DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY, AND PARASITOLOGY, FACULITY OF MEDICINE, ADDIS ABABA UNIVERISTY

THE PREVALENCE OF BACTERIAL URINARY TRACT INFECTION AND ANTIMICROBIAL RESISTANCE PATTERNS OF BACTERIAL UROPATHOGENS ANONG DIABETES MELLITUS PATIENTS AT ZEWDITU MEMORIAL HOSPITAL IN ADDIS ABABA, ETHIOPIA

BY

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The Prevalence of Bacterial Urinary Tract Infection and Antimicrobial Resistance Patterns of Bacterial Uropathogens Among Diabetes Mellitus Patients at Zewditu Memorial Hospital in Addis Ababa, Ethiopia

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List of abbreviations/Acronyms

ASB: Asymptomatic Bacteriuria

CLED: Cysteine Lysine Electrolyte Deficient

CLSI: clinical and laboratory standard institute

DMIP: Department of Microbiology, Immunology and Parasitology

DM; Diabetes Mellitus

MSU: Mid-Stream Urine

NDM: Non Diabetes Mellitus

SB: Symptomatic Bacteriuria

UT: Urinary Tract

UTI: Urinary Tract Infection

WHO: World Health Organazation

ZMH: Zewditu Memorial Hospital

Abstract

Background: Urinary tract infection is caused by colonization and growth of microorganisms such as bacteria, fungi and viruses within genito-urinary system. Most of these infections are acquired through ascending routes from the nearby anal opening. Due to impaired host defense and high glucose concentration in urine, diabetic patients are more prone to bacterial urinary tract infections than non- diabetics patients.

Objective: This study was conducted to determine the prevalence of bacterial urinary tract infection, along with associated factors and antimicrobial resistance patterns of bacterial uropathogens among diabetes patients.

Methodology: Hospital-based cross sectional study was conducted between May and July 2018 at Zewditu Memorial Hospital in Addis Ababa, Ethiopia. Two handred twenty-five mid-stream urine samples collected for culture were inoculated in to Blood and MacConkey agar. Culture results showing significant growth were further tested for biochemical and antibiotic sensitivity.

Results: 150 (66.7%) females and 75 (33.3%) males with age ranges between 20 and 80 years were enrolled in this study**.** The overall prevalence of significant bacteriuria was 9.8%. Five species of bacterial uropathogens were isolated. Among these, *E.coli* (63.6%) was the leading causes of significant bacteriuria followed by *K.pneumoniae* (13.6%). in bivariate logestic regression analysis significant bacteriuria was strongly associated with duration of diabetics, previous urinary tract infections and symptomatic of urinary tract infection. In this study, neither gram-negative nor gram-positive bacteria were resistant to nitrofurantoin. In addition, all isolated gram-negative bacterial uropathogens were 100% sensitive to meropenem. In contrast, all gram-negative bacterial isolates were 100% resistant to ampicillin, doxycycline and cefuroxime. On the other hand, gram-positive bacteria were less resistant against tested antimicrobials

Conclusion and Recommendation: Presence of previous urinary tract infection and duration of diabetes were found as important associated factors that enhance urinary tract infection among diabetes patients. This study also showed high prevalence of drug resistant against common antimicrobials, particularly to co-trimoxazole, ciprofloxacin, doxycycline, ampicillin, augmentin, cefuroxime and penicillin suggesting the need for cautious use of antibiotic therapy for urinary tract infections. Therefore, Therapeutic selection for empirical treatment and management should be based on the knowledge of the local bacterial profile and antimicriobial response.

Keyword: Significant bacteriuria, antibiotic sensitivity, diabetis mellitus, associated factors

1. Introduction

1.1. Background

Urinary tract infection (UTI) is caused by colonization and growth of microorganisms such as bacteria, fungi and viruses within the urinary tract (UT) (1, 2). However, UTI due to virus and fungi contribute low incidence (1). The common uropathogens are *Escherichia coli, Staphylococcus saprophyticus, Klebsiella pneumoniae, Proteus mirabilis, Enterococcus* species, *Pseudomonas aeruginosa, Candida* species (1-4), and group B *streptococcus* (2). Urinary tract infection results in inflammation of both upper and lower parts of the UT and cause asymptomatic, acute or chronic infections (5).

Human urinary system is a continuous hollow organ system, which consists of a pair of kidney and ureter and single structure of bladder and urethra (3, 6). The mucosa of the renal pelvis, ureter and urinary bladder is lined with stratified epithelium (3). The primary function of UT is to collect, transport, store, and expel urine (6). As a result, UT eliminates metabolic products and toxic wastes generated in the kidney, which serve as conducive environment for microbial organisms to temporarily colonize the urinary system (6).

Under normal circumstance, UT is resistant to long term colonization and growth of microorganisms (7). This structure is usually struck by uropathogens like *Escherichia coli, Klebsiella pneumoniae*, and *Proteus mirabilis*, which possess virulence factors that enable them to colonize urinary epithelial (8). However, empting out urine flushes out harmful microbes that temporarily colonize urine in the bladder (3, 6-9). Additionally, innate immunity like cytokines, chemokines (3, 9), secretory immunoglobulin A (3, 7), mucous production, prostatic secretion, barrier formation (9) and high concentration of urea (3, 8, 9) prevent persistent microbial colonization and infection of UT. Nevertheless, structural and functional abnormality that blocks urine flow (6) and other risk factors that break host immunity may lead to UTI (4).

Due to anatomic and physiologic nature, UTI is more common in women than men are (1, 10). Nearly half of all women have been affected by this infection at least once in their lives time (11). Some of the factors that enhance UTI in female include short urethra (1, 10), short distance between the anus and urethral meatus, moist environment around the urethra and lack of

prostatic fluid (10); whereas in healthy men risk factors may be sexual activity with infected women, homosexuality and lack of circumcision (10). Other important risk factors that enhance UTI include: diabetes mellitus (DM), hypertension, allergies, increased sexual activity, catheterization, use of diaphragms, birth control pills and spermicidal agents, age, delays in micturition, abuse of antibiotics and other immune suppressive conditions (4).

Diabetes mellitus is a group of metabolic disorder characterized by hyperglycemic phenomena (9, 12). This chronic disease is caused by the interaction of genetics and environmental factors (9). The disease is mainly divided into two broad groups; type 1 and type 2 DM. Type 1 diabetes mellitus is characterized by complete or nearly total insulin deficiency, whereas type 2 is characterized by inadequate insulin production or insulin resistance (9, 12, 13). The most common type of diabetic is type 2, which accounts for more than 90% of all cases (13). Metabolic disorders associated with DM causes secondary patho-physiologic changes on multiple organs of patients such as cardiovascular disease, neuropathy, nephropathy and eye disease (9, 13) and abnormal host immune system (14).

Host immune system abnormalities such as impaired: migration, chemotaxis, phagocytosis and intracellular killing potential of polymorph nuclear leukocytes; local complications related to neuropathy like impaired bladder emptying and higher glucose concentration of urine in diabetic patients enhance UTI (14, 15). Different documents in the world show that UTI is more common (16-18), sever, and produce serious outcomes on patients with DMs (19-21). According to the study in Damietta Hospital, Egypt, the prevalence of UTI in DM patients was increased by 3.8 times when compared with NDM patients (16). Similarly the odds of getting UTI among DM patients increased by 2.5 times than NDM patients in the study by Worku et al. in Debre Tabor, Ethiopia (17).

1.2. Statement of the problem and justification of the proposed study

Chronic diseases are emerging as one of the leading causes of worldwide morbidity and mortality (22, 23). Among these, diabetes mellitus is one of the dominant non-communicable chronic diseases (22). According to 2017 International Diabetics Federation estimate, globally 451 million (8.4%) people with in the age ranges of 18-99 years were living with diabetes mellitus and 5 million people in the age range of 20-79 years were died due to this disease. However, this disease projected to rise to 693 million (9.9%) by 2045 (13). Diabetes mellitus presents a large social, financial and health system burden across the world (13, 23). The health expenditure for this disease in 2045 is expected to rise to USD 958 billion from the current USD 850 billion (2017) (13). From the African region in 2017, Ethiopia has highest number (2.6 million) of people with diabetes with 5.2% national prevalence (13). Similarly higher prevalence of DM was also found in a retrospective study between January 2010 and December 2013 in Addis Ababa (22).

Urinary tract infection is one of the most prevalent hospital and community acquired infections, which affects all age groups of people and ranges from asymptomatic bacteriuria (ASB) to sever symptomatic bacteriuria (SB) (19). Asymptomatic and symptomatic bacteriuria are more frequent in diabetic patients than the non-diabetes population (20). Comparative study in United Arab Emirates to find the prevalence of UTI among DM and NDM patients reported 35.5% and 12%, respectively (24). Similar study in Uganda documented 31.1% and 11.4% prevalence of UTI among DM and NDM patients, respectively (5). Another study in Nigeria to find ASB from DM patients reported a higher prevalence (36.15%) (25). Therefore, this infection is one of the common health problems causing frequent hospital visit (26). As a result, in countries where many people are affected by this infection, it produces negative impact on economic growth because it increases loss of work time, drugs cost, diagnostic costs and social expense (2).

Studies regarding UTI in DM patients are limited in Ethiopia, particularly in the study area. This research project is therefore proposed to investigate significant bacterial profile in DM patients and their antimicrobial resistance pattern at Zewditu Memorial Hospital (ZMH) in Addis Ababa, Ethiopia.

1.3. Significance of the study

Treatment and management of UTI is complicated in the world especially in the developing countries, due to poor identifications of uropathogenic bacteria; inappropriate drug selections and wide use of anti-microbial drugs. Different studies around the world on uropathogens like *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis,* and other bacterial pathogens reported different level of microbial resistance against common prescribed anti- bacterial drugs.

In developing countries like Ethiopia where most health facilities practice empirical treatment, antibiotic therapy may not be effective due to continuous changing of bacterial pathogens against antimicrobial drugs and inappropriate drug selections. Therefore, regular monitoring of urine culture is very essential to establish reliable information about microbial profile and resistance pattern of urinary pathogens for desirable empirical therapy of diabetic patients with UTI.

Thus, this proposed study is hoped to determine the present situation of bacterial profile, associated factors and antibiotics resistance patterns of bacterial uropathogens among diabetic patients at ZMH in Addis Ababa. It is conceived that the data generated from this study will enrich the already available limited information on bacterial profile and microbial resistance patterns of bacterial uropathogens as well as the associated factors that enhance significant bacteriuria on diabetes patients. So that the result of this research project will serve as additional data source for policy makers and other stake holders to understand the problem and designing appropriate diagnosis and drug selection for improved empirical treatment and management of UTI

2. Literature Review

2.1. Etiological agents of urinary tract infection

From microbiological point of view, UTI exists when pathogenic microorganisms are detected in properly collected mid-stream urine (MSU) samples in bacterial count of 10^5 or more /ml (27). However, significant bacteriuria is lacking in some cases of true UTI, especially in symptomatic patients and urine specimens obtained by suprapubic aspiration or catheterization, fewer bacteria (colony counts of 10^2–10^4/ml) may signify infection (9, 27).

The primary etiological agents of UTIs are gram-negative bacteria; however, gram-positive bacteria may also be involved for infections of UT (9, 27). The majority of uncomplicated UTIs (70-95%) and health care associated UTIs (50%) are caused by uropathogenic *Escherichia coli* (3). Other common uropathogens include *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, and *Proteus mirabilis* (9), *Enterobacter* species, and group B *Streptococci* (28). Infections of UT caused by *Escherichia coli* and other uropathogenic bacteria are complicated by the nature of the bacteria; these uropathogenic bacteria have virulence factors like fimbria and non-fimbrial adhesions that enable them to colonize and produce infection in the UT (7, 8). In addition, other multiple factors help them to survive inside the bladder. These include production of toxins and proteases to release nutrients from the host cells, synthesizing siderophores to obtain iron, capsule and production of biofilm (2).

The common etiologic agents of UTI in diabetic patients are similar to those non-diabetic patients with complicated UTI (28). Etiologic agents involved in complicated UTI include *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus* species, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Serratia marcescens* (9, 29).

Several studies worldwide have confirmed the involvement of the above uropathogens in UTIs. Hospital based research project among DM patients in Romania found major isolates of *Escherichiacoli* (68.9%) and *Klebisella* species (13.9%). The remaining minor isolates were species of *Candida* (6.4%), *Proteus* (3.8%), *Enteroccocus* (2.6%), *Streptococcus* (1.3%), *Pseudomonas* (1.3%), *Citrobacter* (0.6%), *Acinetobacter* (0.6%) and *Staphylococcus* (0.6%) (30). Similar prevalence studies among DM patients in Africa and other parts of the world found

Escherichia coli (50%-72%) and *Klebsiella pneumoniae* (14%-29%) as the dominate uropathogenic isolates (31, 32, 33). The prevalence of other uropathogens in these studies was 6.9% *Acinetobacter baumannii* (31), 14.3% *Staphylococcus aureus* (32), 12.8% *Enterococcus faecalis* (33) and 4%-8% *Proteus mirabilis* (31, 33).

Similarly, different studies among non-diabetic and diabetic patients in Ethiopia confirm the involvement of common uropathogenic bacteria in infections of urinary tract. Microbiological study among general population in Ethiopia found dominate isolates of *Esherichia coli* (49 %-63%) and *Klebsiella* species (7-13%) (34-36). The prevalence of other common uropathogens was more than 8% for *Proteus* species (34-36), about 7% *Pseudomonas* species (34, 35), 0.7%-3% *providential* species (34, 36), 2-6% *Enterobacter* species, 1-5% *Citrobacter* species (34, 36), 4%-5% Coaugulase Negative *Staphylococcus* (34-36), 5%-7% *Staphylococcus aureus* (34, 36), and 2.2% *Enterococcus faecalis* (34). The studies among diabetic patients in Ethiopia found relatively similar bacterial profile among the non-diabetic patients. However, a relatively lower prevalence of *Escherichia coli* was reported from the study done in Hawassa (37) and Gondar (38) where the rates of prevalence were 33.3% and 31.7%, respectively. Other study among diabetic and non-diabetic patients in Debre Tabor, Ethiopia, found *Escherichia coli* (44.4%*)* as the leading bacterial uropathogens in non-diabetic patients, wheras the second prevalent (19.1%) among diabetic patients (17).

