና የጎን ህመም ☐ ☐

የኩላሊት ጠጠር ☐ ☐

ችግሩ ለምን ያህል ጊዜ ቆየበዎት _____ በቀናት/ በሳምንታት / በወራት

ባለፈዉ 72 ሰአታት ዉስጥ ጸረ ባክቴሪያ መደሃኒት ወስደዋል? አዎ _____ አይደለም _____

አዎ ከሆነ መልሱ መደሀኒቱ ምንድን ነዉ _____

አሁን ጸረ ባክቴሪያ መደሃኒት ይወስዳሉ? አዎ _____ አይደለም _____

አዎ ከሆነ መልሱ መደሀኒቱ ምንድን ነዉ? _____

ጸረ ባክቴሪያ የሚወስዱበት ምክንያት _____

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ከዚህ በፊት የሽንት ቱቦ እና የኩላሊት ኢንፊክሽን ህመም ታመዉ ያዉቃሉ አዎ ___አይደለም __

በቅርብ የተሰራ የላብራቶሪ የ CD4 ወጤት ስንት ነዉ _____

Annex VII: LABORATORY RESULT FORMAT

URINALYSIS

Color_____

Appearance_____

CHEMSTRIP SCREEN

PH_____

Protein----------

Albumin +1 +2 +3 +4

Blood +1 +2 +3 +4

Blood +1 +2 +3 + 4

MICROSCOPIC ANALYSIS

WBC_____/HPF

RBC_____/HPF

Casts_____/LPF

Others

CULTURE RESULT

Bacterial Isolated……

Susceptibility Test result

AMP	AMC	CN	CAZ	E	MET	F	TTC	SXT	CRO	NOR	CIP

S=Sensitive I=Intermediate R=resistant

AMP: Ampicillin; AMC: Amoxicillin-Clavulanic acid; CN: Gentamicin; CAZ: Ceftazidime E: Erythromycin; MET: Methicillin F: Nitrofurantion; TTC: Tetracycline; SXT: Trimethoprim-sulphamethoxazole; CRO: Ceftriaxone; NOR: Norfloxacine; CIP: Ciprofloxacin.

Name of principal investigator _____

Signature _____ Date _____

Annex VIII: LABORATORY STANDARD OPRETING PROCEDURS

Gram staining procedures

Purpose: This procedure provides instructions to perform Gram‘s stain.

Materials: Crystal violet, Lugol‘s iodine, Alcohol and Safranin.

Supplies: Disposable plastic loops, Glass microscope slides and Slide warmer, dry heat block, flame, or absolute methanol.

Principle: Gram-positive bacteria have a thick mesh-like cell wall made of Peptidoglycan (50-90% of cell wall), which stains purple while Gram-negative bacteria have a thinner layer (10% of cell wall), which stains pink. Gram-negative bacteria also have an additional outer membrane which contains lipids, and is separated from the cell wall by the periplasmic space.

1. Labeling the slides clearly with the date and patient‘s name and number.
2. Take one colony from the culture, dilute with one drop of sterile saline on the slide and spread smear over an area of the size of a quarter
3. Heat fixation
   a) Pass air-dried smears through a flame two or three times. Do not overheat.
   b) Allow slide to cool before staining.
4. Drying of smears after making smears, the slide should be left in a safe place to air-dry, protected 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. Working with one slide at a time, flood the slide with decolorizer for 5 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 place on a slide warmer or heating block to completely dry.

Limitations:

1. Recovery of organisms not observed on direct Gram‘s stains should prompt a review of both the smear and the culture.
2. Application of excessive heat during fixation of smear may affect the morphologic appearance of host cells and microorganisms.
3. Treatment with antimicrobial agents may cause Gram-positive bacteria to appear Gram-negative.

Result Interpretation

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

Gram-negative bacteria will stain pink to red.

Annex IX LABORATORY PROCEDURE FOR COLLECTION AND CULTURING OF URINE SAMPLE

Purpose: This procedure provides instructions for inoculating, reading and interpreting a culture.

Principle: Urine specimens are submitted for culture from patients with signs and symptoms of urinary tract infection. The culture of urine is performed quantitatively to distinguish urethral and

vaginal flora contaminates from organisms infecting the urinary tract. Although various opinions exist the level of bacterial generally considered to be indicative of infections ≥ 100,000colony forming unites per milliliter of urine

Materials: CLED Agar, BA, MAC, calibrated 0.001ml inoculating loop, 70%alchool, 35-37^{0}C incubator and Safety cabinet.

Sample Type: Clean-catch mid- stream urine specimen: - a specimen obtained from the middle part of urine flow

The best method is properly collected ―clean catch" urine which is collected as follow:

- The patient should urinate a small amount and this is discarded.
- The urine that comes next, the mid-stream specimen, should be collected into a sterile container of 20 to 30ml.
- After obtaining the specimen the patient continues to urinate and this is discarded.

