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SCHOOL OF GRADUATE STUDIES

"Bacterial Uropathogens and their Drug Resistance Pattern in Diabetic Patients Attending Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia"

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List of Abbreviations and Acronyms

AAHB	Addis Ababa Health Bureau
ASB	Asymptomatic Bacteriuria
ATCC	American Type Culture Collection
C_3	Complement 3
CFU	Colony Forming Units
CLED	Cystein-Lactose Electrolyte Deficient medium
CLSI	Clinical Laboratory Standards Institute
CONs	Coagulase Negative Staphylococci
DM	Diabetes Mellitus
FBS	Fasting Blood Sugar
G6PD	Glucose-6-Phosphate Dehydrogenase
HMIS	Health Management Information System
HPF	High Power Field (40x microscope objective)
IL-6	Interleukin-6
KIA	Kligler Iron Agar
NADPH	Nicotinamide-Adenine Dinucleotide (phosphorylated)
NCDs	Non-communicable diseases
SOPs	Standard Operating Procedures
UTI	Urinary Tract Infection
Y12 HMC	Yekatit 12 Hospital Medical College

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Operational Definitions

Diabetes: according to the World Health Organization's criteria, it is defined as a fasting plasma glucose ≥7.0 mmol/l (126 mg/dl) [1].

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

Significant bacteriuria: the presence of $\geq 10^5$ colony forming units (CFU) per milliliter of urine.

Pyuria: the presence of $\geq$10 leukocytes/HPF. An absence of pyuria on microscopic assessment can suggest colonization, instead of infection, when there is bacteriuria [2].

Abstract

Background: Urinary tract infection (UTI) is one of the most common infections encountered and treated worldwide. UTI has been reported to be around four times higher in diabetics compared to non-diabetic patients. Since diabetic patients are at a high risk of development of UTIs, it is recommended that special attention is paid to them, especially for the management of bacterial UTIs.

Objective: The aim of this study was to determine prevalence of significant bacteriuria and assess drug susceptibility pattern of the bacterial isolates in diabetic patients.

Materials and Methods: A cross sectional study was conducted in Yekatit 12 Hospital Medical College from April to July 2015. . In this study, 246 patients diagnosed with diabetes mellitus were recruited consecutively. A standard questionnaire was used to collect information regarding the socio-demographic status and clinical history of the study participants. Mid-stream urine was used for chemical analysis, microscopy and microbiological isolation and identification of bacteria. Standardized disc diffusion method was used for the determination of antibiotic sensitivity testing. Data were entered into and analyzed by SPSS version 20.

Results: Among the 246 study participants, 150 (60%) of them were females and 96 (40%) were males. The mean age of the study population was 51.5 years. Pyuria was detected in 27 (11%) of the urine specimens examined microscopically. Of the 246 urine specimens cultured on CLED and blood agar plate, 17(6.9%) of them showed significant bacteriuria. Among the 17 bacterial isolates, *E. coli* was the most frequently isolated one, accounting 10 (53%), followed by Klebsiella species 4 (21%). All the isolates showed 100% sensitivity to nitrofurantoin, except Proteus species, which exhibited resistance to the antibiotic. The multidrug resistant rate of the isolates in our study was 31.6%

Conclusion and Recommendation: The overall prevalence of significant bacteriuria in our study was 6.9%. Even though Proteus species showed resistance to nitrofurantoin, all the remaining isolates were 100% sensitive to the antimicrobial. Use of antibiotics for treating UTI in DM patients should be supported with periodic culture and drug susceptibility test studies in a given geographical location.

1. Introduction

1.1 Background

Urinary tract infection (UTI) is one of the most common infections encountered and treated worldwide. Despite the widespread availability of antibiotics, urinary tract infection remains the most common bacterial infection in the human population with a high rate of morbidity. It has been estimated that 150 million people are infected with UTI per annum worldwide [3, 4].

Urinary tract infections include a spectrum of clinical entities in which the presence of bacteriuria is common. Other types of microorganisms such as viruses and fungi may also infect the urinary tract but usually do so under special circumstances of systemic infection or decreased host resistance. Asymptomatic bacteriuria, acute pyelonephritis and complications of UTI are reported to be more common in patients with diabetes [5].

Diabetes mellitus (DM) is a group of metabolic disorders characterized by increased blood glucose level resulting from defects in insulin secretion, insulin action, or both. It has become a major public health problem particularly with rapid increase in low and middle income countries [6, 7].

The increased frequency of UTIs in diabetic patients is likely due to several mechanisms including the presence of glycosuria, neutrophil dysfunction and increased adherence of the bacteria to uroepithelial cells [8].

Even though the precise mechanisms for the preference of pathogens to cause UTI in diabetics remains unclear, a few research have revealed that the reasons could be immunological impairments such as impaired migration of neutrophils, intracellular killing, phagocytosis, defects in the local urinary cytokine secretions (IL-8, IL-6), increased adherence of the microorganisms to the uroepithelial cells and chemotaxis of polymorphonuclear leukocytes from diabetic patients and neuropathic complications such as impaired bladder emptying. In addition, a higher glucose concentration in the urine acts as a favorable culture medium for pathogenic bacteria and promotes rapid bacterial colonization and growth [6].

Patients with diabetes are more prone to have resistant pathogens as the cause of their UTI. This might be due to several factors, including multiple courses of antibiotic therapy that are administered to these patients, frequently for asymptomatic or only mildly symptomatic UTI [9].

Antibiotics are usually given empirically before the laboratory results of urine culture are available. To ensure appropriate therapy, current knowledge of the organisms that cause UTI and their antimicrobial susceptibility is mandatory [3].

1.2 Statement of the Problem

Non-communicable diseases (NCDs) were responsible for 68% (38 million) of all deaths globally in 2012, up from 60% (31 million) in 2000. The four main NCDs are cardiovascular diseases, cancers, diabetes and chronic lung diseases. Communicable, maternal, neonatal and nutrition conditions collectively were responsible for 23% of global deaths, and injuries caused 9% of all deaths [10].

Diabetes is one of the most common non-communicable diseases (NCDs). It is the fourth or fifth leading cause of death in most high-income countries and there is substantial evidence that it is epidemic in many economically developing and newly industrialized countries. It is undoubtedly one of the most challenging health problems of the 21st century. Studies continue to confirm that it is the low and middle income countries that face the greatest burden of diabetes [7].

Some 387 million people worldwide are estimated to have diabetes. About 80% live in low- and middle-income countries. If these trends continue, by 2035, some 592 million people, or one adult in 10, will have diabetes. This equates to approximately three new cases every 10 seconds or almost 10 million per year. The largest increases will take place in the regions where developing economies are predominant (for example an increase by nearly 110% is expected in sub-saharan Africa) [7, 11].