2.2. Routes of urinary tract infection

Except for anterior part of urethra, which may contain a few commensals of gram positive and gram negative bacteria, UT is sterile (27) while its neighborhood vagina is rich with lactobacillary and other microbial flora which provides protection against uropathogenic bacteria (10, 27). So urine may be contaminated with these microbial floras when it flows out from bladder to the environment (27). Therefore, finding of bacteria in urine is not always definitive diagnosis of urinary tract infection because vaginal floras may contaminate urine. However, when UTI occurs these normal bacterial floras of vagina may be replaced by uropathogenic microbes (3, 10, 39). Thus, change of vaginal microbial flora and colonization of vagina with uropathogenic bacteria is thought to be a prerequisite for colonization and infections of UT (10). Additionally, structural, functional and physiologic nature of UT also plays a major role for adhesions and colonization of uropathogens to produce disease in the urinary tract (3).

Microorganisms can reach to the urinary system by three mechanisms (3, 26, 29), these mechanisms are discussed and shown in the Figure 2.1 below.

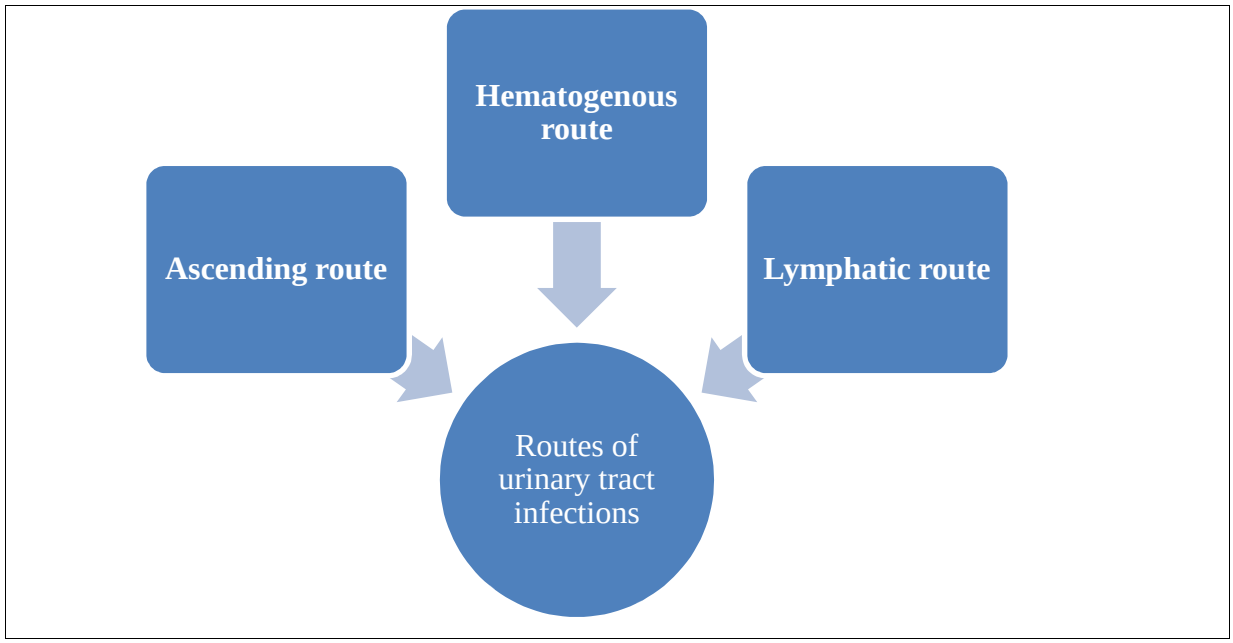

Figure 2.1: Routes of urinary tract infection (data obtained from references 3, 26 and 29)

Ascending route of urinary tract infection: Ascending route is the common pathway where urinary tract infection in healthy women is acquired (10, 26, 29). Uropathogens like *Escherichia coli* colonize vaginal introitus from rectal flora, enter to the bladder via urethra, and produce cystitis (10, 29). Sometimes these uropathogens ascend to the kidney and produce pyelonephritis (3). Successful invasion of urinary tract is determined by bacterial virulence, inoculums size, and host immune system (26). Symptoms of UTIs develop when uropathogens in the bladder or kidney interact and stimulate the release of cytokines because of which inflammatory response is initiated (10).

Hematogenous and lymphatic routes of urinary tract infections: Hematogenous and lymphatic routes of urinary tract infections are uncommon (29). However, patients with persistent blood stream infection and urinary tract obstruction are at risk for infections of renal parenchyma by potential uropathogens like *Staphylococcus aureus*, which is mostly associated with pyelonephritis (9, 10). Additionally, male patients acquire UTI through rectal and colonic

lymphatic vessels to prostate and bladder and females acquire through periuterine lymphatics to urinary tract (29).

2.3. Clinical presentation of urinary tract infection

Even if UTI affects all parts of urinary system, cystitis and pyelonephritis are common (1). Clinically, these infections may be symptomatic or asymptomatic (28). Symptomatic cystitis patients generally have clinical symptoms like dysuria, frequency, urinary urgency, nocturia, hematuria (1, 28), and pressure to lower pelvic (1). On the other hand, symptoms of pyelonephritis include fever, flank pain, chills, and other general symptoms (1, 28). Asymptomatic bacteriuria is defined as the presence of significant number of bacterial counts ($\geq 10^5$ colony count) using properly collected mid-stream urine specimens from patients without any signs and symptoms of UTI (28, 40). Some scholars thought that the presence of ASB is considered as the risk factors for symptomatic UTI in both diabetic and non-diabetic patients (28). On the contrary, others could not believe the assumption of ASB to be risk factors of symptomatic UTI in DM patients so that treatment of asymptomatic UTI among DM patients is not recommended because long-term clearance of bacterial pathogens from urine infection is not achievable through antimicrobial drugs. However, early diagnosis of UTI is important to minimize serious life threatening forms of UTI (41). Despite the fact that treatment is not recommended for patients with ASB under normal conditions, treatment is mandatory for patients with complicating factors like pregnancy, and invasive urological procedures (42).

Diabetes mellitus increases the risk of acquiring acute pyelonephritis in both men and women (43, 44). Recurrent acute pyelonephritis infection in these groups of patients predispose to complicated infection like intrarenal, perinephricabscess, emphysematous pyelonephritis, papillary necrosis and sometimes interstitial nephropathy (42).

Recurrent UTI is defined as a repeated infection of UT after complete resolution of the previous infections, which means three or more episodes of UTI in 12 months or two episodes of UTI in the last 6 months (39, 40, 45). This form of infections may be re-infection or relapse. However, the majority of recurrent UTIs are re-infection from extra urinary source such as rectum and vagina that is common in healthy adult women (39, 46). Risk factors of uncomplicated recurrent UTI would be spermicidal use; contraceptives inserted in the vagina, new sex partners, multiple

sexual partners, and history of sexually transmitted infection (46). Recurrent UTI, which occurs in complicated patients usually due to relapse that is due to treatment failure (1).

Based on anatomic location where the infection occurs, all UTIs can be stratified as lower tract or upper tract infections (1, 9, 29). Lower tract infection includes urethritis (urethral) and cystitis (bladder) (1, 12, 29); whereas upper tract infection includes ureteritis (ureter) (3, 29), pyelonephritis (kidney infection; which may be acute pyelonephritis, chronic pyelonephritis, and interstitial pyelonephritis, intrarenal and perinephric abscesses) (9, 29). However, both upper tract and lower tract infections are further classified as complicated and uncomplicated urinary tract infections (29). Classification of UTI as complicated and uncomplicated is important for: pre and post treatment evaluation, to adjust duration of treatment, and to select the best antimicrobial drugs (10).

Complicated and uncomplicated urinary tract infection: UTI considered as uncomplicated when it affects patients who have normal structural, physiological and neurological conditions (1-3, 29, 40). Although majority of uncomplicated infection is cystitis (3), acute pyelonephritis is also included in these infections (1, 2). Mostly, uncomplicated UTI occurs in females (1, 2), patients with previous UTI (2, 10), patients with sexually active young women, and women who use spermicidal agent (10). On the othe hand, complicated UTI is associated with factors that compromise the UT immunity. These factors would be urinary obstruction, urinary retention caused by neurological disease, immune suppression, diabetes, pregnancy, the presence of foreign bodies such as indwelling catheters and other drainage devices (2, 10, 40, 47), renal failure, renal transplantation (2, 10, 47), and infection with multi-drug resistant uropathogens (10) (Figure 2.2). Complicated UTI occurs in both women and men (1) and produces serious life threatening outcomes because patients with complicated UTI are susceptible to recurrent UTIs, perinephric abscesses, renal failure, urosepsis and finally death (47).

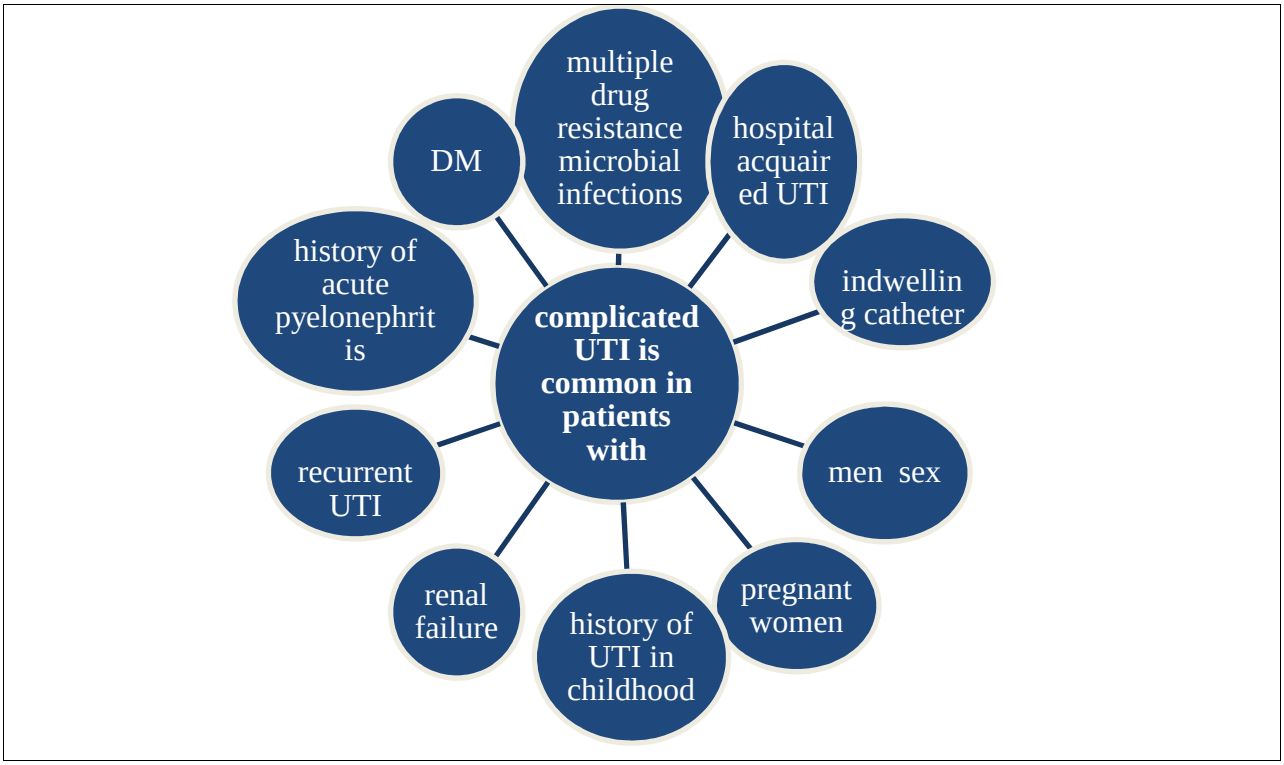

Figure 2.2: patients that commonly show complicated urinary tract infection (data obtained from references 2, 10, 40, 47)

2. 4. Epidemiology of urinary tract infection

Although, the susceptibility of women to UTI are higher than men (1, 11) this infection has significant cause of morbidity in both male and female adult population (1, 48, 49) with an estimated global incidence of around 250 million cases annually (50). Epidemiologically, UTIs are stratified as hospital and community acquired infections (9, 49). Hospital acqured UTI is occurred in patients who attending daily health care service in whom the infection was not present or incubating at the time of admission or health care service (49). Mostly this infection is associated with catheterization (9). On the other hand, community acquired UTI includes all UTIs obtained due to other predisposing factors (49). Globally, the prevalence of hospital acquired UTI (1.4% -3.3 %) is higher than community acquired UTI (0.7%) (49). The main risk factors for community acquired UTI is age, past history of UTI, sexual activities and DM (49). Community acquired UTI is the second prevalent infection next to respiratory infections in people over 65 years because older patients have altered immune functions and host co-morbidities such as diabetes, kidney stones, stroke, dementia, and bowel and bladder incontinence place them at greater risk for developing a UTI (51). Despite the fact that most of

community acquired UTI is caused by microbial strains sensitive to antimicrobials, gradually these strains may develop antibiotic resistance (9).

2.5. Urinary tract infections in relation to diabetes mellitus

Diabetes mellitus is a chronic endocrine disease. This disease produces a number of complicated effects on various organ systems (9, 12, 13). The risk of complication increases as the duration of persistently high blood glucose level increases in the blood (9). Many of patients with diabetes are unaware of their having diabetic complication (13). Usually Type 2 DM has long asymptomatic period of hyperglycemia so that diabetic complication can be present at the moment of diagnosis (9, 13); whereas in type1 diabetes, complication present early on the onset of DM (around 5 years later) (13). When not well controlled, all types of diabetes produce chronic complication, which affects many parts of the body, and resulting in diabetics associated morbidity and mortality (9, 13). These complications may be retinopathy, neuropathy, nephropathy, coronary artery disease (9, 13), diabetic encephalopathy and diabetic foot ulcer and infections (13).

Due to long-term effects of DM in urinary system, individuals with this disease have a greater frequency and severity of UTI (9, 52). Although, the exact mechanism is not well known several postulates have been proposed to describe the association of UTI and DM (53). Some of these postulates may be:

1. Diabetic neuropathy: this is due to long-term hyperglycemia that may lead to genitourinary tract dysfunction including cystopathy, which produces dysfunctional voiding, and urinary retention so physical microbial clearance through micturition decreases, thereby facilitating bacterial growth (43).
2. The presences of hyperglycemia in blood produces high glucose threshold in urinary system that increases glucose concentration in the urine. Higher glucose concentration in the urine may serve as nutrient to bacterial uropathogens as aresult increases colonization and growth of uropathogenic bacteria in UT (53).
3. Persistent hyperglycemia in the circulatory system can also make the blood coagulation system more active (13) and produces impairment in cell-mediated immunity and phagocyte function (9) so that the number of lymphocyte and neutrophil in the blood is reduced (52). Ultimately, these conditions may lead the host to decreased ability to defend against

microbes, thereby increases adherence of microbes to uroepithelial cells, and increased bacterial proliferation and at last increases infection (28, 53).