Procedure:

1. Bring the culture media to RT
2. Gently swirl the container to mix the sample
3. Deposit a 0.001ml sterile loop in to the sample until the top of the loop circle just enters the specimen.
4. Deposit the sample in a single line down the middle of the plate. Crosse-streak the line of material with a series of very close streak lines such that the entire plate surface is utilized
5. Incubate plates 16-18hr at 35-37^{0}C aerobically
6. If there is no growth after 24-48hr of incubation discard the plates and issue final report.
7. If there is growth after 24-48hr of incubation, count the number of colonies of each morph type present. Each colony counted represents 1000CUF in the original sample.
8. If the number of colony is more than 100 that means more than 100,000 organisms/ml perform definitive biochemical identification and sensitivity test

Limitation: Pyuria may result from non- infectious causes

- Treatment of patients with antibiotics prior to collection of specimens.
- Overly diluted urine

- Urethritis than cystitis or infection with organisms not detectable standard methods

Procedural Notes:

A. Do not culture folly catheter tips, urine sediments, voided or catheterized urine anaerobically urine from a folly collection bag
B. Most cases of uncomplicated cystitis are treated empirically and do not require culture.

Clinical Utility: For the diagnosis and appropriate treatment of urinary tract infection caused by bacteria.

Annex X URINE MICROSCOPY PROCEDURE

Purpose: this procedure provides instructions on urine microscopy analysis.

Principle: The urine microscopic examination is a method of identifying and quantifying cells.

Bacteria and other materials in the sediment of centrifuged urine.

Materials: Clean plastic specimen containers, plastic pipette, microscope slide, cover slips, graduated conical centrifuge tubes and cups and Pen/Marker.

Equipment's: Centrifuge and Microscope with 10x and 40x lenses

Sample: 10ml urine

1. Label plastic conical tube with patient`s name /ID
2. Place about 10ml of samples and cover tube with tight fitting cover.
3. Place tube in centrifuge and balance with equal amount water/ urine (with tight fitting cover) directly opposite tube with urine, to act as counter weight.
4. Centrifuge urine specimen at 1200-2000rpm for 5 minutes.
5. After centrifuge has stopped, remove tube and pour off the supernatant and leaving any sediment in the bottom of the tube.
6. Re-suspend the remaining sediment by flicking the bottom of the tube several times.
7. Place one drop of the sediment solution on a glass slide and cover with a cover slip.
8. Examine the sediment using bright light under low (10X) and high (40X)

Result interpretation;

Results reported as number of identified elements per high power (40X) field (HPF) are: WBC`s, RBC`s. And casts crystals epithelial cells report as low power field (LPF) .The casts need to be identified as hyaline, WBC, RBC, epithelial, fatty, or waxy. Results reported as ―few, moderate, or many" are; epithelial cells, bacteria, crystals, and parasites. Identify presence of yeast (hyphae).

Clinical Utility: Help to rule out or diagnose urinary tract infections and / or renal disease.

Limitations: Precision is problem with microscopic examinations of urine specimens. When concentrating and standardizing the aliquot of urine screened, the probability of identifying those urine constituents that occur in low numbers will be significantly increased. An in accurate reading may be caused by one or several of the following errors made in specimen collection or technique:

1. Specimen not obtained by ―clean catch" method and thus contains elements from sources other than the urinary tract (e.g., vaginal discharge, penile discharge).
2. Specimen kept for more than 1hr without refrigeration/ preservative.
3. Specimen not centrifuged long enough.

4. Urine extremely dilute so on sediment obtained, or not enough elements available in amount of urine tested.
5. Specimen not examined with proper lighting or focusing.
6. Microscope not functioning properly, e.g., lens dirty.
7. Examiner fails to recognize the elements on slide.
8. The absence of RBCs in the event of a positive dipstick reflects the absence of free hemoglobin and/or myoglobin. The dipstick does not differentiate between the two molecules.
9. Dipstick portion pad is sensitive starting from 10mg of albumin excreted in the urine, so negative dipstick / positive casts picture, while unlikely, is possible.

Procedural notes

1. Sediment of centrifuged urine may be on side tube, not on bottom of tube observe first before withdrawing sediment specimen.
2. May need to use low light on microscope to see hyaline casts and hyphae forms of yeasts fungi
3. When cells are too numerous to count (over 100HPF) report as many full fields". Also report WBC clumps.
4. Grossly bloody samples should be forwarded to the clinical laboratory for evaluation.

Annex XI CATALASE, COAGULASE AND NOVOBIOCIN

Test principle; it tests the ability of the organism to liberate O_2 from H_2O_2 by the action of catalase.