Currently, an estimated 22 million adults in the Africa Region have diabetes. Some of Africa's most populous countries have the highest numbers of people with diabetes, including: Nigeria (3.8 million), South Africa (2.7 million), Ethiopia (2.1 million), and the United Republic of Tanzania (1.8 million). More than half of all people with diabetes in the Region live in just four of these high-population countries [11].

The incidence of diabetes mellitus throughout the world is increasing strikingly and is becoming a serious public health problem, especially in the developing countries. Diabetes mellitus is associated with many complications and in the long run it has some major effects on the genitourinary system which makes diabetic patients more liable to UTI. UTI has been reported to

be around four times higher in diabetics compared to non-diabetic patients. Patients either with Type1 DM or Type 2 DM are at increased risk for urinary tract infection [6, 12].

Estimates of the global burden of disease indicate that diseases of the kidney and urinary tract account for approximately 830,000 deaths and 18,467,000 disability-adjusted life years annually, ranking them 12th among causes of death (1.4 percent of all deaths) [13].

Urinary tract infection is the most common infectious disease after respiratory tract infection in community. It remains a major public health problem in terms of morbidity and financial cost [14].

Ninety five percent of UTIs are caused by uropathogens. It is a serious clinical problem for people with DM. Individuals with diabetes mellitus are reported to have increased risk of UTI both in frequency and severity. Hospitalization for pyelonephritis occurs 15 times more frequently in diabetic patients [15, 16].

Since diabetic patients are at a high risk of development of UTIs, it is recommended that special attention is paid to them, especially for the management of bacterial UTIs [17].

Many times, physicians resort to prescribing broad-spectrum antibiotics over specific antibiotics in the view of resistance of the causative organism to the antibiotic. Poor patient compliance and incomplete course of antibiotic therapy have resulted in the evolution of resistance to many of these antibiotics [18].

The distribution of antimicrobial susceptibility data of UTI causing microorganisms changes from time to time and from place to place [4].

Since most UTIs are treated empirically, the criteria for the selection of antimicrobial agents should be determined on the basis of the most likely pathogen and its expected resistance pattern in a geographic area. Therefore; there is a need for periodic monitoring of etiologic agents of UTI and their resistance pattern [3].

Therefore, screening for UTI in diabetic patients is very important to enable bacteriuria to be properly treated, and prevent the development of renal complications of diabetes and eventually severe renal damage and failure [19].

The studies regarding uropathogens and their antimicrobial susceptibility patterns in diabetic patients, in Ethiopia, are limited. Thus; this study aims in providing the existing information on bacterial etiologies of urinary tract infection and their antimicrobial susceptibility pattern in diabetic patients attending Yekatit 12 Hospital Medical College.

1.3 Significance of the Study

Investigation of bacteriuria in diabetic patients for urinary tract infection is important for treatment and prevention of renal complications. Increasing multidrug resistance in bacterial uropathogens is an important and emerging public health problem. This needs regular monitoring of the antibiotic susceptibility of uropathogens in a particular area.

The successful management of UTI in diabetics depends on the proper identification of the bacteria responsible and the selection of effective antibiotics against them. The indiscriminate use of antibiotics often results in the increased resistance of urinary pathogens to most commonly used antimicrobials.

This study will help provide the current knowledge about the type of bacteria responsible for UTIs and their susceptibility patterns to common antibiotics in diabetic patients, in the Ethiopian setting. It will also have an immense value for the clinicians to choose the right empirical treatment and manage bacterial UTI in diabetic patients.

2. Literature Review

Urinary tract infections are defined as diseases which are caused by a microbial invasion of the genitourinary tract, which extends from the renal cortex of the kidney to the urethral opening. They represent the most commonly acquired bacterial infections and they account for an estimated 25-40% of the nosocomial infections [20].

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 [21].

The incidence of UTI as a result of viral or fungal infection is considered to be rare phenomena. Though the infection seems to be harmless in the initial stages, the patient shows a variety of symptoms as the stage progresses and can lead to death in severe circumstances. Research studies have defined urinary tract infection as the most common form of bacterial infection [22].

The predominant pathogen responsible for UTI is *E. coli* which constitutes up to 80-85% and is followed by *Staphylococcus saprophyticus* which accounts to 5-10%. In addition to the above mentioned bacterial species, *Klebsiella*, *Proteus*, *Pseudomonas* and *Enterobacter* are associated with UTI. The bacteria enter the bladder through urethra and the infection can also occur through blood and lymph [23].

The clinical manifestation of UTI depend upon the portion of the urinary tract involved, the etiologic organism, the severity of the infection and patients ability to mount an immune response to it. Signs and symptoms include fever, dysuria, and urinary urgency, cloudy or fragrant urine. UTI is an extremely common condition that occurs in both male and female [24].

UTI can affect lower and sometimes both lower and upper urinary tracts. The term cystitis has been used to define the lower UTI infection and is characterized by symptoms such as dysuria, frequency, urgency, and suprapubic tenderness. The presence of the lower UTI symptoms does not exclude the upper UTI which is often present in most UTI cases [4].

Bacteriuria, or the presence of bacteria in urine, is associated with both asymptomatic and symptomatic urinary tract infection and underpins much of the dynamic of microbial colonization of the urinary tract [25].

Urinary tract infection or UTI is said to exist when a significant number of microorganisms, usually greater than 10^5 cells per millilitre of urine, are detected in properly collected mid-stream "clean catch" urine [26].

There is evidence that patients with diabetes have an increased risk of asymptomatic bacteriuria and urinary tract infections (UTIs). UTI is the most common bacterial infection in diabetic patients [27].

An association between urinary tract infection (UTI) and diabetes mellitus was noted in an autopsy series reported in the 1940s. The urinary tract is the principal site of infection in diabetes. Changes in host defense mechanisms, the presence of diabetic cystopathy and of microvascular disease in the kidneys may play a role in the higher incidence of UTI in diabetic patients [28].

Diabetes mellitus has a number of effects on genitourinary system. Urinary Tract Infection (UTI) is more common in diabetics because of a combination of host and local risk factors. Under some circumstances urine may be inhibitory or even bactericidal against uropathogens. Modification of chemical composition of urine in diabetes mellitus can alter the ability of urine and support the growth of microorganisms. Autonomic neuropathy in diabetes mellitus impairs bladder emptying and subsequent urological manipulation predispose to UTI [29].

