URINARY TRACT INFECTION: BACTERIAL ETIOLOGIES, ANTIMICROBIAL SUSCEPTIBILITY PROFILE AND ASSOCIATED RISK FACTORS IN DIABETIC PATIENTS ATTENDING HAWASSA UNIVERSITY COMPREHENSIVE SPECIALIZED HOSPITAL, HAWASSA, SOUTH ETHIOPIA

BY: - ALEY MOHAMMED (BSc)

A THESIS SUBMITTED TO SCHOOL OF MEDICAL LABORATORY SCIENCES, FACULTY OF HEALTH SCIENCES, INSTITUTE OF HEALTH, JIMMA UNIVERSITY; IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE IN MEDICAL MICROBIOLOGY

SEPTEMBER, 2018
JIMMA, ETHIOPIA

JIMMA UNIVERSITY

INSTITUTE OF HEALTH

FACULTY OF HEALTH SCIENCES

SCHOOL OF MEDICAL LABORATORY SCIENCES

URINARY TRACT INFECTION: BACTERIAL ETIOLOGIES, ANTIMICROBIAL SUSCEPTIBILITY PROFILE AND ASSOCIATED RISK FACTORS IN DIABETIC PATIENTS ATTENDING HAWASSA UNIVERSITY COMPREHENSIVE SPECIALIZED HOSPITAL, HAWASSA, SOUTH ETHIOPIA

BY: ALEY MOHAMMED (BSc)

ADVISORS:

1. **Dr. GETNET BEYENE (PhD), ASSOCIATE PROFESSOR**: JIMMA UNIVERSITY, INSTITUTE OF HEALTH, SCHOOL OF MEDICAL LABORATORY SCIENCES, JIMMA, SOUTHWEST ETHIOPIA

2. **Mr. LULE TESHAGER (MSc, PhD CANDIDATE)**: JIMMA UNIVERSITY, INSTITUTE OF HEALTH, SCHOOL OF MEDICAL LABORATORY SCIENCES, JIMMA, SOUTHWEST ETHIOPIA

3. **Mr. DERESSE DAKA (MSc, ASSISTANT PROFESSOR)**: HAWASSA UNIVERSITY COLLEGE OF MEDICINE AND HEALTH SCIENCES DEPRTMENT OF MEDICAL LABORATORY SCIENCES, HAWASSA ETHIOPIA

SEPTEMBER, 2018

JIMMA, ETHIOPIA

ABSTRACT

Back ground: *Urinary Tract Infections are one of the most prevalent extra-intestinal bacterial infections, and is responsible for considerable morbidity, particularly if it is unrecognized or untreated. Diabetes mellitus causes several abnormalities of the host immune system that may result in a higher risk of infections like urinary tract infections. The improper and irrational use of many antibiotics resulted in antimicrobial resistant strains to become a major health problem throughout the world including Ethiopia.*

Objectives: *The aim of this study was to assess etiology, risk factors and antimicrobial susceptibility pattern of uropathogenic bacteria isolated from diabetic patients.*

Methods: *A hospital based prospective cross sectional study was conducted on diabetic patients from March to May, 2017. Demographic and clinical data were collected by using questionnaires. Clean catch mid-stream urine samples were collected and isolation, identification, and antimicrobial susceptibility tests were done using standard bacteriological procedures. Data entry and statistical analysis were performed by using SPSS version 21 statistical software package.*

Results: *Two hundred forty seven patients were included in this study and the overall prevalence of significant bacteriuria was 10.5%. Significant bacteriuria was significantly associated with age and body mass index. The predominant bacteria isolate was E. coli 12(46.2%) followed by Coagulase negative staphylococcus 7(26.9%). Gram negative bacteria showed high rate of sensitivity (94.1%) to Nitrofurantoin and Norfloxacine. Gram positive bacteria showed 100% sensitive for Amoxacillin-Clavunic acid. Multidrug resistance to two or more drug was observed in 19 (73.1%) of bacterial isolates.*

Conclusion and Recommendation: *Significant bacteriuria had been observed from 10.5 % of diabetic patients. Nitrofurantoin, Norfloxacine and Amoxacillin-Clavunic acid can be used for empiric treatment. Regular monitoring of susceptibility pattern of uropathogens should be essential for optimal empirical therapy of diabetic patients with urinary tract infections.*

Key words: *Diabetes, UTI, Uropathogens, Antimicrobial susceptibility, Hawassa*

ACKNOWLEDGEMENTS

First, I would like to express my gratitude to Jimma University, School of Medical Laboratory Sciences for giving me the chance to conduct this thesis with financial support.

My thanks are extended to my advisors Dr. Getnet Beyene, Mr. Lule Teshager and Mr. Derese Daka for their valuable advises starting from proposal development, actual work, and to the final write up.

I am greatful to Butajira General Hospital for sponsoring my study.

I also thank Hawassa University Compressive Specialized Hospital to allow me to undertake this work, and microbiology laboratory staff who assisted me during laboratory work and the study participants for their willingness to participate in the study.