Method;

1. Place a small amount of growth from your culture onto a clean microscope slide. If using colonies from a blood agar plate, be very careful not to scrape up any of the blood agar—blood cells are catalase positive and any contaminating agar could give a false positive.
2. Add a few drops of H_2O_2 onto the smear. If needed, mix with a toothpick. DO NOT use a metal loop or needle for H_2O_2; it will give a false positive because it degrades the metal.
3. A positive result is the rapid evolution of O_2 as evidenced by bubbling.
4. A negative result is no bubbles or only a few scattered bubbles.
5. Dispose of your slide in the biohazard glass disposal container. Dispose of any toothpicks in the Pipet Keeper.

Coagulase test procedure

Principles; Coagulase is a protein enzyme produced by several microorganisms that enables the conversion of fibrinogen to fibrin. Coagulase binds plasma fibrinogen, causing the organisms to agglutinate or plasma to clot.

1. Place two drops of distilled water on a clean glass slide. Identify where the test strain (T) and the control(C) will be placed by labeling the slide. An additional slide will be required for the control strains and this should be clearly labeled. Set up the positive and negative control organisms on the same slide to be tested simultaneously.
2. Emulsify the test strain to obtain a homogenous thick suspension. False negative reactions will occur if the bacterial suspension is not heavy enough.
3. Observe for auto agglutination. Strains which auto agglutinate must be tested by an alternative procedure.
4. Dip a straight wire or loop in the plasma and stir gently with the homogenous suspension. If using a reusable loop, sterilize the loop before proceeding.

Note: Plasma is added only to the test strain and the control organisms but not the control (C) as it serves as an auto agglutination control.

5. Observe for immediate formation of white clumps

Result interpretation

Positive Result; - Visible clumping within 10s

Negative Result; - No visible clumping within 10s.

Note: The positive control species should show clumping only when emulsified in the plasma and the negative control species should not show clumping in either water (saline) or plasma.

Novobiocin test procedure

Principle; the mechanisms of novobiocin resistance includes inhibitions of protein and nucleic acid synthesis. It helps to differentiate *S.saprophyticus* from other coagulase negative *staphylococci* by the overnight disk test methods.

Procedures;

1. The test isolate should be 18-72hrs in pure culture. prepare a suspension of the test isolate equal to a McFarland standard 0.5 standard or equivalent
2. Immerse striate swabs in to the suspension and rotate it against the side of the tube above the fluid level to remove excise inoculum.
3. Using the swab inoculate in a Muller Hinton agar plate by streaking the swab over the entire agar surface.
4. Allow the agar surface to dry no more than 15minutes before applying a novobiocin disk.
5. Invert and incubate at 35 -37°C for 18 – 24 h.
6. Measure the zone of inhibition, if present, with a ruler or caliper.

Result interpretation

Inhibition zone ≤16mm = *Staphylococcus saprophyticus*

Inhibition zone >16mm = *Staphylococcus epidermidis*

Annex XII ANTIMICROBIAL SENSITIVITY TEST

Purpose: This procedure provides instructions to determine the drug sensitivity pattern of bacteria using Kirby-Bauer disk diffusion method.

Principle: the antibiotics will diffuse in a radial manner from the disc and will inhibit bacterial growth around it.

Materials: 0.5 McFarland standards, Muller Hinton agar, Normal saline, Test tube, sterile wooden applicator sticks with cotton, Antimicrobial disks, Measuring caliper/ruler and Candle jar.

Sample: 2ml pure colony equivalent to 0.5 McFarland.

Procedure:

1. Prepare pure colony suspension into normal saline equivalent to 0.5 McFarland standards.
2. Streak on appropriate media the entire surface.
3. Select antimicrobial agents according to the CLSI guideline & Put the disc on the plate aseptically
4. Incubate for 16 – 24 hrs at 35^0C +/- 2^oC
5. Measure zone of inhibition and interpret the result based on CLSI break point.

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 site of infection.
2. **Intermediate (I)** the ‗intermediate' category includes isolates with antimicrobial agent minimum inhibitory concentrations (MICs) that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates. The intermediate category implies clinical efficacy in body sites where the drugs are physiologically concentrated or when a higher than normal dosage of a drug can be used. This category also includes a buffer zone, which should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretation, especially for drugs with narrow pharmacotoxicity margin.
3. **Resistant (R)** the ‗resistant' category implies that isolates are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules, and/or that demonstrate MICs or zone diameters that fall in the range where specific microbial resistant mechanisms are likely, and clinical efficacy of the agent against the isolate has not been reliably shown in treatment studies.

Limitations: the response to antimicrobial therapy in vivo may not always reflect results of in vitro.

Procedural Notes: make sure the turbidity is equivalent to 0.5 McFarland and the thickness of the Mueller Hinton agar is 4 mm.

Clinical Utility: to detect the in vitro relationship between an organism and an antibiotic to predict the failure or success of therapy in vivo (in patient).

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