Several epidemiological studies worldwide have acknowledged the importance of UTIs among DM patients. For example, a study conducted on DM patients in Tamilnadu tertiary care Hospital, India, showed 12.2% significant bacteriuria. In this report, females were more affected (73.91%) than males (15). Similarly, 10.7% of prevalence significant bacteriuria was found among DM patients in Romania also. In this study, majority (78.4%) of the cases were ASB (30). Other similar studies found higher prevalence of significant bacteriuria among DM patients 54.8% in Nepal (54) and 49.3% in Pakistan (31). Studies on DM patients in Africa also reported variable overall prevalence rate of bacterial uropathogens: 13.3% in Uganda (32); 17.3 % in Nigeria (55) and 19.5% in the Sudan (33).

The prevalence of UTI among DM patients in Ethiopia ranges from 10.9% to 17.8% (17, 37, 38, 56). A report from Gondar University Hospital showed 51.4% and 14.7% significant bacteriuria among symptomatic and asymptomatic patients, respectively. In terms of associated factors, significant bacteriuria was strongly associated with history of previous UTI, antibiotic treatment, type of diabetes, and blood glucose level (38). Other similar studies in Ethiopia also found higher signignificant bacteriuria in symptomatic patients than asymptomatic patients (37, 56). On the contrary, a study in Debre-Tabor found higher magnitude (80.9%) of significant bacteriuria among asymptomatic UTIs patients than symptomatic UTI among DM patients (17).

2.6. Treatment of urinary tract infection and antimicrobial resistance

To be effective in the treatment of UTI, many antibacterial drugs should be partially or completely execrated through glomerular filtration and others like ciprofloxacin and co-trimoxazole should be secreted through tubular part of urinary tract (57). Generally, the following list of antimicrobial drugs are used in the treatment of UTI: trimethoprim, co-trimoxazole, norfloxacin, ciprofloxacin, levofloxacin, erythromycin, clarithromycin, doxycycline, tetracycline, nitrofurantoin, fosomycin, penicillin, oxacillin, ampicillin, amoxicillin, ampicillin/sulbactam, amoxicillin/clavulanic-acid, cefazolin, cefuroxime, cefotaxime, ceftriaxone, ceftazidime, Imipenem, meropenem, gentamicin, amikacin, vancomycin, and linezolid (44). Antimicrobial selection and duration of therapy depends on types of UTI whether

it is complicated or uncomplicated (58). Treatment option and duration antimicrobial drugs used in Ethiopia are discussed in the Table 2.1 bellow.

Despite the availability of these drugs in the treatment of UTI, worldwide resistance of uropathogens is alarming (44). The changing trend of antibiotic resistance worldwide complicates treatment and managements of UTI (59). These resistances against broad-spectrum antibiotics particularly fluoroquinolones and cephalosporin are acquired due to wide use of consumption (44). Additionally, emergence of plasmid encoded Extended-spectrum β-lactamase (ESBL) and carbapenem-producing Enterobacteriaceae such as *Escherichiacoli* and *Klebsiella* species confer resistance against several antimicrobials like 3rd and 4th generation cephalosporin and monobactams (59). Therefore, emergence of drug resistance is a serious public health problem all over the world. particularly, in developing countries because acquired antimicrobial resistance is facilitated due to poor identification of definite etiologic agents and their antibiotic choices, circulations of fake and expired drugs, using incorrectly prescribed drugs, self-treatment, using low drug dose, treatment for insufficient duration, over use of broad spectrum antibiotics, high level of poverty, overcrowding, ignorance and poor hygiene practices (27). Hence, an antimicrobial resistance pattern of uropathogens assessment is necessary.

Table 2.1: Recommended drug list used for treatment of urinary tract infection in Ethiopia (data obtained from reference 58)

Type of UTI	1st line drugs		Alternative drugs	
	drug name	consumption mode	drugs name	consumption mode
Acute uncomplicated in woman	Ciprofloxacin 500mg	P.O., BID, for 3 days	Nitrofurantoin 50mg	P.O., QID for 7 days
	Norfloxacin 400mg	P.O., BID, for 3 days.	Cefpodoxime proxetil 100mg	P.O, BID for 3 days
			Co-trimoxazole 960 mg	P.O, BID for 3 days
Acute uncomplicated Pyelonephritis in non-pregnant woman	Ciprofloxacin 500mg	P.O., BID, for 7-10 days	Co-trimoxazole 960mg	P.O, BID for 14 days
			Cefpodoxime proxetil 200mg	P.O., BID for 10 days
Severe acute uncomplicated pyelonephritis	Ciprofloxacin 400mg	I.V, BID until patient improvement and then continue	Ceftriaxone2gm	I.V daily or
			Ceftriaxone 1gm	I.V, BID till improves and then continue
	Ciprofloxacin 500mg,	P.O., BID to complete 10-14 days	Ciprofloxacin 500mg	PO, BID to 2gm complete 10-14 days
Recurrent UTI in women	Primarily no therapeutic drugs used but if she is sexual active, she used postcoital voiding and have liberal fluid intake.		Antibiotics recommended (single dose daily at night /after coitus) for 6 months or 1-2 years if symptoms of UTI exist 3 times in 12 months or 2 times in 6 months.	

BID: twice a day; I.V. intravenous; P.O. Per mouth; QID: four times a day

a. The resistance of gram-negative uropathogens against antimicrobials

Studies done for evaluation of antimicrobial susceptibility of gram-negative uropathogens in the world indicates that imipenem, ertapenem (31), nitrofurantoin (15, 37), gentamicin (32) and ceftriaxone (17) were the antibiotics with the highest activity against most of uropathogenic isolates. On the contrary, the resistances of common gram-negative uropathogens against the following list of antimicrobials were potentially high; cefuroxime, nalidixic acid (31), ampicillin, co-tromoxazole (15, 32, 37, 38), tetracycline (38), ciprofloxacin and norfloxacin (31).

b. The resistance of gram-positive uropathogens against antimicrobials

Research documents around the world on the resistances patterns of gram-positive uropathogens against common antimicrobial drugs show better alternatives. For example, gentamicin, co-trimoxazole, ceftriaxone (32), nitrofurantoin, amoxicillin- clavulanic-acid (33), ciprofloxacin and ampicillin (32, 33) were a drug of choice for the treatment of UTI caused by gram-positive uropathogens. Studies in Ethiopia also found similar antimicrobial resistance against gram-positive uropathogens (17, 37). On the contrary, these uropathogens were highly resistant to penicillin (17, 37).

3. Objectives

3.1. General objective

To determine the prevalence of bacterial urinary tract infection, along with the associated factors and antimicrobial resistance patterns of bacterial uropathogens among Diabetes Mellitus patients at Zewditu Memorial Hospital in Addis Ababa.

3.2. Specific objectives

- To determine the prevalence significant culture positive bacterial uropathogens among diabetic patients
- To identify the most prevalent bacterial uropathogens among diabetic patients
- To assess antimicrobial resistance patterns of bacterial isolates from diabetic patients
- To assess the associated factors that enhance urinary tract infection among diabetic patients

4. Methods and Materials

4.1 Study design

Hospital based cross sectional study was conducted to determine the prevalence of urinary tract infection and antimicrobial resistance patterns of bacterial uropathogens among diabetes mellitus patients at Zewditu Memorial Hospital in Addis Ababa, Ethiopia.

4.2. Study area and period

The study was conducted in Addis Ababa, where ZMH is located. Addis Ababa is the capital city of Ethiopia. According to the 2007 census report (60), the city has a total population of 2,738,248 with growth rate of 2.1%. Based on this figure central statically agency of Ethiopia estimates that the population of Addis Ababa is projected to reach around 3.95 million in 2018. The city has 13 governmental and 36 private hospitals. Of the 13 hospitals, six are under Addis Ababa Health City Administration. The rest 1, 4 and 2 hospitals are under Addis Ababa University, Federal Ministry of Health and Ministry of Defense, respectively. The city also has 96 health centers and over 700 different level private clinics (data obtained from Addis Ababa health bureau).

Zewditu Memorial Hospital is one of the biggest hospitals in Ethiopia, which is located in central Addis Ababa. It was built, owned and operated by the Seventh–Day Adventist Church, but was nationalized during the Derg regime in 1976. Currently, this hospital is operated under Addis Ababa Health Bureau. It provides all round health care service. It is also the leading hospital in treatment of ART patients and other chronic disease including DM (data obtained from Zewditu Memorial Hospital). The study was conducted on DM patients attending the hospital for treatment follow up during sample collection period of between May to July 2018.

4.3. Source population

All new and follow up DM cases visiting ZMH

4.4. Study population

Selected DM cases visiting ZMH during the study period

4.5. Inclusion criteria

DM patients who were visiting this Hospital for treatment follow up as well as those who were willing to be part of the project during the study period.

4.6. Exclusion criteria

DM patients who have been taking antibacterial drugs for the last two weeks, DM women with pregnancy and DM patients previously exposed to chatetrization.

4.7. Study variables

4.7.1. Independent variables

Age, sex, types of DM, duration of DM, previous UTIs, signs and symptoms of UTI.

4.7.2. Dependent variables

Significant bacteriuria

4.8. Sample size calculation, sampling methods & procedures

Sample size was calculated based on single population proportion formula using 95% confidence interval (α= 0.05) and margin of error 5%. Prevalence of bacterial profile was estimated from the study at Gondar University Hospital report that is 17.8% (38).

$$\mathbf{n = (Z_{\alpha/2})^2 P\ (1\text{-}P)/d^2}$$

Where *n* = sample size,

$\mathbf{Z_{\alpha/2}}$ **(**statistic for a level of confidence) =1.96

P - (Expected prevalence) = 0.178

d - (Precision) =0.05

$n = (1.96)^2(0.178)\ (1\text{-}0.178) / (0.05)^2$

n= 224.83501824 ≈ 225

The total DM patients expected to visit ZMH for treatment follow up during the sample collection period was estimated to be about 750. Therefore, 225 MSU samples were collected using systematic random sampling technique (every third patients (750/225=3.3)) from DM patients with both symptomatic and asymptomatic UTIs.

4.9. Data collection techniques and laboratory procedures

4.9.1 Data collection tools

Information concerning socio- demographic characteristics and clinical history of DM patients was collected by using interview (for patients who were unable to read and write) or self-administered structured questionnaire (for patients able to read and write) prepared in English

language and translated into Amharic. This questionnaire contains information about socio-demographics characteristics and the possible associated factors (such as duration of DM, previous UTIs, signs and symptoms of UTI, etc) which predispose DM patients to UTIs; and laboratory investigations of the respondents were recorded in laboratory result delivery formats.

4.9.2. Specimen collection, bacterial isolation and identification

4.9.2.1. Specimen collection

Mid-stream urine (MSU) specimen was collected from both symptomatic and asymptomatic study population during the study period. The patients were given sterile, dry, wide-necked, and leak-proof container and explained how to collect to bring about 10–20 ml urine specimens. A specimen container was labeled with date, time of collection and a unique identification number. The collected urine specimen was delivered to microbiology unit of Addis Ababa Regional Laboratory and part of this sample was inoculated within 2 hours of collection (27, 61).

4.9.2.2. Bacterial isolation and identification

All collected urine specimens were examined microscopically for pyuria; and urine biochemical tests was also done by reagent strip (mission®, India). Using calibrated loop 0.001ml of well mixed urine sample was inoculated onto MacConkey agar (HKM, China) and Blood agar (Biomark, India) (61). The inoculated media was incubated aerobically at 35–37^{o}C overnight. Then the inoculated plate was inspected for significant growth; a pure colony count yielding bacterial growth of 10^5 CFU or more per ml of urine (pure colony from Blood agar plate were counted and plates containing 100 or more colony) was regarded as significant for bacteriuria (62). Then identification of bacteria from pure colony was done using colony characteristics and gram stain. Further identification was done using different biochemical tests including catalase, manitol salt agar and PYR test for gram-positive bacteria and manitol utilization, hydrogen sulphide production (H_2S), indole production, citrate utilization, lysine iron agar test, gas production, hydrolysis of urea, and motility tests and carbohydrate metabolism for gram-negative bacteria (27).

4.9.2.3. Antimicrobial sensitivity test

After microbial identification, antimicrobial resistance pattern was determined using Kirby-Bauer disc diffusion method on Mueller Hinton Agar (Biomark, India) according to CLSI, 2016 guideline (63). Pure colonies of the test organism were taken using a sterile wire loop and

emulsified in sterile saline. Turbidity of this suspension was adjusted by Comparing with 0.5 McFarland standards. Then a sterile cotton swab was dipped into the suspension and inoculated onto the Muller-Hinton agar. Selected antimicrobial discs (oxoid, UK) were placed on to the surface of inoculated media by using disc dispenser and incubated for 16-18 hours at 35^{o}C-37 ^{o}C (27, 61). Based on recommended drugs for treatment of UTI from Basic laboratory procedures in clinical bacteriology, 2003 (61), guidelines from European associations of urology, 2013 (44) and Ethiopian hospital treatment guideline, 2014 (58), the following list of antimicrobial discs were used.

- nitrofurantoin, ciprofloxacin, doxycycline, ampicillin, vancomycin and erythromycin for *Enterococcus* species
- nitrofurantoin, co-trimoxazole, gentamycin, ciprofloxacin, doxycycline, erythromycin, clindamycin, cefoxitin and penicillin for CoNS
- meropenem, nitrofurantoin, co-trimoxazole, gentamycin, ciprofloxacin, doxycycline, ampicillin, ceftazidime, cefuroxime, cefotaxime, cefepime, and amoxicillin-clavulanic acid for gram-negative bacteria.

Results were read by measuring the diameter of inhibition zone to the nearest millimeter and interpreted after comparing with the standards. Finally, isolates were classified as susceptible or resistant to the tested antibiotics (61).

4.9.3. Data management and quality assurance

Data quality was maintained by checking completeness of questionnaire. Specimen collection containers were labeled and the collected urine specimens were transported carefully, which means the specimens were collected using sterile leak proof containers and inserted in plastic bag and then put into vaccine carrier. Data was entered in IBM SPSS software version 20, cleaned and crosschecked. The qualification of data collector was a diploma nurse. The data collector was trained on data collection procedures; and during data collection, the principal investigator supervised the data collection process. In addition, pre-test was done to check the correctness of the prepared questionnaires. The data collected at pre-test was not used for analysis. Sterility and performance of culture media was tested prior to the actual work. Sterility of media was checked by incubating overnight at 37^{o}c. In addition, *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 700603), *Proteus mirabilis* (ATCC 35699) and *Staphylococcus aureus*

(ATCC 25923) were used as reference strains for potency of culture media and antimicrobial resistance testing.