I would like to thank my family for their unlimited support and motivation.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS AND ACRONYMS

ASB	Asymptomatic bacteriuria
BMI	Body mass index
CFU	Colony forming units
DM	Diabetes Mellitus
ESCMID	European Society for Clinical Microbiology and Infectious Diseases
HUCSH	Hawassa University Comprehensive Specialized Hospital
IDF	International Diabetes Federations
IDSA	Infectious Diseases Society of America
IGT	Impaired Glucose Tolerance
IL	Interleukin
LPS	Lipopolysaccharide
OPD	Out Patient Department
SXT	Trimethoprim–Sulfamethoxazole
UTI	Urinary Tract Infection
UTS	Urinary Tract System
WHO	World Health Organization

1. INTRODUCTION

1.1. Back ground

Urinary tract infection (UTI) is the commonest bacterial infectious disease in community practice with a high rate of morbidity and financial cost. It has been estimated that 150 million people were infected with UTI per annum worldwide costing global economy more than 6 billion US dollar (1). In humans, urinary tract is the second commonest site after the respiratory tract, for bacterial infection (2).

Urinary tract infection is an infection of the lower (urethra, bladder) or upper (ureter, kidney) urinary tract system, caused by the presence and growth of microorganisms anywhere in the urinary tract. It is usually due to bacteria from the digestive tract which climbs the opening of the urethra and begins to multiply to cause infection (3, 4).

Urinary tract infections are either complicated and difficult to treat, or uncomplicated, easy to be treated and occur mostly in young women. Many conditions enhance susceptibility for the development of a UTI with complication. Amongst these conditions are age and diabetes mellitus (5).

Urinary tract infection is more common in diabetics because of a combination of host and local risk factors. Under some circumstances urine may be inhibitory or even bactericidal against uropathogens. Modification of chemical composition of urine in diabetes mellitus can alter the ability of urine and support the growth of microorganisms. Autonomic neuropathy in diabetes mellitus impairs bladder emptying and subsequent urological manipulation predispose to UTI (6, 7).

Many different microorganisms can cause UTIs though the most common pathogens causing the simple infections in the community are *Escherichia coli* and other Enterobacteriacae, which accounts approximately 75% of the isolates (8). Gram-positive bacteria such as *Enterococcus spp*. and *Staphylococcus spp*. can also cause UTIs (9).

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (10, 11). Based on pathogenic process, DM has two broad categories. Type 1 DM is the result of complete or near-total insulin deficiency. Type 2 DM is a heterogeneous group of disorders characterized by variable degrees of insulin resistance, impaired insulin secretion, and increased glucose production (12). The chronic hyperglycemia in diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels (6, 11). Over time, patients with diabetes may develop cystopathy, nephropathy, and renal papillary necrosis, complications that predispose them to urinary tract infections. Susceptibility increases with the longer duration and great severity of diabetes (13, 14) .

Diabetes mellitus has a number of effects on urinary system. Patients either with Type 1 or Type 2 DM are at increased risk for urinary tract infection (15). Poor circulation of blood in diabetes reduces the ability of infection fighting white blood cells to get to their target site, even when they get there, they are less able to ingest the offending bacteria and kill them than normal white blood cells (16). A characteristic feature observed in UTI in diabetic patients in the presence of asymptomatic bacteriuria, more in female patients than in male patients. The exact reasons for these is not clear but may be attributed to a number of factors. These include impairment of granulocyte function, increased adherence of uropathogens to uroepithelial cells, dysfunctional bladder and increased in sugar content of urine (7). Various risk factors such as sexual intercourse, age, duration of diabetes, glycemic control, and complications of diabetes are associated with UTI (17).

The mechanisms which potentially contribute to urinary tract infection in diabetic patients are defects in local urinary cytokine secretions (IL-6, IL-8), increased adherence of microorganisms to uroepithelial cells, partly due to changed and lowered Tamm-Horsfall protein, and granulocyte dysfunction possibly as a result of an abnormal intracellular calcium metabolism. In addition hyperglycemia facilitates the colonization and growth of variety of organisms (6, 18). Nerve damage caused by high blood glucose levels, affecting the ability of the bladder to sense the presence of urine and thus allowing urine to stay for a long time in the bladder and increasing infection probability could also be another factor (18, 19).

Emphysematous complications in the kidney or the bladder are presumed to be due to the presence of organisms that rapidly ferment glucose and produce carbon dioxide. However, it is also possible that in the presence of diabetes, there is impaired transport of metabolic end products perhaps due to impaired tissue perfusion (20).

Antibiotic resistance of uropathogens is increasingly being reported in diabetic patients with high occurrence of multiple drug resistance. Higher percentage of resistance to the most commonly prescribed antimicrobial such as Ampicillin, Tetracycline, and Trimethoprim-sulphamethoxazole are reported in isolates from diabetic patients (9, 21, 22).