The reason for this predisposition is not completely understood. Studies are limited, however, evidence of some reports have shown that diabetes affects many systems that protect against infection in general and against urinary tract infections specifically. Poor circulation of blood in diabetes reduces the ability of infection fighting white blood cells to get to their target site, even when they do get there, they are less able to ingest the offending bacteria and kill them than

normal white blood cells. Many people with diabetes also have dysfunctional bladders that contract poorly. This allows urine to remain in static pools for long periods of time, providing ponds for bacteria to grow in [30].

Weakened leukocyte phagocytosis has been found in both Type 1 and Type 2 DM and explains the diminished infecting bacterial clearance during hyperglycemia as observed in mouse models. Phagocytosis was reduced by 50% in the leukocytes in diabetic mice infected by *Staphylococcus aureus*. The main factor of neutrophil dysfunction is the hyperglycemia, which alters neutrophil chemotaxis, phagocytic action and intracellular killing of the bacteria. Respiratory burst by monocytes from hyperglycemic patients has been affected. Humoral immunity in diabetic patients can be affected as well, which can be shown by a shorter duration of the protective antibodies after tetanus vaccination and lower levels of tetanus antitoxin compared with those in non-diabetic subjects. Alterations in levels of complement fractions can also be found. Hyperglycemia suppresses activation of C3 fraction, which results in changed C_3 tertiary structure and impairs the interactions of C3 with the bacterial pathogens [31].

Decreased mobilization of polymorphonuclear leukocytes, chemotaxis, and phagocytic activity may occur during hyperglycemia. The hyperglycemic environment also blocks the antimicrobial function by inhibiting glucose-6-phosphate dehydrogenase (G6PD), increasing apoptosis of polymorphonuclear leukocytes, and reducing polymorphonuclear leukocyte transmigration through the endothelium. In tissues that do not need insulin for glucose transport, the hyperglycemic environment increases intracellular glucose levels, which are then metabolized, using NADPH as a cofactor. The decrease in the levels of NADPH prevents the regeneration of molecules that play a key role in antioxidant mechanisms of the cell, thereby increasing the susceptibility to oxidative stress [32].

Various studies done worldwide have shown changing patterns in the etiology of UTIs in diabetic patients [18].

In a cross sectional study conducted in Nepal to assess the spectrum of uropathogens and their antibiotic sensitivity pattern in diabetic patients, the overall culture positivity rate was 34.5%. *Escherichia coli* was the most frequent organism (64.5%) followed by Klebsiella sps (22.6%). *E.*

coli was highly sensitive to gentamicin and nitrofurantoin among the tested antimicrobials followed by cotrimoxazole, norfloxacin and ciprofloxacin. Least sensitivity rate was observed with ampicillin and cephalexin [33].

In another study conducted in Nepal to determine the prevalence of UTI among diabetic patients, the causative pathogens & their antimicrobial susceptibility pattern the overall prevalence of UTI was found to be 54.76% from the total of 462 diabetic patients. The gram positive isolates and *Escherichia coli* were found to be sensitive to nitrofurantoin [6].

A prospective study done in India to know the bacteriological and resistance profile of isolates obtained from diabetic patients showed that 18 of 65 (27.7%) had UTI. Twenty six bacterial species were isolated from urine samples. The most frequently isolated species was *E.coli* followed by *K. Pneumoniae* [34].

In a study conducted, for two years in Mysore (Southern India), to determine the prevalence and incidence of urinary tract infection among diabetic patients, about 900 samples were culture positive and 936 isolates were obtained out of 1085 urine samples. *Escherichia coli* was the major cause for urinary tract infection in both type 1 (34%) and type 2 (32%) diabetic patients, followed by Methicillin resistant *Staphylococcus aureus* (11.4% and 12.6%) [35].

In a study conducted in Karnataka, India, to determine the frequency of uropathogens and their antibiotic susceptibility in different gender of diabetic patients found that from the total of 44 urine samples 68% of the patients were having asymptomatic bacteriuria. Biochemical characterization revealed prevalent gram negative organisms and *E. coli* as the predominant isolate. Among the antibiotics tested, trimethoprim was found to be effective for empirical treatment of UTI and has covered the majority of urinary pathogens followed by nalidixic acid, Chloramphenicol and kanamycin. Most of the isolates were resistant to oxytetracycline [36].
In a prospective study carried out in Government Medical College Amritsar-India to find out the prevalence of uropathogens in diabetic patients and to study their antibiotic susceptibility pattern bacterial growth was obtained in 118 (43%) cases out of the 270 urine specimens from diabetic patients. Among these 50.84% (60/118) had asymptomatic bacteruria while symptomatic infection occurred in 49.15 % (58/118) patients. *Escherichia coli* was the most frequent isolate

(41.5%), followed by *Klebsiella species* (14.4%), *Proteus species* (10.1%), *Enterococci* (8.4%), *Pseudomonas aeruginosa* (6.7%), Staphylococcus species (10.1%) and *Candida albicans* (8.4%). The isolates showed highest resistance against amoxicillin and ciprofloxacin, moderate resistance against norfloxacin, nitrofurantoin, gentamicin and cefotaxime while resistance against amikacin was low [37].

In a study done to assess prevalence of urinary tract infection and risk factors among Saudi patients with diabetes, the prevalence of UTI was 25.3 % from the total of 1,000 diabetic patients and 7.2 and 41.1 % in males and females, respectively [38].

In another study conducted in National Center of Diabetes- Baghdad, Iraq on 122 patients with diabetes mellitus to determine the prevalence of uropathogens and their antibiotic susceptibility significant bacteriuria was seen in 60(49.1%) patients. Among the isolates 45 were gram negative bacilli and 15 were gram positive cocci. Among the gram negative bacilli *E. coli* w*as* the predominant isolate (60%) while *Pseudomonas aeruginosa* was the least (1.66%). *E.coli* and *Klebsiella pneumoniae* showed highest sensitivity to piperacyclin followed by nitrofurantoin. *Staphylococcus aureus* had highest sensitivity to ciprofloxacin and cefoxitin (75%) and the highest resistance to chloramphenicol (91.7%). Most bacterial isolates exhibited complete resistance to Tetracyclin (100%), only *Enterobacter* species had sensitivity to Tetracyclin (33.3%) [16].