4.10. Data processing and analysis

Statistical analysis was done using SPSS software version 20. Frequency and percentage was calculated to summarize the results and presented in Tables and Graphs. Dependent and independent variables association and strength of associated factors was determined using Chi-square ($\chi 2$), bivariate logistic regression and variables showing statistically association were further analyzed for multivariate logistic regression and a P value of less than 0.05 was considered as statistically significant.

4.11. Operational definition

Asymptomatic bacteriuria: the presence of significant bacteriuria ($\geq 10^5$cfu/ml) in urine culture without signs and symptoms of urinary tract infection (28, 40)

Bacteriuria: the presence of significant bacteria in urine culture (27)

Complicated Urinary Tract Infection: the occurrence of urinary tract infection in patients with abnormal structural or functional urinary tract (2, 10, 47).

Diabetes mellitus: heterogeneous metabolic disorder characterized by hyperglycemia due to due to either impaired insulin secretion or impaired insulin action (12, 13)

Mid-stream urine: the portion of urine specimen collected at the middle part of urination (27).

Multi drug resistance: resistance to one antibiotic in three or more classes of drugs (59)

Resistant: the growth of bacterial isolates are not inhibited by the usually concentrations of antimicrobial agent (63).

Sensitive: the growth of bacterial isolates is inhibited by the usual achievable concentration of antimicrobial agent (63).

Symptomatic bacteriuiria: the presence significant bacteria in urine culture ($\geq 10^5$cfu/ml) accompanied with at least two complains of UTIsymptoms such as dysuria, urgency urination, frequent urination,suprapubic pain, flank pain, fever and chills (38)

Uncomplicated Urinary Tract Infection: the occurrence of urinary tract infection in the absence of structural and functional abnormality of urinary tract (1-3)

4.12. Ethical considerations

Ethical clearance was obtained from Department of Microbiology, Immunology, and Parasitology (DMIP), College of Health Sciences, Addis Ababa University. Ethical clearance was also secured from Addis Ababa public health research and emergency management core process; and support letter was provided by the same asking ZMH for cooperation which it granted the permission to do the study in the Hospital. In addition, written consent was obtained from the study participants before being included in the study. The confidentiality of collected data was maintained and names of the study participants were omitted from the data collection format.

4.13. Information dissemination

The finding of the study was submitted in the form of thesis for partial fulfillment of Master degree to Addis Ababa University, DMIP and Addis Ababa Health Bureau, Public Health Research and Emergency Management Core Process as well as other concerned organizations**.** The finding will also be disseminated to the national and international academic community through publications and oral or poster presentations.

5. Results

5.1. Socio- demographic characteristics

A total of 225 diabetes mellitus patients who had DM follow up in ZMH were enrolled in this study during May to July, 2018. No new DM patients were presented at the target clinic during the study period, although the original plan was to include these groups also. The minimum and maximum ages of the patients were 20 and 80 years and the mean age was 45.52 years. Out of the 225 patients, 150 (66.7%) were females. Majority of the study participants (70.2%) were married. Regarding diabetic types, 91.6% of the patients had type 2 and the remaining 8.4% had type 1. Duration of diabetes ranges from 1-30 years with mean value of 10.28 years. Fasting blood glucose level ranges between 63 mg/dl and 400mg/dl with mean value of 165.97 mg/dl. All of the study participants were urban dwellers and majority (79.1%) of the participants had primary or more modern schooling. Regarding average monthly income, 61.2% of the study participants had more than 1,650 birr. Among the 225 patients, 69 (30.7%) were previously ill with urinary tract infection at least once (Table 5.1).

Table 5.1: Socio demographic characteristics of diabetes mellitus patients investigated for significant bacteriuria at Zewditu Memorial Hospital, Addis Ababa, Ethiopia May- July 2018.

Variables		Frequency	Percent
Gender	Female	150	66.7
	Male	75	33.3
Marital status	Single	28	12.4
	Married	158	70.2
	Separated/divorced	39	17.3
Educational status	No modern schooling	47	20.9
	Primary schooling	71	31.6
	Secondary schooling	71	31.6
	Above secondary schooling	36	16.0
Average monthly income in Birr	<=1650	86	38.2
	1651-5250	111	49.3
	>5250	28	12.4
Types of DM	Type 1	19	8.4
	Type 2	206	91.6
Previous UTI	Yes	69	30.7
	No	156	69.3
Frequency of previous UTI	Once	38	55.1
	Twice	16	23.2
	Three and more times	15	21.7
UTI symptomatic	Symptomatic	97	43.1
	Asymptomatic	128	56.9

5.2. Clinical features of urinary tract infection

Among the 225 study participants, symptoms suggestive of UTI were observed in 97 (43.11%) of participants. A symptom of UTI is defined as the presence of at least two of the following complaints: dysuria, urgent urination, frequent urination, suprabic pain, fever, vomiting, and nausea and / or flank/loin pain (38). The most frequently observed complaint in the patients was flank/loin pain followed by frequent urination (Table 5.2).

Table 5.2: Frequency of symptoms suggestive UTI in diabetes mellitus patients in Zewditu Meemorial Hospital, Addis Ababa, Ethiopia May-July 2018

Symptoms	Frequency	Percentage %
Dysuria	42	18.67
Urgent urination	66	29.33
Frequent urination	80	35.56
Suprabic pain	38	16.89
Fever	52	23.11
Nausea	26	11.56
Vomiting	5	2.22
Flank/loin pain	90	40.00

5.3. Significant bacteriuria and etiologic agents

From the total 225 diabetes patients with both symptomatic and asymptomatic UTI enrolled in the study, 22 bacterial uropathogens were isolated. The overall prevalence of significant bacteriuria in this study was 9.8%. Out of the total 22 significant bacteriuria cases 15 (68.2%) were isolated from symptomatic UTI and the remaining seven (37.8%) were isolated from asymptomatic UTI patients (Figure 5.1). From the total 22 isolates, five species of bacterial uropathogens were identified. These were *Escherichia coli*, *Klebsiella pneumoniae*, *Providencia rettgeri*, *Enterococcus* species and Coagulase negative *Staphylococcus* species (CoNS). Gram-negative bacteria were more prevalent (81.8%) than gram positive (18.2%). Among the 22 bacterial isolates, *Escherichia coli* was the most frequently isolated one accounting for 14 (63.6%) followed by *Klebsiella pneumoniae* for three (13.6%) (Figure 5.1).

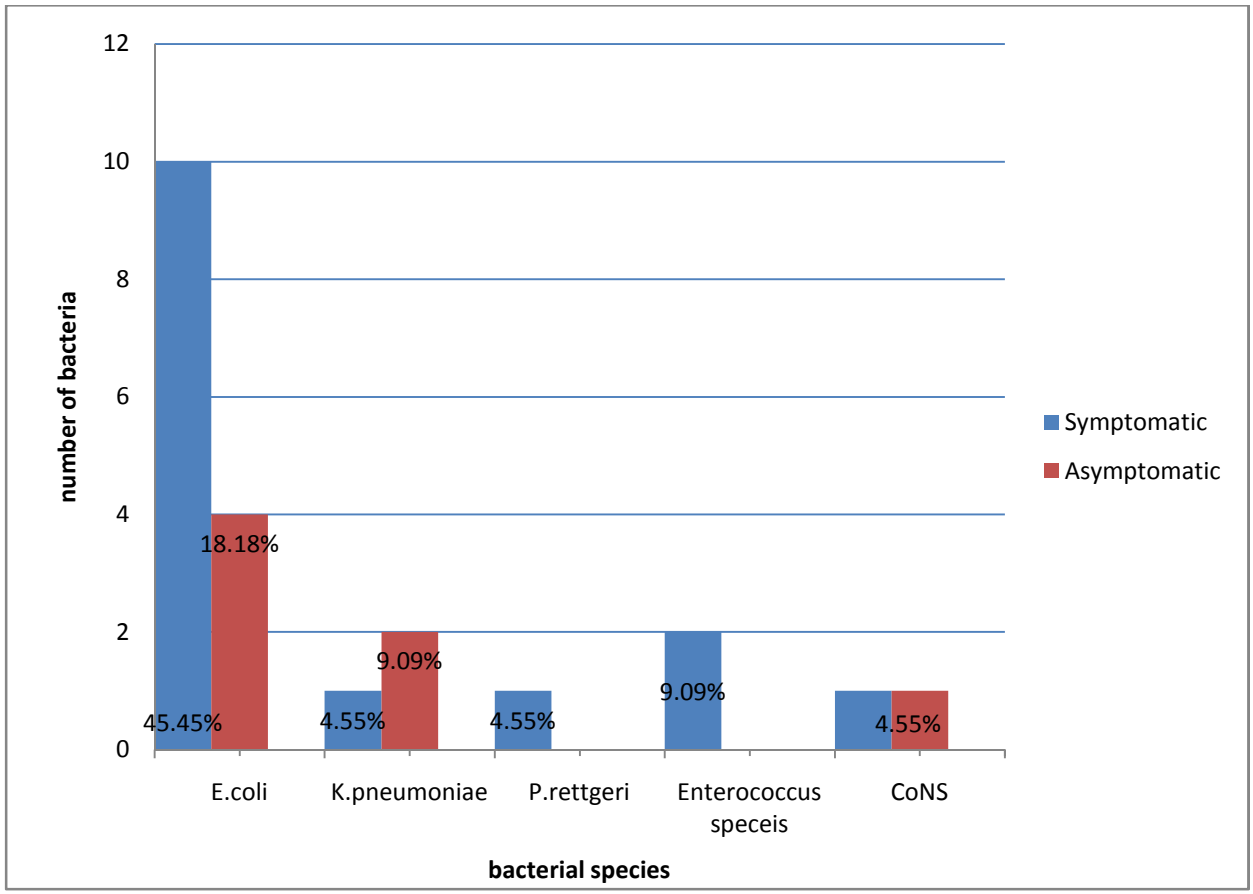

Figure 5.1: Frequency and types of bacterial uropathogens isolated from symptomatic and asymptomatic urinary tract infections among Diabetic patients at Zewditu Memorial Hospital, Addis Ababa, Ethiopia May- July 2018

5.4. Associated factors that enhance the presence of significant bacteriuria

In bivariate logistic regression analysis significant bacteriuria was strongly associated with duration of diabetics, previous UTI and current symptoms of UTI (P<0.05). As shown from Table 5.3 statistical significance difference was observed in relation to duration of diabetics, previous UTI and symptomatic UTI and significant bacteriuria with crude odd ratio (C**OR)** (95% **CI**) 4.364 [1.637-11.629], 4.709 [1.873-11.842] and 3.162 [1.235-8.094], respectively. The results of the study indicates that the chance of getting UTI among DM patients who have long duration of diabetics (for more than 10 years) was more than 4 fold (COR; 4.364 [95 % CI**,** 1.637- 11.629] than DM patients who have 10 or less years of diabetics. Similarly, DM patients with previous UTI have 4.709 times more chance of developing UTI (COR; 4.709 [95% CI**;** 1.873- 11.842]) than those patients without previous UTI; and those patients with symptomatic

UTI were 3 times at risk of having significant bacteriuria than those asymptomatic patients (COR; 3.162 [95% CI, 1.235- 8.094]) (Table 5.3).

However, other factors like gender, educational status, DM types, marital status, and frequency of previous UTI were not statistically associated with significant bacteriuria. Significant bacterial isolates were distributed in all age groups of the study participants. However, the highest (20.0%) and the lowest (4.7%) prevalent of significant bacteriuria were observed among the age groups ≥64 years and 50-64 years respectively. The odds of getting significant bacteriuria were no statistically significant between age groups (P> 0.05) (Table 5.3). Regarding marital status, the highest (14.3%) significant bacteriurias were isolated from single DM patients but there was no statically significant association between marital status and significant bacteriuria (P> 0.050) (Table 5.3). Similarly, 19/22 (86.4%) of bacteria were isolated from female diabetics. Even though, the risk of getting UTI in female patients was higher (3.481 times) than male, no statistically significant association was observed between gender and significant bacteriuria (Table 5.3).

In multivariate logistic regression analysis, duration of diabetics and previous UTI were statistically significant associated with significant bacteriuria with adjusted odd ratio (AOR) (95%CI) 3.477 [1.266-9.554] and 3.645 [1.403-9.473], respectively, whereas symptomatic condition was not showed significant association in multivariate logistic regression analysis with AOR (95%CI) 2.354 [0.881, 6.294] (Table 5.3).

Table 5.3: Association of significant bacteriuria with associated factors among diabetes patients visiting diabetic clinic in Zewditu Memorial Hospital, Addis Ababa, Ethiopia May- July 2018

variables	Results of UTI		Crude		Adjusted	
	Yes N (%)	No N (%)	P value	Odd ratio (95% CI)	P value	Odd ratio (95% CI)
Gender						
Male	3 (4.0)	72 (96.0)		1		
Female	19 (12.7)	131 (87.3)	0.051	3.481 [0.974-1.036]		
Age in years						
20-34	5 (9.4)	48 (90.6)		1		
35-49	9 (10.8)	74 (89.2)	0.792	1.168 [0.369-3.695]		
50-64	3 (4.7)	61 (95.3)	0.320	0.472 [0.107-2.075]		
>64	5 (20.0)	20 (80.0)	0.202	2.40 [0.625-9.210]		
Marital status						
Single	4 (14.3)	24 (85.7)		1		
Married	14 (8.9)	144 (90.1)	0.376	0.583 [0.177-1.922]		
divorced	4 (10.3)	35 (89.7)	0.617	0.686 [0.156-3.012]		
Educational status (modern schooling)						
No schooling	7 (14.9)	40 (85.1)	0.098	6.125 [0.718-52.259]		
Primary	9 (12.7)	62 (87.3)	0.131	5.081 [0.618-41.786]		
Secondary	5 (7.0)	66 (93.0)	0.382	2.652 [0.298-23.592]		
Above secondary	1 (2.8)	35 (97.2)		1		
Average monthly income in birr						
<=1650	14 (16.3)	72 (83.7)	0.118	5.250[0.658-41.873]		
1651-5250	7 (6.3)	104 (93.7)	0.584	1.817[0.214-15.409]		
>5250	1 (3.6)	27 (96.4)		1		
Types of DM						
Type 1	3 (15.8)	16 (84.2)		1		
Type 2	19 (9.2)	187 (90.8)	0.363	0.542 [0.145-2.029]		
Durations of DM						
<=10 years	6 (4.5)	126 (95.5)		1		1
>10 years	16 (17.2)	77 (82.8)	**0.003**	4.364 [1.637-11.629]	**0.016**	3.477 [1.266- 9.554]
Fasting blood glucose						
<126 mg/dl	3 (5.4)	53 (94.6)		1		
>=126 mg/dl	19 (11.2)	150 (88.8)	0.209	2.238 [(0.636-7.868]		
Previous UTI						
no	8 (5.1)	148 (94.9)		1		
yes	14 (20.3)	55 (79.7)	**0.001**	4.709 [1.873-11.842]	**0.008**	3.645 [1.403-9.473]
Frequency of previous UTI						
Once	6 (15.8)	32 (84.2)		1		
Twice	2 (12.5)	14 (87.5)	0.757	0.762 [0.137-4.251]		
Three and above	6 (40.0)	9 (60.0)	0.066	3.556 [0.920-13.740]		
Present symptoms of UTI						
Asymptomatic	7 (5.5)	121(94.5)		1		1
Symptomatic	15 (15.5)	82 (84.5)	**0.016**	3.162 [1.235-8.094]	0.088	2.354 [0.881-6.294]

5.5. Microscopy and dipstick results of urine analysis

Microscopic and urine dipstick results of the collected urine samples were also assessed for association of significant bacteriuria with UTI cases. In this study all mid- stream centrifuged urine sample were examined microscopically for pyuria in which 38/225 (16.9%) of DM patients were found to have significant pyuria (>10 WBCs/hpf) which is suggestive of infection (27). In addition, urine dipstick tests were done for assessing urine nitrite and hematuria, which showed 16/225 (7.1%) of the samples to be nitrite positive and 50/225 (22.2%) to have had hematuria. Statistically significant association was found between results of significant bacteriuria and pyuria (P=0.000), nitrite positivity (P=0.000), and hematuria (P= 0.000) (Table 5.4)

Table 5.4: Association of significant bacteriuria in relation to pyuria, urine nitrite and haematuria among Diabetes Mellitus patients at Zewditu Memorial Hospital, Addis Ababa, Ethiopia May-July 2018.

Variables		culture result of patients		Total N (%)	$\chi 2$	P value	Odd Ration [95%CI]
		Positive N (%)	Negative N (%)				
white blood cells / Hpf	<10	1 (0.5)	186 (99.5)	187 (83.1)	101.11	**0.000**	0.004 [0.001-0.034]
	>=10	21 (55.3)	17 (44.7)	38 (16.9)			
	Total	22 (9.8)	203(90.2)	225 (100.0)			
urine nitrite	yes	16 (100.0)	0 (0.0)	16 (7.1)		**0.000**	
	no	6 (2.9)	203(97.1)	209 (92.9)			
	Total	22 (9.8)	203 (90.2)	225 (100.0)			
blood in urine	yes	21 (42.0)	29 (58.0)	50 (22.2)	71.038	**0.000**	126.0 [16.31-973.14]
	no	1 (0.6)	174 (99.4)	175 (77.8)			
	Total	22 (9.8)	203 (90.2)	225(100.0)			

5.6. Antimicrobial resistance patterns among isolated bacterial uropathogens

The resistance patterns of uropathogen isolates from the significant bacteriuria were determined against twelve drugs for the gram-negative bacteria and six drugs against *Enterococcus* species while nine drugs were used against CoNS. Among antimicrobials tested, neither gram-negative nor gram-positive bacteria were resistant to nitrofurantoin. In addition, all isolated gram-negative bacterial uropathogens were 100% sensitive to meropenem. In contrast, all gram-negative bacterial isolates were 100% resistant to ampicillin, cefuroxime and doxycycline. On the other

hand, gram-positive bacteria were less resistant against tested antimicrobials. *Enterococcus* species were 100% sensitive to ampicillin and vancomycin but 100% resistances were observed against doxycycline, whereas CONS showed 100% sensitivity to all tested antimicrobials except penicillin, which showed 100% resistance. Antimicrobial activities of gram-negative and gram-positive bacteria are presented in the Table 5.5 and Table 5.6 below, respectively.

Table 5.5: Antimicrobial resistance patterns gram-negative bacterial isolates from mid-stream urine samples among diabetes mellitus patients at Zewditu Memorial Hospital, Addis Ababa, Ethiopia (May –July 2018).

Antimicrobials	*E.coli* (n=14)		*K.pneumoniae* (n= 3)		*P.rettgeri* (n=1)		Overall gram-negative (n=18)	
	Sensitive N (%)	Resistance N (%)	Sensitive N (%)	Resistance N (%)	Sensitive N (%)	Resistance N (%)	Sensitive N (%)	Resistance N (%)
Meropenem	14 (100)	0	3 (100)	0	1 (100)	0	18 (100)	0
Nitrofurantoin	14 (100)	0	3 (100)	0	1 (100)	0	18 (100)	0
Co-trimoxazole	4 (28.6)	10 (71.4)	1 (33.3)	2 (66.7)	0	1(100)	5 (27.8)	13 (72.2)
Gentamicin	12 (85.7)	2 (14.3)	3 (100)	0	1(100)	0	16 (88.9)	2 (11.1)
Ciprofloxacin	6 (42.9)	8 (57.1)	0	3 (100)	1(100)	0	7 (38.9)	11 (61.1)
Doxycycline	0	14 (100)	0	3 (100)	0	1(100)	0	18 (100)
Augmentin	1 (7.1)	13 (92.9)	0	3 (100)	0	1(100)	1(5.6)	17 (94.4)
Ampicillin	0	14 (100)	0	3 (100)	0	1(100)	0	18 (100)
Cefuroxime	0	14 (100)	0	3 (100)	0	1(100)	0	18 (100)
Cefotaxime	8 (57.1)	6 (42.9)	1 (33.3)	2 (66.7)	1(100)	0	10 (55.6)	8 (44.4)
Ceftazidime	10 (71.4)	4 (28.6)	0	3 (100)	1(100)	0	11(61.1)	7 (38.9)
Cefepime	11 (78.6)	3 (21.4)	2 (66.7)	1(33.3)	1(100)	0	14 (77.8)	4 (22.2)

Table 5.6: Antimicrobial resistance patterns gram-positive bacterial isolates from mid-stream urine samples among diabetes mellitus patients at Zewditu Memorial Hospital, Addis Ababa, Ethiopia (May –July 2018).

Antimicrobials	*Enterococcus* species (n=2)		Coagulase Negative *Staphylococcus* (n=2)		Overall gram-positive	
	Sensitive N (%)	Resistance N (%)	Sensitive N (%)	Resistance N (%)	Sensitive N (%)	Resistance N (%)
Nitrofurantoin	2 (100)	0	2 (100)	0	4 (100)	0
Ciprofloxacin	1 (50)	1 (50)	2 (100)	0	3 (75)	1 (25)
Doxycycline	0	2 (100)	2 (100)	0	2 (50)	2 (50)
Ampicillin	2 (100)	0	-	-	2 (100)	0
Vancomycin	2	0	-	-	2 (100)	0
Erythromycin	1 (50)	1 (50)	2 (100)	0	3 (75)	1 (25)
Co-trimoxazole	-	-	2 (100)	0	2 (100)	0
Gentamicin	-	-	2 (100)	0	2 (100)	0
clindamycin	-	-	2 (100)	0	2 (100)	0
Cefoxitin	-	-	2 (100)	0	2 (100)	0
Penicillin	-	-	0 (100)	2 (100)	0	2 (100)

5.7. Multiple drug resistances patterns of isolates

The frequency of multiple drug resistances to one antibiotic in three or more classes of drugs (59) was found in all gram-negative bacteria (100%), whereas only one (25%) out of the total four gram-positive bacteria showed multiple drug resistance (Figure 5.2).

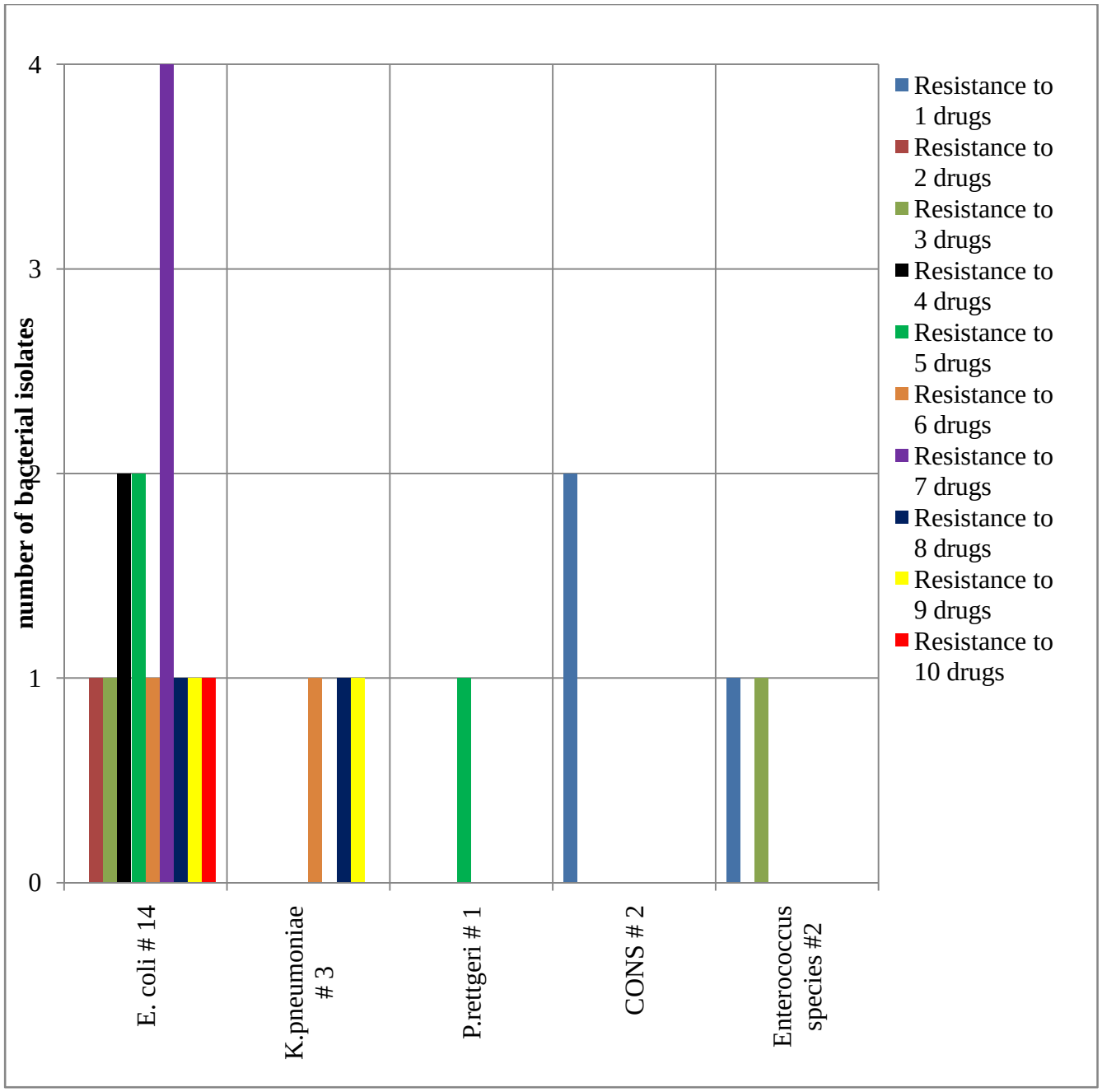

Figure 5.2: Multiple antibiotic resistance patterns of bacterial uropathogen from mid-stream urine culture among diabetic patients in Zewditu Memorial Hospital, Addis Ababa, Ethiopia (May –July 2018).

6. Discussion

6. 1. Prevalence of significant bacteriuria among diabetic patients

Urinary tract infection arises due to imbalance between host protective mechanisms and uropathogens virulence (49). One of the common causes of protective imbalance that causes UTI is DM (9, 12). These protective imbalances in diabetic patients may be due to complication of diabetes like nephropathy and neuropathy, which are the major risk factors for developing urinary system disorders (9, 13). In addition to these, uncontrolled hyperglycemic condition increases the presence of high glucose in urine that may create favorable environment for growth of bacterial uropathogens (13). Urinary tract infection in these groups of patients may ranges from simple cystitis to sever urosepsis (49).

In our study, the overall prevalence of significant bacteriuria among both symptomatic and asymptomatic diabetes patients was 9.8%. Similar findings have been found in the previous studies conducted in Ethiopia. For example, 10.9% of significant bacteriuria was found in Debre Tabor (17) and Addis Ababa (56). This finding is also supported by findings from other studies elsewhere like India, 12.2% (15) and Romania, 10.7% (30). However, result from our study was lower than other similar studies both in Ethiopia like Harar, 15.4% (64), Gondar, 17.8% (38), and abroad like studies from Nigeria, 17.3% (55), Sudan, 19.5% (33), Uganda, 27% (5) and India, 22% (18). In addition to the above mentioned studies, our finding was much lower than other similar studies conducted in Iraq, 35% (65), Pakistan, 49.25% (31), Egypt, 52.2% (16) and Nepal, 54.25% (54). This much difference may be due to the facts that more than 50% of our study participants were asymptomatic for UTI including exclusion of patients with co-morbidities like persons with catheterization, unlike the other studies mentioned above which included symptomatic patients with clinical features of UTI (16, 31, 54).

6.2. Etiological agents of urinary tract infections

In our study, five species of 22 uropathogens isolates from mid-stream urine samples of significant bacteria were isolated from the total 225 diabetes patients investigated for bacteriuria. Gram-negative bacteria were more prevalent (81.8%) than gram positive (18.2%). Our finding is in concordance with similar higher gram-negative uropathogens reports from Nigeria 80.3% (55), Iraq 85% (65), and Sudan 87.2% (33), but higher (88% in Pakistan (31) and lower (74.7% in Nigeria (19) and (57.3% in Gondar (38).

In addition to host immunity and environmental conditions, pathogenic factors determine pathogenesis and the courses of urinary tract infections (66). Globally, *Escherichia coli* have been demonstrated as the most common bacterial uropathogens (1-3). Similarly in our study, from the total 22 bacterial isolates *Escherichia coli* was the most frequently isolated uropathogens accounted for 14 (63.6%) followed by *Klebsiellia pneumoniae* accounting for 3 (13.6%). This higher prevalence of *Escherichia coli* may be due to the possession of such virulence factors like fimbrial adhesions, afimbrial adhesions, capsules and lipopolysaccharide, which may enable to resist wash out effects of urine, escape phagocytosis and ascend to bladder and other parts of the urinary tract (66). Our study was confirmed with similarly higher prevalence of *Escherichia coli* (60.6%- 69.4%) and *Klebsiellia pneumoniae* (13.9%-18.3%) from Romania (30), India (15, 18) and Egypt (16). However, relatively lower percentage 45.5% and 54.6% of *E. coli* and higher percentage of *Klebsiella pneumoniae*, 26.2% and 23.0%, were reported from Nigeria (55) and Sudan (33), respectively.