A study conducted at Buea and Limbe Regional Hospital Diabetic and Hypertensive Clinic in Cameroon, to investigate the prevalence of asymptomatic bacteriuria and UTIs in clinically diagnosed patients with diabetes and to determine the uropathogens responsible for ASB (asymptomatic bacteriuria) and UTIs as well as their antimicrobial susceptibility pattern, revealed that one hundred and two (81.6%) of the total 125 urine samples had significant bacteriuria. Eight different bacteria were isolated from the study participants: *Escherichia coli* (48.0%) were the most prevalent, followed by *Staphylococcus aureus* (19.6%) and *Proteus mirabilis* (8.9%). Most of the bacterial isolates were highly sensitive to gentamicin (88.6%), imipenem (87.9%), nitrofurantoin (79.5%) and amikacin (88.3%). Some of the bacterial isolates

showed 53.3% resistance rate while all the bacterial isolates were highly resistant (96.3%) to amoxicillin [39].

A cross sectional study conducted at Muhimbili National Hospital- Dar es Salaam, Tanzania to determine the prevalence and risk factors of bacteriuria in diabetic women and antimicrobial resistance pattern of the isolates revealed 13.7% (41/300) significant bacteriuria, of which 13.4% (31/231) and 14.5% (10/69) were asymptomatic and symptomatic bacteriuria, respectively. The isolated pathogens were *Escherichia coli* (39.0%), *Klebsiella pneumoniae* (22.0%), coagulase negative Staphylococci (14.65%) and *Proteus spp* (12.2%). Both Gram positive and negative bacteria showed high rate of resistance towards co-trimoxazole (55.6% and 50.0%, respectively). Gram negative bacteria showed high rate of resistance to ampicillin (62.55%), penicillin (53.1%) and moderate resistance to cefotaxime (18.8%). Most uropathogens were resistant to co-trimoxazole, ampicillin and ciprofloxacin [40].

A prospective cross sectional study conducted in Gondar University Hospital, Ethiopia, on a total of 422 diabetic patients with asymptomatic UTI (n=387) and symptomatic UTI (n=35), to investigate for urinary tract infection showed 17.8% significant bacteriuria, of which 14.7% were asymptomatic and 51.4% were symptomatic. Out of the total 82 bacterial isolates, *E. coli* (31.7%), coagulase negative staphylococci (CONs) (22%), *Klebsiella spp*. (14.6%), *Enterococcus spp*. (11%) and *S. aureus* (8.5%) were the commonest bacterial uropathogens in both groups. All gram negative isolates showed intermediate level of resistance (60-80%) against ampicillin and chloramphenicol. Low level of resistance (<60%) was observed against amoxicillin-clavulanic acid, ciprofloxacin, ceftriaxone, gentamicin and trimethoprim-sulphamethoxazole. High level of resistance (>80%) was observed against tetracycline. Gram-positive bacteria showed low level of resistance (<60%) to all antimicrobials tested except for tetracycline [41].

3. Objectives

3.1 General Objective

To determine the prevalence of significant bacteriuria and assess the antimicrobial susceptibility pattern of the isolates in diabetic patients at Yekatit 12 Hospital Medical College from April to July 2015.

3.2 Specific Objectives

- To determine the prevalence of significant bacteriuria in diabetic patients.
- To assess the antimicrobial susceptibility pattern of bacterial isolates in diabetic patients.
- To determine the prevalence of pyuria in diabetic patients.

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4. Materials and Methods

4.1 Study area

The study was conducted in Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia. It is located in the Arada Sub-City of City Government of Addis Ababa. The hospital is established in 1915 with a total of 25 beds and 37 health professionals. According to the data obtained from the hospital, currently the hospital has 507 health professionals, 375 administrative staff and around 272 beds, and provides different medical services for around 4 million population. It's the only hospital under the City Government of Addis Ababa where there is culture and drug susceptibility test service. According to the HMIS data obtained from the hospital, about 943 patients were diagnosed with diabetes mellitus in the year 2006 E.C (2013/2014).

4.2 Study design and period

A cross sectional study was conducted from April to July 2015.

4.3 Source population

The source population was all diabetic patients visiting the study area.

4.4 Study population

All diabetic patients visiting the study area during the study period and fulfilled inclusion criteria were the study population.

4.5 Patient inclusion and exclusion criteria

4.5.1 Inclusion criteria

Confirmed diabetic patients who visited the diabetic center of Yekatit 12Hospital Medical College, for their follow up, during the study period were included as study subjects.

4.5.2 Exclusion criteria

Those diabetic patients who refused to give their consent were excluded. Diabetic patients who were on antibiotic treatment in the last two weeks of their visit at enrolment to the study were also excluded.

4.6 Study variables

4.6.1 Dependent variable

- Prevalence of significant bacteriuria
- Antimicrobial susceptibility pattern
- Prevalence of pyuria

4.6.2 Independent variable

- Diabetes mellitus
- Type of diabetes mellitus
- Duration of diabetes
- Comorbidity
- Fasting blood glucose level
- Sign and symptom of urinary tract infection
- Age
- Gender

4.7 Sample size determination

The sample size was calculated using the formula for single proportion sample size calculation. Taking a 95% confidence level and a 5% margin of error the sample size was calculated as follows.

$$n = \frac{Z^2_{\alpha/2}\, p(1-p)}{d^2}$$

$$= \frac{(1.96)^2\, 0.178(1-0.178)}{(0.05)^2}$$

$$n = 224$$

Where; n = sample size

P = estimated prevalence of significant bacteriuria (obtained from previous study) [41]

Z = standard normal value at 95% confidence interval=1.96

d = margin of error

Taking 10% non-response rate into account, the overall sample size was 246.

4.8 Sampling technique

A convenient sampling technique was employed. Study participants were recruited consecutively until the required sample size was achieved.

4.9 Data collection and processing

4.9.1 Socio-demographic Information

A standard questionnaire was used to collect information regarding the socio-demographic status and clinical history of the study participants.

4.9.2 Specimen collection

Midstream urine sample was collected from diagnosed diabetics attending outpatient diabetic clinic at Yekatit 12 Hospital Medical College from June to July, 2015. Patients were consulted to bring “mid-stream” urine of 15–20 ml volume to the microbiology unit of Yeketit 12 Hospital Medical College Laboratory. All urine specimens were obtained in a well labeled, sterile, dry, wide-necked, leak proof and screw capped universal container.

4.9.3 Specimen processing

Chemical test and microscopical examination of urine

After inoculating onto a culture, about 10 mL of each urine sample was taken for chemical test and microscopic examination. Chemical test was carried out using dipstick method. Wet preparation for the presence of pyuria was examined microscopically after centrifugation at 3000 revolutions per minute for 5 minutes.

Culture

Using a calibrated loop, a volume of 0.001 mL, the un-centrifuged well mixed urine specimen was streaked onto cystein-Lactose electrolyte deficient medium (CLED) and blood agar plate and was incubated at 37°C for 24 hrs. A specimen was considered positive for UTI if a single organism was cultured at a concentration of $\geq 10^5$ colony-forming units/mL. The gram positive and gram negative bacterial isolates were further identified by using various biochemical reactions.