In addition, our finding indicated lower cases of *Providencia rettgeri* (4.5%), *Enterococcus* species (9.1%) and CoNS (9.1%). The finding on the prevalence of *Providencia rettgeri* was in agreement with the study in Nigeria (4.1%) (19). Similarly, our report on the prevalence of *Enterococcus* species was supported by studies done in Nigeria (10%) (55) and Sudan (12.8%) (33). However, slightly higher prevalence rates of *Enterococcus* species (13.8-14.1%) in India (18) and Nepal (54) and *Staphylococcus* species (15.3%) in Egypt (16) were reported.

6.3. Associated factors of urinary tract infections among diabetic patients

Several factors are thought to increase the risk of urinary tract infection among diabetic patients. Female gender (65, 67), previous UTI (67, 68), duration of diabetics (68), older age, duration of comorbidity, and poor glycemic control (67) are among the common associated factors that enhance UTI in diabetic patients.

In our study, the prevalence of culture confirmed significant bacteriuria in relation to duration of diabetics showed significance difference between DM with long and short duration of diabetes. In this study, the prevalence of significant bacteriuria among patients with more than 10 years of diabetic was 17.2 %, whereas in patients with 10 or less years of duration of diabetic was 4.5 %. The chance of getting culture confirmed significant bacteriuria was 4.4 fold in patients having more than 10 years of diabetes when compared to those patients having 10 years or less durations

of diabetes, the difference being statistically significant with p value 0.003 (Table 5.3). This significance difference may be due to progressive nature of diabetics that may damage genitourinary system because of diabetic neuropathy, which may lead to dysfunctional bladder thereby creating micturition abnormality that is important for the developments of UTI (16, 53). Our result was in agreement with similar studies in Egypt (16), United Arab Emirates (24) and India (68) which showed statistically significant association between longer DM duration and high rate of significant bacteriuria. However, our study result was contradicted with studies done by Raoofi et al (69) in Iran and Janifer et al in India (68), where no statistically significant association between duration of diabetics and significant bacteriuria were reported.

Sixty-nine out of 225 diabetic patients in this study had history of previous UTI at least once in the past. The prevalence of UTI among diabetic patients with previous history of UTI was 4.7 time higher (20.3%) than those without previous history of UTI (5.1%) ($P<0.05$) (Table 5.3). The reason for these finding might be due to the low dose of antibiotics administration to eradicate bacterial uropathogens (27, 58) as well as the presence of resistant bacterial strains to common antimicrobial drugs or it might be due to re-infection because of DM patients' poor immune response (10). Similarly, other studies in Ethiopia also found higher prevalence of significant bacteriuria among patients having previous history of UTI compared to DM patients having no previous history of UTI (30.2% vs. 15.6% from Gondar, (38); and 20.6% vs. 13.5% from Harar (64)). However, Nigussie et al in Hawassa, Ethiopia (37) contradicted our finding, where higher statistically significant bacteriuria was found in patients with no previous history of UTI.

The prevalence of UTI in relation to current symptoms suggestive of UTI was also analyzed. In this study, 97 (43.1 %) study participants were identified to have had symptoms of UTI. The prevalence of significant culture positive bacteriuria was higher (15.5%) in symptomatic patients than asymptomatic patients (5.5%), which shows a strong statistically significant association ($P<0.050$) (Table 5.3). The reasons for this high prevalence in symptomatic patients might be due to longer durations of diabetics in association with asymptomatic UTI and delayed medical interventions resulting in renal defects, which lead to progress to symptomatic bacteriuria (1). Our finding agrees with other studies which reported higher prevalence among symptomatic patients compared to asymptomatic patients: 56.7% symptomatic versus 13.7% asymptomatic in Nigeria (55); 13.6% symptomatic versus 10.4% asymptomatic in Addis Ababa, Ethiopia (56);

51.4% symptomatic versus 14.7% asymptomatic in Gondar, Ethiopia (38); 20% symptomatic versus 12.4% asymptomatic in Harar, Ethiopia (64). On the contrary, our finding disagrees with the studies in Sudan (33) where 20.9 % asymptomatic versus 17.1% symptomatic and India (18) where 22.3 % asymptomatic versus 22% symptomatic were reported.

The prevalence of significant bacteriuria in relation to gender among DM patients in our study found 12.7% in female and 4.0% in male patients. In this study the odds of getting UTI for females was 3.5 times more than for male patients but no statistically significant association was observed (P>0.05) (Table 5.3). Similar higher prevalence in women than men were reported in Romania (female 15.3% and male 4.5% (30)); .in Gondar (female 21.2% versus male 14.0% (38)); Hawassa (female 20.2% versus male 9.6% (37)); and Harar (female 19.2% and male 10.1%) (64)). Contrary to these observations, however, UTI prevalence in the Sudan was higher in males (22.3%) than females (15.1%) (33). The overall observed difference in the prevalence between higher in females versus to males may be explained by anatomic, and/or physiologic factors, including short and wide urethra, proximity of urethra to anus; and moist environment around vaginal surface in women (9, 10).

The prevalence of culture confirmed UTI in relation to age shows differences, although not statistically significant (P>0.050), with age group>64 years having the highest prevalence of 20.0% followed by 35-49 years (10.8%) and 20-34 (9.4%) while the least prevalence was recorded within the age group 50-64 (4.7%). Similar report was found from the study in Hawassa where age group 61-86 years had the highest rate (22.6%) as compared to the other age groups while the least was in the age group 42-60 years (9.2%) (37). But our finding disagrees with report from Debre Tabor, Ethiopia, where the highest prevalence was observed in age groups 46-55 years (17.1%) (17). Prevalence of UTI in older groups of patients may be related to menopause in women and kidney stones and prostatic problems in men. Menopause is usually associated with losses of estrogen, which increases bacterial uropathogen colonization due to loss of resident lactobacilli (70).

Prevalence of UTI in relation to marital status reveals that DM patients who were single (unmarried ever before) showed higher prevalence of significant bacteriuria (14.3%), whereas 8.9% and 10.3% of significant bacteriuria were recorded among married and separated/ divorced

diabetic patients, respectively, although the association was not statistically significant (P>0.050) (Table 5.3). The finding from a study conducted in Harar, Ethiopia supports this result, where higher rate of significant bateriuria was documented among singles (21.2%) as compared to married ones (13.2%) (64). Probably, this can be related to increased number of sexual partners since these people may not be committed to a single partner.

Prevalence of significant culture positive bacteriuria in relation to educational status was also analyzed. In this study, the prevalence of significant culture positive bacteriuria and the odds of getting UTI decreased as the educational status of diabetic patients increased, though not statistically significant. Such observed trend might be explained by the fact that people may become more and more hygiene-conscious and their treatment seeking behavior improves as their educational status improves. Our finding was supported by similar studies from Ethiopia by Abate et al (64) and Iran by Raoofi et al (69), which showed that as educational status grows from lower to higher the prevalence of UTI decreases. Another important inverse association was observed between average monthly income and culture positive significant bacteriuria, in which the risk of getting UTI decreased as the patients' average monthly income increased (Table 5.3). Our finding was supported by similar study from America by Boyko et al, which showed that patients with higher yearly income had low prevalence of UTI (14). This high prevalence of UTI among patients having low average monthly income may be due to poor hygienic conditions.

With respect to DM type, of the total 225 study participants majority of them were type 2 DM patients accounting for 206 (91.6%), whereas the remaining 19 (8.4%) were type 1 DM patients. However, the prevalence of culture confirmed UTI among type1 DM patients was slightly higher (3/19; or 15.8%) than type 2 (19/187; or 9.2%), with no statistical significance (P>0.050) (Table 5.3). Our finding was in agreement with report from similar study in Romania where culture positive UTI was observed among 12.8% of type 1 and 10.5% of type 2 DM patients (30). In our study most of type 1 DM patients showing culture positive significant bacteriuria, had long duration of diabetes and history of previous UTI. The presence of these significant associated factors may cause higher prevalent of bacteriuria in type1 DM than type 2 DM. On the contrary, Yismaw et al in Gondar found higher prevalence of significant bacteriuria in patients with type 2 diabetic (28.3%) than type 1 (10.4%) (38).

With regard to the association of fasting blood glucose and significant bacteriuria, the prevalence of significant bacteriuria in patients who had less than 126 mg/dl fasting blood glucose was lower (5.4%) than patients who had ≥126 mg/dl (11.2%); however, there was no statistically significant association (Table 5.3). Yismaw et al reported similar lower prevalence (5.7%) of significant bacteriuria among DM patients who had fasting blood glucose less than 126 mg/dl than DM patients with fasting blood glucose 126 mg/dl or more (20.9%) (38). The finding that higher prevalence of significant bateriuria among those DM patients with more than 126 mg/dl cannot be surprising because hyperglycemia is one of the associated factors for developing bacterial UTI (9).

Multivariate analysis on duration of diabetics, previous history of UTI and current symptoms of UTI was analyzed in relation to significant bacteriuria in our study. In this analysis, duration of diabetics and previous history of UTI showed statistically significant association with significant bacteriuria. Our result was contradicted with previous similar study in Sudan (33), where no multivariate analysis showed duration of diabetics and previous history of UTI in relation to significant bacteriuria. Multivariate analysis of previous UTI with other factors such as gender in Harar (64) and educational status in Hawassa (37) showed statistically significant association with significant culture positive bacteria in diabetic patients.

6.4. Significant bacteriuria in relation to pyuria, nitrite positive and haematuria among diabetes mellitus patiemts

It is possible to diagnosis and treat acute uncomplicated cystitis based on clinical features and urine dipstick. However, urine culture is recommended for patients with complicating risk factors as well as patients suspected with complicated UTI (71). Additionally, macroscopic and microscopic examination of urine can provide crude indicators for the presence of uropathogens (66). Specifically, microscopic examination of centrifuged urine for pyuria (27, 66) and rapid dipstick urine test for nitrite and hematuria provides positive predictive value for the presence of significant bacteriuria (27, 62, 66). The presence of more than ten pus cells per high power field in wet mounted urine sample is suggestive of significant pyuria (27). Many species of gram negative-bacteria have the enzyme reductase that enables them to reduce nitrate to nitrite so that the presence of nitrite during urinalysis provides 45-60 percent sensitivity and 85-98 percent specificity for the presence of UTI (72). Table 5.4 showed our results of analysis on significant culture positive bacteriuria in relation to pyuria, positive to nitrite and hematuria, which showed

strong statistically significant association (P< 0.050) between significant bacteriuria and pyuria, positive nitrite and hematuria. In Nigeria, Johan et al also found statistically significant association between significant bacteriuria and nitrite positivity (73). However, our result contradicted with the study results obtained by Danjuma et al in Nigeria where no statistically significant association was observed among nitrite and pyuria and significant bacteriuria (74); and another result by Adeyeba et al in Nigeria where their results showed no growth in urine samples that yielded six or more pus cells per high power field (75). These statically significant differences may be due to the presence of proportionally many gram-negative bacteria in our result, which metabolized nitrate to nitrite in UTI, and/or with prolonged DM duration, the bacteria might have gotten enough duration to metabolize nitrate to nitrite in bladder.

6.5. Antimicrobial resistances patterns of isolated bacterial uropathogens

Innate immunities like normal urine flow, mucosa production and barrier formation contributes to host protection against bacterial uropathogens (41). However, increased susceptibility of host and bacterial virulence like adhesions, toxins, siderophores, biofilms formation and others virulence factors enable uropathogens to colonize and persist in upper and lower parts of urinary tract to produce both acute and chronic UTI (76, 77). Treatment and management options of UTI depend on its site of infections and complication so that the appropriate drug of choice to manage UTI needs careful selection (71). Empirical treatment of simple acute cystitis can well respond to oral antibiotics but pyelonheritis should be early diagnosed and correctly differentiated whether it is complicated or uncomplicated to prevent antimicrobial resistance and other life threatening UTI like urospsis (71).

Emergence of antimicrobial resistance against important antimicrobial drugs left health care systems with limited options for the treatment and control of infectious diseases including UTI (58). The in vitro antimicrobial sensitivity studies showed that organisms react differently to various antibiotics, as demonstrated by their sensitivity patterns. In our study, a large number of gram-negative uropathogenic bacteria showed more than 60 % resistance to the common prescribed drugs, ampicillin, cefuroxime, doxycycline, augmentin, co-trimoxazole and ciprofloxacin. Consistent resistance against cefuroxime (31), co-tromoxazole, ciprofloxacin, augmentin (31, 64) and ampicillin (15, 37, 38, 56) were reported in Ethiopia and elsewhere aboard. The remarkably higher prevalence of resistance to the commonly prescribed antibiotics

such as ampicillin, doxycycline, co-trimoxazole and ciprofloxacin noticed in the present study may be due to the easy access, availability and indiscriminate use of the drugs without prescription (58). However, our finding on meropenem, nitrofurantoin, cefepime and gentamycin were a treatment of choice for gram-negative bacteria. Our result was supported by similar study results from Ethiopia and other parts of the world: nitrofurantoin (15, 38) and gentamicin (32).

In our study, the resistance patterns of gram-positive bacterial uropathogens against antimicrobial drugs were not as intensive as gram negative-bacterial uropathogens. Isolates of *Enterococcus* species were 100% sensitive to nitrofurantoin, ampicillin and vancomycin but 100% resistances were observed against doxycycline. Similar antimicrobial response against nitrofrurantoin (33, 38) and ampicillin (17) were reported from Sudan and Ethiopia. On the contrary, our result on the resistance of *Enterococcus* species disagrees with other studies; for example, ampicillin (80%-100%) in Nepal (54) and Ethiopia (64), nitrofurantoin (50%), and doxycycline (0%) in Debre Tabor, Ethiopia (17).

In this study, CoNS showed better option for empiric treatment. Since all isolates of CoNS were 100% sensitive to all tested antimicrobial drugs except 100% resistance to penicillin. This finding was supported by other previous studies in Ethiopia, where 100% sensitivity were observed against erythromycin, doxycycline (17), gentamicin (37) and ampicillin (33). Similarly, 100% resistance was observed against penicillin (100 %) (37, 64).

In the present study, *K.pneumoniae* was not appreciably susceptible to most antimicrobial agents and it showed 100% resistant to cefuroxime, ceftazidime, ampicillin, ciprofloxacin, agumentin and doxycycline. Resistance against ampicilin (37, 38) and agumentin (64) from Ethiopia, and cefuroxime and ciprofloxacin from Pakistan (31) were similar to our finding.

The high frequency of multiple antibiotics resistance might be a reflection of inappropriate use of antimicrobials obtained outside treatment center, which may circulate fake drugs, sub-standard or expired drugs, self-treatment; frequent use of broad-spectrum antibiotics for prophylactics and lack of laboratory tests (27). In our study, Multidrug resistance was seen in all gram-negative bacterial uropathogens. This result is comparable with reports from Hawassa 88.2 % (37) and from Addis Ababa 77.6 % (36). This multidrug resistance to the common used antibiotics frustrating the health care system.

7. Limitation of this study

Due to budget constraint

- the study did not include control groups
- the study was conducted only at a single hospital which may not represents the Ethiopian DM patients at large
- the study was limited to aerobic bacterial uropathogens
- This study did not address UTI caused by other bacterial pathogens that unable grow in the ordinary culture media such as, *Mycoplasma hominis*, *Mycoplasma genitalium*, *Ureaplasma urealyticum*, and Chlamydia *trachomatis*

8. Conclusion and recommendation

Conclusion

About 43% of the diabetic study participants had symptoms suggestive to UTI and the rest were asymptomatic patients, of whom culture positive significant bacteriuria was 9.8%. The prevalence of significant bacteriuria was higher in diabetic patients with symptoms suggestive to UTI than asymptomatic. In this study, dominant bacterial uropathogens were *E.coli*, which account for 63.6% followed by *K. pneumoniae* (13.6%). Culture positive significant bacteriuria showed statistically significant association with history of previous urinary tract infection, duration of diabetes and presence of UTI symptoms. In addition, positive urine for nitrite and pyuria also showed strong statistically significant association with culture positive significant bacteriuria. This study also showed significantly higher prevalence of drug resistant isolates, particularly to cotrimoxazole, ciprofloxacin, doxycycline, ampiciline, augmentin, Cefuroxime and penicillin, which suggests the need for cautious use of antibiotic therapy for urinary tract infections. However, response rate for nitrofrantoin and gentamicin among both gram-positive and gram-negative bacterial uropathogens was excellent.

Recommendations:

- Early diagnosis and antimicrobial resistance testing of bacterial uropathogens among diabetic patients is important to protect patients from serious complication and drug résistance.
- Therapeutic selection for empirical treatment should be based on the knowledge of the locally prevalent bacterial uropathogens.
- The impact of DM on UTI patients would be clearer if the study included non-diabetic UTI patients as controls. Therefore, there should be more studies in the future that consider inclusion of control groups.
- Since only a fraction of symptomatic UTI patients have demonstrated significant bacteriuria, other possible causes for the rest of UTI symptoms must be investigated, as other etiologic agents such as *Mycoplasma hominis*, *Mycoplasma genitalium*, *Ureaplasma urealyticum*, Chlamydia *trachomatis*, viruses and fungi should considered.
- Further study also needed to establish clear association between urine culture and microscopic examination for pyuria along with urine dipstick test for nitirite.

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Annex I: Questionnaire

1. Information sheet for study participants

Hello, my name is---------- I am working (with the principal investigator) to gather data for conducting a research on prevalence of urinary tract infection and bacterial resistance among Diabetics patients.

Purpose of questionnaire: The aim ofthis questionnaire is to gather data from Diabetes Mellitus patients as part of a research on the prevalence of urinary tract infection and anti-microbial resistance patterns of bacterial uropathogens among Diabetes Mellitus patients at Zewditu Memorial Hospital in Addis Ababa, Ethiopia.

Purpose of the Research: The purpose of this research is to study the prevalence of urinary tract infection and anti-microbial resistance patterns of bacterial uropathogens among Diabetes Mellitus patients. Chronic diseases like Diabetics Mellitus are emerged as the leading cause of death and morbidity worldwide. According to 2013 estimation, more than382 million people were affected with Diabetics worldwide. In Ethiopia this disease has 6.5% prevalence. Due to impaired host defense, patients with Diabetes Mellitus are more prone to urinary tract infection. The rate of infection in this group of patients increases by 2 to 3 times when compared with non diabetics. This infection affects all age groups of people and ranges from asymptomatic bacteriuria to sever symptomatic bacteriuria. Therefore this infection is one of the common health problems causing hospital visit. As a result, urinary tract infection has great impact on country economic growth because it causes social expense, loss of work time, drugs and associated costs.Studying the prevalence of bacterial uropathogens and their antimicrobial resistance pattern helps to implement appropriate drug selection and better urinary tract infection management during empiric treatment. Unfortunately, Studies regarding bacterial profile and their antimicrobial resistance pattern among Diabetic patients is not adequate in Ethiopia. Therefore, this research project is developed with the aim of finding the problem of bacterial uropathogens and their antimicrobial resistance pattern among Diabetes Mellitus patients in this Hospital. So you are kindly requested to participant voluntarily in this study.

Procedure: If you agree to be a part of this study, you will be asked some questions about socio-demographics characteristics and your general health condition which might be associated with suspected risk factors for bacterial urinary tract infections, and you will also be asked to bring about 10- 20 ml of urine specimen.

Risk and Discomfort: participating in this research will not cause any harm up on you.

Benefits: You will gain both direct and indirect benefit by participating in this research project. Direct benefit: your laboratory result will be delivered to your physician for better treatment. Indirectly: the information obtained from this research project helps to understand the problem of bacterial uropathogen profile and their antimicrobial resistance pattern among Diabetes Mellitus patients. Based on this information and other similar findings throughout the country, stakeholders will implement effective empiric management practice.

Confidentiality: Any information collected from you for this research will be kept confidential. It will be stored in a file, which will not have your name on it, but a code number assigned to it. It will not be revealed to anyone except the principal investigator and your physician.

Participant right: You have full right to refuse from participating in this research at any time without giving any reason. Withdrawing from this study will not have negative impact on your future treatment follow up in this hospital or elsewhere.

If you have any questions about this research, you can contact the principal investigator directly.

Name: Gebremedhin Yenehun; Mobile: 0910301669; Email. gmedhinyenhun@gmail.com

2. Consent form for study participants

I read the information sheet (or it has been read to me). I have understood that this study is about "prevalence of urinary tract infection and anti-microbial resistance patterns of bacterial uropathogens among Diabetes Mellitus patients at Zewditu Memorial Hospital in Addis Ababa, Ethiopia". The principal investigator/ data collector has briefed me about the purpose of the study. I have asked some questions and clarification has been given to me for my questions. I have been informed that I will be asked some questions about my socio demographics characteristics and possible sources of urinary tract infection risk factors. In addition to this, urine specimens will be collected from me. I have been informed that I will get both direct and indirect benefit from the study. The investigator /data collector also informed me that all the laboratory results and all private information about me will be kept confidential. Moreover, I have been well informed of my right to withdraw from the study at any time and my withdrawal from the study will not have any negative impact on future treatment follow up in this hospital or elsewhere. After taking enough time to think over the benefit of the study to me as well as my country, I have decided to be part of study participants. Therefore I have given my informed consent and approved agreement with my signature hereunder.

Participant		witnesses				Data collector	
Code		1st witness		2nd witness			
Signature		Signature		Signature		Signature	
Date		Date		Date		Date	

3. Questionnaires for study participants

This questionnaire has two parts. The first part deals with socio demographics characteristics whereas the second part focuses on the possible risk factors that promote urinary tract infection among Diabetes Mellitus patients.

Part one: socio demographic characteristics

Code of study participant_____

1.1. sex □ male □ female

1.2. Age in year _____

1.3.Resident □ urban □ rural

1.4. Marital status □ single □ married □ separated /divorced

1.5.Educational status □ no modern schooling □ Primary schooling □ secondary schooling□ above secondary school

1.6. Average monthly income in birr. □ ≤ 1650 □ 1,651-3,200 □ 3,201-5,250 □ >5,250

Part II. Possible risk factors for urinary tract infection among Diabetes Mellitus patients

2.2. Duration of diabetics in years? _____

2.2. Have you got urinary tract infections previously? □ yes □ no

2.3. If your answer is yes in question number 2.2, frequency of previous UTI in the past □ Once □ twice □ 3 times and above

2.4. Do you have the following urinary tract infection symptoms currently?

2.4.1. Dysuria □ yes □ no

2.4.2. Urgent urination □ yes □ no

2.4.3. Frequent urination □ yes □ no

2.4.4. Suprapubic pain □ yes □ no

2.4.5. fever □ yes □ no

2.4.6. Nausea □ yes □ no

2.4.7. Vomiting □ yes □ no

2.4.8. Flank pain □ yes □ no

መጠይቅ

1. ለጥናቱተሳታፊዎችማብራያለመሰጠትየተዘጋጀመረጃ

ጤና ይስጥልኝ፥ ስሜ ---------- ይባላል፡፡ የኩላሊት ና ሽንት ቧንቧ በባክቴሪያል ተህዋስያን መመርቀዝ (ኢንፌክሽን) ስርጭት ና የነዚህ የባክቴሪያል ተህዋስያን ፀረ-ባክቴሪያል መድሃኒትን የመቋቋም አቅም በስኳር በሽተኞች ላይ ለሚካሔደው ጥናትና ምርምር መረጃ ሰባሳቢ ነኝ፡፡

የመጠይቁ ዓላማ፡-ይህ መጠይቅ የተዘጋጀበት ዋና ዓላማ በዘዉዲቱ መታሰቢያ ሆስፒታል የስኳር በሽታ ታማሚዎች ላይ ኩላሊት ና የሽንት ቧንቧ በባክቴሪያል ተህዋስያን መመርቀዝ ሰርጭትን ና የእነዚህ ተህዋስያን ፀረ-ባክቴሪያል መድሃኒት የመቋቋም አቅም ላይ ለሚካሔደው ጥናት ና ምርምር መረጃ ለመሰብሰብ ነው፡፡

የጥናት ና ምረምሩ አስፈላጊነት፡-ይህ ጥናት ና ምረምር ያስፈለገበት ምክንያት በዘዉዲቱ መታሰቢያ ሆስፒታል የስኳር በሽታ ታማሚዎች ላይ ኩላሊት ና የሽንት ቧንቧ በባክቴሪያል ተህዋስያን የመመርቀዝ ሰርጭትን ና የእነዚህ ተህዋስያን በፀረ-ባክቴሪያል መድሃኒት የመጠቃት አቅም ለመጥናት ነዉ፡፡ በዓለም ላይ እነደ ስኳር ህመም ያሉ በቀላሉ ሊድኑ የማይችሉ በሽታዎች ለከፍተኛ ደረጃ ህመም ና ሞት ምክንያት ግንባር ቀደም ተጠሽ ናቸው፡፡ በዓለም ላይ ከ384 ሚሊዮን በላይ ሕዝብ በ2013 የስኳር በሽታ ታማሚ አንደነበር ይገመታል፡፡ በአገራችን ኢትዮጵያ የዚህ በሽታ ስርጭት እስከ 6.5% ይደርሳል፡፡ የስኳር ታማሚ የሆኑ ሰዎች በሽታን የመከላከል አቅማቸው በመዳከሙ ምክንያት ለኩላሊት ና የሽንት ቧንቧ መመርቀዝ ተጋለጭ ናቸው፡፡ በስኳር በሽታ የተያዙ የህብረተሰብ ክፍሎች ካልተያዙት ጋር ሲነጻጸር በኩላሊት ና የሽንት ቧንቧ መመርቀዝ የመያዘ ዕድላቸው ከ 2 እስከ 3 እጥፍ ይበልጣል፡፡ ይህ መመርቀዝ በሁሉንም የዕድሜ ደረጃ የሚገኙ የህብረተሰብ ክፍሎች የሚያጠቃ ሲሆን ከምልክት አልባነት እስከ ከፍተኛ የህመም ደረጃ ሊደርስ ይችላል፡፡ ስለዚህ ይህ ህመም የሰውን ልጅ ወደ ህክምና ተቋማት እንዲመላለስ ከሚያደርጉት የጤና ችግሮች አንዱ ነው፡፡ በዚህም የተነሳ የኩላሊት ና ሽንት ቧንቧ መመርቀዝ በአንድ አገር የኢኮኖሚ ዕድገት ላይ አሉታዊ ተጽዕኖ ይኖርዋል ምክንያቱም ለማህበራዊ ተዋጽዖ፣ ለመድኃኒት ና ተያያዥነት ላላቸው ጉዳዮች የሚወጣን ወጪ ከማናሩ ባሻገር ለስራ የሚውለውን ጊዜ ያባክናል፡፡ ስለዚህ ኩላሊትን ና የሽንት ቧንቧን የሚያመረቅዙ የባክቴሪያል ተህዋስያንን ስርጭት ና ፀረ-ባክቴሪል መድኃኒትን የመቋቋም አቅማቸዉን ማጥናቱ በላብራቶሪ ምርመራ ያልተደገፈውን የኩላሊት ና ሽንት ቧንቧ መመረቀዝ ህክምና በተሻለ ሁኔታ ለመቆጣጠር ብሎም በፈዋሽነታቸው የተሻሉ መድኃኒቶችን ለተጠቃሚዎች ለማቅረብ ይረዳል፡፡ በአገራችን ኢትዮጵያ ይህን መሰል ጥናትና ምርምር በበቂ ሁኔታ አይገኝም፡፡ በመሆኑም የዚህ ጥናት ና ምርምር ዓላማ በዘዉዲቱ መታሰቢያ ሆስፒታል ያለውን የባክቴሪያል ተህዋስያን ኩላሊትን ና የሽንት ቧንቧን የማመርቀዝ ስርጭት ና የነዚህን ተህዋስያን መድኃኒት የመቋቋም አቅም በስኳር ህመምተኞች

ላይ በምን ደረጃ እንዳለ ለመረዳት ነው። ስለሆነም ለዚህ ጥናት ና ምርምር መሳካት የበኩሎዉን አስተዋጽዖ እነዲያደርጉ ስል በትህትና እጠይቃለሁ።

የጥናቱ ሂደት፡- በጥናቱ ተሳታፊ ለመሆን ፍቃደኛ ከሆኑ ስለ እርስዎ አጠቃላይ ማህበራዊ ና አካባቢያዊ መረጃዎች እንዲሁም የስኳር ህመምተኞችን ለኩላሊት ና ሽንት ቧንቧ መመርቀዝ ሊያባብሱ ስለሚችሉ አጠቃላይ የጤና ሁኔታ በተመለከተ የተዘጋጁ ጥያቄዎችን ይሞላሉ። በተጨማሪም ለላበራቶሪ አገልገግሎት የሚዉል ከ 10-20 ሚሊ ሊትር ሽንት የሰጣሉ።