Identification of uropathogens

Identification of the isolated bacterial pathogens was done on the basis of culture morphology and biochemical characters. Kligler iron agar (KIA), indole test, citrate, urea, coagulase, catalase, motility agar and lysine iron agar were used. Novobiocin disc was used to differentiate between *S. saprophyticus* and *S. epidermidis*.

Antimicrobial susceptibility testing

For the positive cultures, a suspension, which is equivalent with 0.5 MacFarland's standard, from pure colony was prepared by using a 0.5 MacFarland's standard densitometry. Using a sterile swab the Muller Hinton agar plates were inoculated. Appropriate antimicrobial discs were put on the agar. The antimicrobial discs used were Ampicillinn 10 µg, Amikacin 30 µg, Ceftazidime 30 µg, Cefepime 30 µg, Cefixime 5 µg, Cefotaxime 30 µg, Ciprofloxacin 5 µg, Cotrimoxazole 25 µg, Gentamycin 10 µg, Meropenem 10 µg, Nitrofurantoin 300 µg, Norfloxacin 10 µg, Tobramycin 10 µg and vancomycin 30 µg.

The plates were incubated at 37^0C for 16 to 18 hours. The antimicrobial susceptibility of the isolated pathogens was determined by using Kirby Bauer Disc Diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guideline [42].

4.10 Quality control

The urine collection container was labeled and dated. Patients were well instructed to collect a midstream urine sample. The collected urine specimen was transported promptly to the microbiology laboratory. Sterility and performance of culture media were tested prior to the actual work. Sterility of the media was checked by incubating overnight at 37^0C. Performance of CLED medium and blood agar plate was tested using the control strains *E. coli* ATCC25922, *P. mirabilis* ATCC35659 and *S. aureus* ATCC25923, *P. pneumonia* (patient strain); respectively. For biochemical test, the media were inoculated with bacterial species of known positive and negative reactions. In the antimicrobial susceptibility testing the reference strains used as control were *E. coli* ATCC25922 and *S. aureus* ATCC25923. Moreover; Culture growth, biochemical test and antimicrobial susceptibility test results were confirmed by an experienced medical laboratory technologist working in the microbiology laboratory unit of the study area.

4.11 Data management

Completed questionnaires, microscopic examination, culture and antimicrobial susceptibility test results were coded by numbers and entered in a computer software SPSS version 20. Cross-checking and data cleaning was done. During data cleaning and cross checking, missing information was obtained by going back to the questionnaire and laboratory records. The data was also stored in a CD as a backup.

4.12 Statistical analysis

Data were entered into and analyzed by SPSS version 20. Simple tables and descriptive statistics using frequency were used to present different findings. Chi-square and multivariate logistic regression analysis was also used to see an association with different variables. Probability values (P) of < 0.05 were considered statistically significant.

4.13 Dissemination of results

The findings of this study were forwarded to the Department of Medical Laboratory Sciences, School of Allied Health Sciences, Addis Ababa University. And an attempt will be made to present the findings in different conferences and will be sent for publication.

4.14 Ethical consideration

Before starting the study, ethical clearance was obtained from Departmental Research and Ethics Review Committee (DRERC) of Medical Laboratory Sciences, Addis Ababa University and Addis Ababa Health Bureau (AAHB). Informed consent from Yekatit 12 Hospital Medical College and written consent from the study participants was also obtained. The confidentiality of the information collected was maintained by using code numbers for participants. In addition, the clinical specimens collected during the study period were used only for the stated objectives. For those participants found to have significant bacteriuria, results were sent to the responsible clinician as soon as possible.

5. Results

5.1 Study subjects

From the total of 246 study participants, 150 (61%) of them were females and 96 (39%) were males. The mean age of the study population was 51.5 years. Greater numbers of them (40%) were in the age group of 47 to 62 years. About 90% of the participants were type 2 diabetes mellitus patients. The mean duration of diabetes mellitus was 8.9 years. 59% of the participants did not complain for the signs and symptoms of urinary tract infection.

Table 1: Characteristics of Study Participants, Yekatit 12 HMC, June to July, 2015

Variable	Categories	Number	Percent
Gender			
	Male	96	39
	Female	150	61
Age (in year)			
	15 - 30	33	13.4
	31 - 46	53	21.5
	47 - 62	98	39.8
	> 62	62	25.2
Education			
	No school	36	14.6
	Religious school only	30	12.2
	Primary school	75	30.5
	High school	62	25.2
	Higher education	43	17.5
Marital status			
	Single	37	15
	Married	164	66.7
	Divorced	45	18.3
Type of DM			
	Type 1	25	10.2
	Type 2	221	89.8
Blood glucose level			
	<126 mg/dl	76	30.9
	≥126 mg/dl	170	69.1
Duration of DM			
	≤5 years	100	40.7
	> 5 years	146	59.3
Type of drug			
	Oral drug	103	41.9
	Insulin	143	58.1
Co-morbidity			
	None	149	60.5
	Hypertension	96	39

	Goiter	1	0.4
Signs and symptoms of UTI			
	No	146	59.3
	Yes	100	40.7

5.2 Urinary tract infection

5.2.1 Culture

Of the 246 urine specimens cultured on CLED and blood agar plate 17(6.9%) of them showed significant bacteriuria. From these significant bacteriuria culture results, female diabetic patients account 14 (82.4%).

Table 2: **Distribution of Significant Bacteriuria by Study Participant Characteristics, Yekatit 12 HMC, June to July, 2015**

Variables	Bacterial Culture Growth		Total
	No Significant Bateriuria	Significant Bateriuria	
Gender			
Male	93	3	96
Female	136	14	150
Total	229	17	246
Age			
15 - 30	31	2	33
31 - 46	49	4	53
47 - 62	91	7	98
> 62	58	4	62
Type of Diabetes			
Type 1	23	2	25
Type 2	206	15	221
Duration of Diabetes			
Up to 5	94	6	100
> 5	135	11	146
Blood Glucose Level			
< 126 mg/dl	68	8	76
≥ 126 mg/dl	161	9	170

Presence of Comorbidity			
No	141	8	149
Yes	88	9	97
Signs and Symptoms of UTI			
No	137	9	146
Yes	92	8	100

5.2.1.1 Bacterial isolates

Among the 19 bacterial isolates, *E. coli* was the most frequently isolated one accounting 10 (53%), followed by Klebsiella species (21%).