በጥናቱ መሳተፍ የሚያስከትለዉ ጉዳት፡- በዚህ ጥናት ና ምርምር በመሳተፍዎ በእርስዎ ላይ የሚስከትለዉ ምንም ጉዳት የለም።

በጥናቱ መሳተፍ የሚያሰገኘዉ ጥቅም፡- እርስዎ በዚህ ጥናት ላይ በመሳተፍዎ በቀጥታ ይሁን በተዘዋዋሪ የሚያገኙት ጥቅም ይኖራል። በቀጥታ የሚጠቀሙት፡- የተሸለ ህክምና ያገኙ ዘንድ የእርሰዎ የላብራቶሪ ውጤት ለሃኪምዎት ይሰጣል። በተዘዋዋሪ የሚያገኙት ጥቅም፡- ከዚህ ጥናት ና ምርምር የሚገኘዉ መረጃ የባክቴሪያል ተህዋስያን ኩላሊትን ና የሽንት ቧንቧን የመመርቀዝ ስርጭት ና የነዚህን ተህዋስያን መድኃኒት የመቋቋም አቅም በስኳር ህመምተኞች ላይ በምን ደረጃ እንዳለ ለመረዳት ሲሆን ይህን ና ሌሎች በአገራቱ የሚካሄዱ መሰል ጥናት ና ምርምሮችን መሠረት በማዱረግ ባለድርሻ አካላት የተሻለ የኩላሊት ን ሽንት ቧንቧ መመርቀዝ ህክምና ለስኳር ታማሚዎች እንዲሰጡ ያግዛል።

ምስጥራዊነት፡-የጥናቱን ምስጥራዊነት ለመጠበቅ ሲባል መጠይቁ በሚሞላበት ጊዜ ስምዎት አይገለጽም። ነገር ግን የመለያ ቁጥር ይጻፋል። በተጨማሪም የሞሉት መጠይቅ ና የላቦራቶሪ ውጤት ከዋናው የጥናት ና ምርምር ባለሙያ ና ከሃኪምዎት በስተቀር ምስጢራዊነቱን በጠበቀ መልኩ ማንም በማይደርስበት ቦታ ይቀመጣል።

የጥናቱ ተሳታፊዎች መብት፡- በጥናቱ ላለመሳተፍ ከወሰኑ በማንኛዉም ሰዓት ጥናቱን ማቋረጥ ይችላሉ። ከዚህ ጥናት ና ምርምር በመውጣትዎ የተነሳ በእርስዎ የወደፊት ህክምና ክትትል ላይ የሚያስከትለው አሉታዊ ተጽዕኖ አይኖርም።

ስለጥናቱ ተጨማሪ ማብራሪያ ከፈለጉ፡- ዋናውን የጥናቱን ባለሙያ በሚከተለዉ አድራሻ ማግኘት ይችላሉ።

ስም፡ ገ/መድህን የኔሁን፣ ስልክ -0910301669፣ ኢሜል- gmedhinyenhun@gmail.com

2. ለጥናቱተሳታፊዎችየተዘጋጀየስምምነትመግለጫቅጽ

የጥናቱን ማብራሪያ አንብቤ (ተነቦልኝ) በዘዉዲቱ መታሰቢያ ሆስፒታል የስኳር በሽታ ታማሚዎች ላይ ኩላሊት ና የሽንት ቧንቧ በባክቴሪያል ተህዋስያን የመመርቀዝ ሰርጭትን ና የእነዚህ ተህዋስያን ፀረ-ባክቴሪያል መድሃኒት የመቋቋም አቅም ላይ ጥናት ማካሔድ መሆኑን ተረድቻለሁ።ዋናው የጥናቱ ባለሙያ (መረጃ ሰብሳቢው) ስለጥናቱ ጥቅም ማብራሪያ ሰጥቶኛል። ስለ ጥናቱ የተወሰኑ ጥያቄዎችን አንስቼ ማብራሪያ አግኝቻለሁ። ለጥናቱ አስፈላጊ የሆኑ መረጃዎችን ለማሰባሰብ ሲባል ስለ እኔ ማህበራዊ ና አካባቢያዊ ሁኔታዎች እና ሌሎች የኩላሊት ና ሽንት ቧንቧ መመርቀዝን ሊያባብሱ ስለሚችሉ ሁኔታዎች መረጃ እንደምሰጥ፤ በተጨማሪም የሽንት ናሙና ተወስዶ ጥናትና ምርምር እነደሚካሔደበት ተገልጾልኛል። ከዚህ ጥናት ና ምርምር በቀጥታም ይሁን በተዘዋዋሪ ተጠቃሚ እንደምሆን ተነግሮኛል። በተጨማሪም የጥናቱ ባለሙያ (መረጃ ሰብሳቢዉ) የሰጠሁት መረጃ ና የላቦራቶሪ ውጤት ከዋናው የጥናት ና ምርምር ባለሙያ ና ከሃኪሜ በስተቀር የጥናቱ ሚስጥራዊነት የተጠበቀ እንደሚሆን ገልጾልኛል። በመጨረሻም በጥናቱ ለመሳተፍ ብወስንም በማንኛዉም ሰዓት ማቋረጥ እንደምችል እና ጥናቱን በማቋረጤ ምክንያት ወደፊት በሆስፒታሉ በሚኖረኝ የህክምና ክትትል ላይ አሉታዊ ተጽዕኖ እንደማያስከትልብኝ ተነግሮኛል። በቂ ጊዜ ወስጄ ጥናቱ ለእኔም ሆነ ለአገሪቱ ያለዉን ጠቀሜታ ከግምት ዉስጥ በማስገባት በዚህ ጥናት ና ምርምሩ ለመሳተፍ የወሰንኩ መሆኑን በፊርማየ አረጋግጣለሁ።

የጥናቱ ተሳታፊ		ምስክሮች				የመረጃ ሰብሳቢ	
		1 ኛ ምስክር		2 ኛ ምስክር			
ፊርማ		ፊርማ		ፊርማ		ፊርማ	
ቀን		ቀን		ቀን		ቀን	

3. ለጥናቱተሳታፊዎችየተዘጋጀመጠይቅ

ይህ መጠይቅ ሁለት ክፍሎች ሲኖሩት የመጀመሪያው ክፍል ማህበራዊ ና አካባቢያዊ ሁኔታዎችን ሲያትት ሁለተኛዉ ክፍል ደግሞ በስኳር ህመምተኞች ላይ የኩላሊትን ና ሽንት ቧንቧ መመርቀዝን ሊያባብሱ ይችላሉ ተብለው በሚታሰቡ ሁኔታወች ላይ ያተኮረ ነዉ።

ክፍል አንድ፡-ማህበራዊ ና አካባቢያዊ ሁኔታዎችን(socio demographic characteristics)

የተሳታፊዉ መለያ ቁጥር፡ ----------

የሚከተለዉን ምልክት በመልስ መስጫው ሳጥን ዉስጥ ያስቀምጡ "X"

1.1. ጾታ □ ወንድ □ ሴት

1.2. እድሜ በ አመት _____

1.3. የመኖሪያ አድራሻ □ ከተማ □ ገጠር

1.4. የጋብቻ ሁኔታ □ ያላገባ/ያለገባች □ ያገባ/ያገባች □ የተለየ/የተለየች

1.5. የትምህርት ሁኔታ □ዘመናዊ ትምህረት ያልተማረ/ች□የመጀመሪያ ደረጃ □ሁለተኛ ደረጃ □ከሁለተኛ ደረጃ በላይ

1.6. አማካኝ ወርሃዊ ገቢ በብር □≤ 1650 □ 1,651-3,200 □ 3,201-5,250 □>5,250

ክፍል ሁለት፡- በስኳር ህመምተኞች ላይ የኩላሊትን ና ሽንት ቧንቧ መመርቀዝን ሊያባብሱ የሚችሉ ምክንያቶች

2.1. በስኳር በሽታ ከተያዙ ስንት ዓመት ሆነዎት? _____

2.2. ከዚህ በፊት በኩላሊት ና ሽንት ቧንቧ መመርቀዝ ታመዉ ያዉቃሉ? □ አዎ □ የለም

2.3. ለተራ ቁጥር 2.2 መልስዎ አዎ ከሆነ ስንት ግዜ ታመዉ ነበር?□ አንድ □ ሁለት □ ሶስት ና ከዚያ በላይ

2.4. የሚከተሉት የኩላሊትን ና ሽንት ቧንቧ መመርቀዝን ምልክቶች በአሁኑ ግዜ አለብዎት?

2.4.1. ሽንት በሚሸኑበት ግዜ የማቃጠል ስሜት □ አዎ □ የለም

2.4.2. ሽንት ማጣደፍ □ አዎ □ የለም

2.4.3. ቶል ቶል መሽናት □ አዎ □ የለም

2.4.4. የብልት አካባቢ ህመም □ አዎ □ የለም

2.4.5. ትኩሳት □ አዎ □ የለም

2.4.6. ማቅለሽለሽ □ አዎ □ የለም

2.4.7. ትዉከት □ አዎ □ የለም

2.4.8. የወገብ ና ሽንጥ ህመም □ አዎ □ የለም

2. **Patient medical history record (to be filled by physician/nurses)**

1. Types of Diabetics ☐ type 1 ☐ type 2

2. Fasting blood glucose level in mg/dl_____

Annex II: Laboratory procedures

Laboratory procedures performed includes: macroscopic inspection of urine, urine dipstick test, wet mount, gram stain of centrifuged of urine, urine culture and antimicrobial sensitivity test.

A. Media Preparation

1. Media preparation was strictly based on the manufacturer's instructions.

2. Culture plate was prepared in quantity that was used up before the shelf-life

B. Storage of prepared media

1. Media was stored in areas which provide protection against sunlight.

2. It was protected against heat and stored in the refrigerator.

C. Quality control of prepared media

1. **Sterility testing:** A Sterility test was done after autoclaving. 3 % of each batch was taken and incubated at **37 ^{o} c** for overnight. The rest was be refrigerated.
2. **Performance testing**: Performance of media was monitored with laboratory stock strains.

D. Specimen collection

1. sterile urine container was labeled with patient code and date
2. patients were instructed to discard the first few flow of urine and collect the next 10-20 ml urine
3. after the specimen received, the time of collection was recorded on urine container
4. without delaying, the collected specimens were transported to microbiology units of Addis Ababa Regional Laboratory
5. collected urine specimens physical conditions like turbidity and color were inspected
6. Urine reagent strip test was done and centrifuged urine microscopy was done for pyuria and bacteria.

E. Procedures of wet preparation

1. conical test tubes were labeled with unique patients ID provided by data collectors

2. Urine specimens were well mixed and aseptically about 10 ml of urine was transferred to the labeled test tubes.
3. Urine specimens were centrifuged at 500-1000 g for 5 minutes and supernatants were discarded completely.
4. sediment was remixed by taping the bottom of the tubes
5. one drop of well mixed sediment was transferred to a slide and covered with cover glass
6. these preparation were examined microscopically using 10X and 40X objective
7. microscopic findings were recorded (bacteria, pus cell, yeast cells and red blood cells)

F. urine Culture

1. Urine was gently mixed, and then it was slanted and tipped with a 1µl inoculating Loop touched the surface so that the urine sucked up into the loop.
2. a loop of urine was inoculated in a half plate of Blood agar and MacConkey agar
3. these plates were incubated aerobically over night at 37 ^{O}C
4. overnight incubated plates were examined for significant bacterial growth

Count the approximate number of colonies. Estimate the number of bacteria, i.e. colony-forming units (CFU) per ml of urine. Report the bacterial count as:

- Less than 10 000 colony count/ml - not significant.
- 10 000–100 000 colony count /ml, doubtful significance (suggest repeat specimen)
- More than or equal to 100 000 colony count /ml, significant bacteriuria.

5. Pure colonies were examined by Gram's staining and further identification was made by using different biochemical tests including catalase and coagulase tests for Gram-positive bacteria and hydrogen sulphide production (H_2S), oxidase test, indole test, citrate utilization, lysine decarboxylase (LDC) test, gas production, and carbohydrate metabolism for Gram-negative bacteria
6. After all identifying the bacteria antimicrobial sensitivity test was done.

G. Antimicrobial sensitivity test

1. Take pure colonies of the test organism was taken by using a sterile plastic loop and emulsified in a sterile saline.
2. Turbidity of the suspension was adjusted with 0.5 McFarland standards.

3. A sterile cotton swap was dipped into the suspension, and then this suspension was inoculated onto Muller-Hinton agar.
4. The selected antimicrobial discs were placed onto the surface of inoculated media using disc dispenser and incubated 16-18 hours at $37^{O\,c}$.
5. After 16-18 hours of incubation inhibition zone of isolate was measured by the zone of caliper.
6. Result of was recorded as Sensitive (S) and Resistant (R) according to the CLSI break point.

H. Gram staining procedures

1. the slide was labeled with patient code and date
2. one pure colony from the culture was taken and diluted with one drop of sterile saline on the slide a
3. the smear was spread over the slide
4. the smear was allowed to air dry
5. dried smear was fixed with heat (pass the smear 3 times over flame for 30 seconds)
6. the smear was covered with crystal violet for 30-45 seconds and Washed of the smear with tap water and drain
7. then it was covered with legouils iodine for 30-45 seconds and washed of the smear with tap water and drain
8. it was covered with acetone alcohol until decolorize and washed of the slide with tap water and drain
9. the smear was covered with safrannin for 30-45 seconds and washed of the smear with tap water and drain
10. the stained slide was dried with air
11. Finally, the stained smear was examined under an oil-immersion lens for gram reaction and bacterial morphology.

I. Waste Management

1. The workplace was ensured safe; by cleaning before and after work

2**.** Waste containers of different type with labeled biohazard signs in the waste generating area of the laboratory was availed at all time

3. Wase segregation was maintained by dispensing accordingly

5. Solid wastes were sealed by biohazard bag and incinerated

6. Any liquid infectious waste in the laboratory was decontaminated with 10% sodium hypochlorite before disposing it in the water sewage

7. Any accidental spillage of waste material also decontaminated using 10% sodium hypochlorate

8. Reusable materials were washed properly and sterilized with autoclave. However, those potential infectious wastes was decontaminated and incinerated.

Laboratory investigation recording format

Date _____

Unique ID number _____

Urinalysis							
Color							
Appearance							
PH				Protein			
SG.				Glucose			
Nitrite				Blood			
				Leucocytes			
Urine microscopy							
Culture result (isolated species)							
Name of drugs	**Anti microbial activities**						
	S	I	R	Name of drugs	S	I	R

S: Sensitive, I: Intermediate, R: Resistance

DECLARATION

I, undersigned, declare that this M.Sc. research thesis is my original work, has not been presented for a degree in any other University.

M.Sc. candidate: Gebremedhin Yenehun, B.Sc

Signature _____

Date of submission _____

Advisor: Woldeargay Erku, PhD

Signature: _____

Date and place _____

Addis Ababa, Ethiopia