Table 3: Bacterial Isolates, Yekatit 12 HMC, June to July, 2015

Bacteria	Number	Percent
E. coli	10	53
Klebsiella spp	4	21
Proteus spp	1	5
Citrobacter spp	1	5
S. saprophyticus	1	5
Enterococcus spp	2	11
Total	19	100

5.2.2 Microscopy

Pyuria was detected in 27 (11%) of the total 246 urine specimens examined microscopically. Most of (77.8%) the pyuria detected was from female participants.

Table 4: Distribution of Pyuria by Study Participant Characteristics, Yekatit 12 HMC, June to July, 2015

Variables	White Blood Cells per HPF		Total
	Below 10 WBCs	≥ 10 WBCs	
Gender			
Male	90	6	96
Female	129	21	150
Age			
15 - 30	30	3	33
31 - 46	49	4	53
47 - 62	86	12	98
> 62	54	8	62
Type of Diabetes			
Type 1	23	2	25
Type 2	196	25	221
Duration of Diabetes			
Up to 5	88	12	100
> 5	131	15	146
Blood Glucose Level			
< 126 mg/dl	70	6	76
≥ 126 mg/dl	149	21	170
Presence of Comorbidity			
No	132	17	149
Yes	87	10	97
Signs and Symptoms of UTI			
No	135	11	146
Yes	84	16	100

5.2.3 Chemical test

From the 246 urine specimens tested chemically for leukocyte, nitrite and protein, 24 (9.8%) of them were either leukocyte esterase positive or nitrite positive.

Table 5: Urine Chemical Characteristics of Study Participants, Y12 HMC, June to July, 2015

Variable	Categories	Number (%) n = 246
Nitrite test	Negative	238 (96.7)
	Positive	8 (3.3)
Leukocyte esterase test	Negative	230 (93.5)
	Positive	16 (6.5)
Protein	Negative	234 (95.1)
	Positive (1^+ to 3^+)	12 (4.9)

5.3 Association of UTI with some variables

UTI showed a statistically significant association with nitrite (χ^2 = 31.29; df = 1; p = 0.00), leukocyte (χ^2 = 20.06; df = 1; p = 0.00) and pyuria (χ^2 = 28.5; df = 1; p = 0.00).

Table 6: Association of UTI with some Variables, Yekatit 12 HMC, June to July, 2015

Variable	¡ ^{2}	df	P value
Gender	2.61	1	0.106
Type of DM	0	1	1.0
Blood glucose level	1.5	1	0.22
Duration of diabetes	0.04	1	0.83
Protein	3.80	1	0.051
Nitrite	31.29	1	0.00
Leukocyte	20.06	1	0.00
Pyuria	28.5	1	0.00

5.4 Antimicrobial susceptibility testing

5.4.1 The overall sensitivity rate of isolates

Among the antimicrobials tested, both gram negative (93.8%) and gram positive bacteria (100%) showed high sensitivity to nitrofurantoin.

Table 7: The overall sensitivity rate of isolates, Yekatit 12 HMC, June to July, 2015

Antimicrobials	Bacterial isolates	
	Gram negative (n = 16)	Gram positive (n = 3)
Ampicillin	5 (31.3%)	3 (100%)
Cefotaxime	13 (81.3%)	1 (100%) n = 1
Ceftazidime	12 (75%)	0 n = 1
Ciprofloxacin	13 (81.3%)	3 (100%)
Cotrimoxazole	13 (81.3%)	1 (100) n = 1
Gentamicin	15 (93.8%)	
Amikacin	16 (100%)	-
Cefixime	14 (87.5%)	
Cefepime	14 (87%)	
Meropenem	16 (100%)	
Nitrofurantoin	15 (93.8%)	3 (100%)
Norfloxacin	-	2 (100%) n = 2
Tobramycin	15 (93.8%)	-
Vancomycin	-	1 (50%) n = 2

5.4.2 Sensitivity rate of individual isolates

E. coli was 100% sensitive to nitrofurantoin, meropenem and amikacin, but showed only 20% sensitivity to ampicillin. All the isolates showed 100% sensitivity to nitrofurantoin, except Proteus species, which exhibited resistance to the antibiotic.

Table 8: The Sensitivity Percent of Isolates to Different Antibiotics, June to July, 2015

Antibiotic Discs	E. coli (n = 10)	Klebsiella spp (n = 4)	Proteus spp (n = 1)	Citrobacter spp (n = 1)	S. saprophyticus (n = 1)	Enterococcus spp (n = 2)
Ampicillin	2 (20)	1 (25)	1 (100)	1 (100)	1 (100)	2 (100)
Cefotaxime	8 (80)	3 (75)	1 (100)	1 (100)	1 (100)	-
Ceftazidime	8 (80)	2 (50)	1 (100)	1 (100)	0	-
Ciprofloxacin	7 (70)	4 (100)	1 (100)	1 (100)	1 (100)	2 (100)
Cotrimoxazole	8 (80)	3 (75)	1 (100)	1 (100)	1 (100)	-
Gentamicin	9(90)	4 (100)	1 (100)	1 (100)	1 (100)	-
Amikacin	10 (100)	4 (100)	1 (100)	1 (100)	-	-
Cefixime	8(80)	4 (100)	1 (100)	1 (100)	-	-
Cefepime	8(80)	4 (100)	1 (100)	1 (100)	-	-
Meropenem	10(100)	4 (100)	1 (100)	1 (100)	-	-
Nitrofurantoin	10(100)	4 (100)	0	1 (100)	1 (100)	2 (100)
Norfloxacin	-	-	-	-	-	2 (100)
Tobramycin	9(90)	4 (100)	1 (100)	1 (100)	-	-
Vancomycin	-	-	-	-	-	1 (50)

The numbers in parentheses represent percent

5.4.3 Multidrug resistance pattern of isolates

Among all the isolates tested only *E. coli* and Klebsiella spps showed resistance to two or more antimicrobials.

Table 9: Distribution of Multidrug Resistant Bacterial Uropathogens, June to July, 2015

Bacterial isolates	Number of isolates	Multi-drug resistant isolates	
		Number	Percent
E. coli	10	4	40
Klebsiella spp	4	2	50
Proteus spp	1	0	0
Citrobacter spp	1	0	0
S. saprophyticus	1	0	0
Enterococcus spp	2	0	0
Total	19	6	31.6

6. Discussion

In our study the prevalence of significant bacteriuria was 6.9%. Similar prevalence (10.9%) was reported by Yeshitela et al, conducted in Tikur Anbessa Specialized University Hospital (43), and by Chita et al (10.7%) (27). The finding of our study is lower than those reported by Gizachew Y et al (17.8%), conducted in Gondar University Hospital, in Ethiopia (41) and many other studies (16, 29, 30, 33 and 34) which revealed a UTI prevalence of 49.1%, 45%, 21%, 34.5% and 27.7%; respectively. The difference in the prevalence rate may be due to sample size variation. Even though it was not statistically significant, in our study significant bacteriuria was detected more frequently in females (82.4%) than in males (17.6%). Other studies (16, 17, 29 and 30) reported similar results.

The most frequently isolated uropathogen in our study was E. coli 10(53%), followed by Klebsiella species 4(21%). Our finding is in agreement with other studies done in Ethiopia and many other countries (16, 17, 30, 33, 43), but differs from the finding recorded by Gizachew et al done in Northwest Ethiopia, which revealed CONs as the second most frequently isolated bacteria (41). Other bacterial isolates such as Enterococcus spp, *S. saprophyticus*, Proteus spp, and Citrobacter spps accounted 2(11%), 1(5%), 1(5%) and 1(5%); respectively. Similar findings were reported by different studies (19, 27, and 37).

The present study revealed a pyuria prevalence of 11%. No studies were found, which were done on both male and female diabetic patients, but when compared to findings specific to diabetic females our finding is lower than previous studies by Hussein et al (44) and the one conducted by Manhal et al (45) in Iraq, which reported a prevalence of 32% and 30%; respectively. In this study pyuria was more prevalent in females (14%) than in males (6.2%). The signs and symptoms of UTI were found to have a marginal statistical significant association with pyuria ($\chi^2 = 3.53$; df = 1; p = 0.06).

In this study any of the possible factors such as gender, type of DM, duration of diabetes mellitus and blood glucose level did not show statistically significant association with the UTI. A study by Al-Rubeaan et al (38) revealed that blood glucose level and duration of DM had no statistically significant association with UTI, which is in agreement with our study. However;

our finding differs from a study conducted by Gizachew et al (41) which recorded a statistically significant association of UTI with type of diabetes mellitus and blood glucose level, and another study by Sowmya et al (35) which found a significant association of UTI with gender and type of DM. Even though no similar studies on diabetic patients were found to compare with, our study showed the presence of statistically significant association of UTI with proteinuria (χ^2 = 3.80; df = 1; p = 0.05), nitrite (χ^2 = 31.29; df = 1; p = 0.00) leukocyte esterase (χ^2 = 20.06; df = 1; p = 0.00) and pyuria (χ^2 = 28.5 df = 1; p = 0.00).

In our study, all the bacterial isolates (both gram negative and gram positive) were 100% sensitive to cotrimoxazole, except *E. coli* and Klebsiella species, which were 80% and 75% sensitive; respectively. Results from a study done by Getachew et al has agreed with our finding in that sensitivity rate of *E. coli* to cotrimoxazole was 76.9%, but revealed Klebsiella species having sensitivity rate of 16.7% to the antibiotic (41) which is much lower than our finding. Even though Proteus species showed resistance to nitrofurantoin, all the remaining isolates were 100% sensitive to the antimicrobial. In our study *E. coli* was found to be only 20% sensitive to ampicillin. Study done by Getachew et al also reported similarly low sensitivity level (38.5%) (41). A study conducted by Aswani et al in India (19) also found the same low level sensitivity rate of 16.7%. The multidrug resistant rate of the isolates in our study was 31.6%, which is different from those reported by other studies conducted in Ethiopia (41, 43) as 59.8% and 71.7%; respectively.

7. Limitations of the Study

- The study was conducted in a single hospital based center which may not necessarily represent the Ethiopian diabetics at large.
- Only bacterial pathogens were addressed.
- The cut-off point for significant bacteriuria was not based on the state of the patient.

8. Conclusion

The overall prevalence of significant bacteriuria in our study was 6.9%. Bacteriuria was more prevalent in females than males. Pyruia was prevalent in 27 (11%) of the total 246 DM patients. From the antimicrobial susceptibility test, all the other bacterial isolates (both gram negative and gram positive) were 100% sensitive to cotrimoxazole, except *E. coli* and Klebsiella species, which were 80% and 75% sensitive; respectively. Even though Proteus species showed resistance to nitrofurantoin, all the remaining isolates were 100% sensitive to the antimicrobial.

9. Recommendations

- Use of antibiotics for treating UTI in DM patients should be supported with periodic culture and drug susceptibility test studies in a given geographical location.
- Besides the culture and drug susceptibility test, urinalysis test may help health care providers for the better care and management of diabetic patients.
- Similar studies need to be conducted at a larger scale in different health care settings across the country to get a better picture of the magnitude of the problem.

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Annexes

Annex I: Informed Consent Sheet

1. Study Information Sheet

Hello, my name is ----------and I am working with Tebarek Lega on this study. The study aims in identifying the bacterial pathogens and determining their antimicrobial susceptibility causing urinary tract infection in diabetic patients. Hereby, provides useful information for the management of urinary tract infection in these patients. Currently; I am here & would like to ask you some questions and request you to bring a small amount of urine specimen (15-20mL). I will tell you how you can collect the urine and bring safely. Though it seems something procedural the study will help improve health of diabetic patients.

Your name will not be asked and unique identification is not required. If you want to withdraw from the study anytime along the process, you will not be obliged to continue or give reasons for doing so.

Refusing to participate or withdrawing from the study along the process will not have any consequences on you and the services provided to you. I would like to appreciate your help. If you have any question or anything that is not clear, please direct to Tebarek Lega, School of Medical Laboratory Sciences, AAU. Cell phone 0912-01-34-42; e-mail: tebarekk@gmail.com

If you are clear with the information provided and agree to participate please sign on the consent form attached.

2. Consent Form

I, the undersigned individual, am oriented about the objective of the study. I have informed that all of my information will be kept confidential and used solely for this study. In addition, I have been well informed that my name will not be asked and unique identification is not required. If I want to withdraw from the study anytime along the process, I will not be obliged to continue or give reasons for doing so. However, my agreement to participate in this study is with the assumption that, the information and the specimen that I provide will help greatly to the management of diabetic patients.

Signature:-_____ Date:-_____

Annex II: Amharic Version of Study Information and Consent Form

1. መረጃ ለጥናቱ ተሳታፊዎች

ጤና ይስጥልኝ! _____ እባላለሁ፡፡ በአዲስ አበባ ዩኒቨርሲቲ በተባረክ ለጋ በኩል በሚደረገው ጥናት አብሬ እሰራለሁ፡፡ የዚህ ጥናት ዋና አላማ የስኳር ህመምተኞች ብዙውን ጊዜ ለህመም የሚዳረጉበት የሆነው የሽንት ትቦ ኢንፌክሽን አምጪ ረቂቅ ተህዋሳትን በመለየት በሽታ አምጪ ተህዋሲያኑ ሊያክም የሚችል መድሃኒት መምረጥ በዚህም የስኳር ህመምተኞች አንዱ የጤና እክል የሆነውን የዚህን ኢንፌክሽን በአግባቡ መቆጣጠር እንዲቻል ማድረግ ነው፡፡ እዚህ አጠገባችሁ የሆንኩት የተወሰኑ ጥያቄዎች ለመጠየቅና መጠነኛ የሆነ የውሃ ሽንት ናሙና (15-20mL) እንድትሰጡኝ ነው፡፡ የውሃ ሽንቱ ለዚሁ ጥናት በተዘጋጀ እቃ ውስጥ አድርጋችሁ በጥንቃቄ የምታመጡበትን መንገድ እነግራችኋለሁ፡፡ ይህን ስናደረግ የርስዎ ስምም ሆነ የተለየ እርስዎን የሚለይ ሚስጥራዊ ቁጥር አንጠቀምም፡፡ በዚህ ጥናት እየተሳተፉ ባሉበት ድንገት ማቋረጥ ቢፈልጉ የማቋረጥ መብትዎ የተጠበቀ ነው፡፡ ለምን ማቋረጥ እንደፈለጉ ምክንያት እንዲያቀርቡም ሆነ ጥናቱ እንዲቀጥሉ አይገደዱም፡፡ በጥናቱ መሳተፍ ባለመፈለግዎ በእርስዎ ላይም ሆነ በሚያገኙት አገልግሎት ላይ የሚያመጣው ምንም አይነት ችግር አይኖርም፡፡ የእርስዎ በጥናቱ መሳተፍ ግን ለሚደረገው ጥናት ትልቅ እገዛ እንደሚሆን ሳልጠቀምዎት አላልፍም፡፡ ስለትብብርዎ ከልብ አመሰግናለሁ፡፡ ስለጥናቱ ለሚኖሮት ማንኛውም አይነት ጥያቄ አቶ ተባረክ ለጋን በስልክ ቁጥር 0912-01-34-42 ደውለው መጠየቅ ይችላሉ፡፡

ስለጥናቱ የተሰጠው መረጃ ግልፅ ከሆነልዎ እና በጥናቱ ለመሳተፍ ፈቃደኛ ከሆኑ እባክዎን ከዚህ ወረቀት ጋር በተያያዘው የስምምነት መግለጫ ፎርም ላይ ይፈርሙ፡፡

2. የስምምነት ማረጋገጫ ፎርም

እኔ ፊርማዬ በስተመጨረሻው ላይ የሚገኘው ግለሰብ የዚህ ጥናት አላማ ተገልፆልኛል፡፡ በተጨማሪም እኔ የምሰጠው መረጃም ሆነ ናሙና ለዚህ ጥናት ብቻ እንደሚዉልና በሚስጥር እንደሚያዝ ተገልፆልኛል፡፡

በዚህ ጥናት ለመሳተፍ ስምና ሌላ አድራሻ መግለፅ እንደማያስፈልገኝ ተረድቻለሁ፡፡ ከዚህ በተጨማሪም በጥናቱ ላለመሳተፍ መወሰን ወይንም በፈለግኩት ጊዜ ማቋረጥ እንደምችልና ሳቋርጥም ለማቋረጥ የፈለግኩበትን ምክንያት ለማስረዳት እንደማልገደድ እንዲሁም በጥናቱ ለመሳተፍ ፈቃደኛ አለመሆኔ ወይም በጥናቱ ሂደት ላይ ተሳታፊ ከሆንኩ በኋላ አቋርጬ መውጣቴ በእኔ ላይ የሚደርሰው አንዳችም ተፅእኖ እንደሌለ ተረድቻለሁ፡፡

ሆኖም እኔ በዚህ ጥናት ላይ ተሳታፊ ለመሆን ስስማማ በሚገኘው ጠቃሚ መረጃ የሽንት ትቦ ኢንፌክሽን በስኳር ህመምተኞች ላይ እያመጣ ያለውን ጫና ለመቀነስ የሚረዳ መሆኑን ተስፋ ለማድረግ ነው፡፡

ፊርማ፡_____ ቀን፡_____

Annex III: Questionnaire

General and socio-demographic questions

Date: _____

Code: _____

Gender:

☐ Male ☐ Female

Age _____

Education:

☐ No school ☐ Primary

☐ High school ☐ Higher education

☐ Religious school only

Marital status:

☐ Single ☐Married ☐ Divorced

Annex IV: Amharic Version of General and Socio-demographic Questions

ፆታ፡ ☐ **ወንድ** ☐ **ሴት** **እድሜ፡** _____

የትምህርት ደረጃ፡-

☐ **ማንበብና መፃፍ የማይችል** ☐ 1ኛ **ደረጃ ት/ት**

☐ **የሃይማኖት ት/ት የወሰደ/ች** ☐ ከፍተኛ የት/ት ተቋም የተከታተለ/ች

የትዳር ሁኔታ፡-

☐ **ያላገባ/ች** ☐ **ያገባ/ች** ☐ **የተፋታ/ች**

Annex V: Patient History Recording Format

Date: _____

Code: _____

Sex: ☐ Male ☐ Female Age: _____

- Last fasting blood glucose level check up: _____
- Type of DM: ☐ Type I ☐ Type II
- Duration of diabetes mellitus:_____
- Type of the drug that the patient takes : _____
- Other clinical condition (co-morbidity): _____
- Does the patient show signs and symptoms of urinary tract infection? ☐ Yes ☐ No

Annex VI: Laboratory Request Form

Urinalysis, Culture and Antimicrobial Susceptibility Test Request Form

Code: _____ Sex: _____ Age: _____

Specimen: **Urine** Date of collection: _____ Time: _____

Physicician: _____

Laboratory Report

Protein: _____ Leukocyte esterase: _____ Nitrite: _____ WBC/HPF: _____

Bacteria Isolated	Antimicrobial Susceptibility Reading														
	Ampicillinn	Ceftazidime	Cefixime	Cefepime	Amikacin	Cefotaxime	Ciprofloxacin	Cotrimoxazole	Gentamicin	Meropenem	Nitrofurantoin	Norfloxacin	Tobramycin	Vancomycin	S= Sensitive I= Intermediate R= Resistant

Date of report: _____ Signature: _____

Declaration

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

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   Signature: _____

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   E-mail: tedlakgb@yahoo.com Phone: +251-911-22-58-60/0912-93-54-81

   Signature: _____

Date of submission: _____