Antibiotics are usually given empirically before the laboratory results of urine culture are available. To ensure appropriate therapy, current knowledge of the organisms that cause UTI and their antibiotic susceptibility is mandatory. Prevalence and type of etiological agent as well as drug susceptibility pattern may vary from time to time or from place to place. The main objective of this study is therefore to study bacteria isolates causing UTI in diabetic patients, determine antimicrobial susceptibility pattern and associated factors.

1.2. Statement of the problem

Urinary Tract Infections (UTIs) are one of the most prevalent extra-intestinal bacterial infections. Nowadays, it represents one of the most common diseases encountered in medical practice affecting people of all ages from the neonate to the geriatric age group (23). Ninety five percent of UTIs are caused by uropathogens which multiply at the notch of the urethra and migrate towards the bladder. UTI is a result of various factors which may trigger infection (24).

Worldwide about 150 million people are diagnosed with UTI each year. Urinary tract infection continues to be an important and frequent cause of morbidity and mortality in the community and mainly women are predisposed. Urinary tract infections are the most commonly found bacterial infections, accounting for nearly seven million OPD visits and one million emergency department visits, resulting in 100,000 hospitalization of women, the elderly and patients with spinal cord injuries and / or catheters, multiple sclerosis`, HIV and diabetes (6, 25).

Diabetes mellitus is a worldwide health problem. In 2013, 382 million people had diabetes in the age of 20-79 ; this number is expected to rise to 592 million by 2035. (26) According to WHO, diabetes mellitus is the ninth leading cause of death worldwide (27). An association between UTI and diabetes was noted in an autopsy series reported in the 1940 (4). Many studies have shown that diabetes mellitus has a long term deleterious effect on genitourinary system that causes significant morbidity and mortality (10).

The incidence of diabetes is ever-increasing throughout the world and is becoming a serious public health threat particularly in the developing countries (6, 10, 18). Urinary tract infection is the most common infection among patients with DM and is responsible for considerable morbidity, particularly if it is unrecognized or untreated (22).

Patients with diabetes have a 10-fold increased risk of UTI when compared to non-diabetics and diabetics have a longer hospitalization than non-diabetics (11). Diabetes mellitus alters the genitourinary system where UTI can be a cause of severe complications ranging from dysuria (pain or burning sensation during Urination) organ damage and sometimes even death due to complicated UTI (pyelonephritis) (24).

Diabetes mellitus causes changes in host defense mechanisms, neuropathy which impair bladder emptying and the presence of diabetic cystopathy and micro-vascular disease in the kidneys play a significant role in the higher incidence of UTIs in diabetic patients (17).

People with diabetes can develop acute and chronic complications of diabetes. Acute complications like diabetic ketoacidosis and serious long-term complications include diabetic retinopathy, cardiovascular disease, chronic renal failure, perirenal abscess, emphysematous cystitis, emphysematous pyelonephritis, fungal infections, xantho granulomatous pyelonephritis, and papillary necrosis (14).

In sub-Saharan Africa, over 14.2 million people are estimated to have DM in 2015, and associated with the highest rate of morbidity and mortality in the world, particularly in the population who are able to work (28). In Ethiopia, although a nationwide surveillance on occurrence of DM has not been made, International Diabetes Federations (IDF) 2012 report indicated an estimated DM prevalence of 3.32 % (29). However, DM prevalence of as high as 8% has been reported in 2013 on HIV/AIDS patients taking HAART (30). On the other hand, a study conducted in Jimma reported 15.4% Impaired Glucose Tolerances (IGT) prevalence (31).

In Ethiopia, studies reported the prevalence of significant bacteriuria among diabetic patients at 10.9 % and 17.8%, and among the isolates the rate of resistance to two or more antimicrobials was 59.8% and 71.7% (9, 21).

Current management of UTIs is usually empirical, without the use of a urine culture or susceptibility testing to guide therapy. However, as with many community acquired infections, antimicrobial resistance among the pathogens that cause UTI is increasing and it is a major health problem in the treatment of UTI (4).

In summary the studies regarding bacterial uropathogens and their antimicrobial susceptibility patterns in diabetic patients, in Ethiopia, are limited. Thus; this study aims to provide additional information on bacterial etiologies of urinary tract infection, risk factors and their antimicrobial susceptibility pattern in diabetic patients attending Hawassa University comprehensive specialized Hospital.

1.3. Significance of the study

Several studies conducted elsewhere in the world indicated that diabetic patients are at greater risk of UTI than non-diabetic patients (32-34). However, in our country, there is limited information regarding bacterial uropathogens, risk factors and their resistance pattern to the commonly used anti-microbial agents among diabetic patients.

Information for empirical treatment of UTI in diabetic patients is essential, because of the emergence of resistant bacterial strains due to indiscriminate used of antimicrobial agents resulting in increased resistance to the commonly used antimicrobial agents.

Therefore, the current study will help to provide the current knowledge about the type of bacteria, risk factors responsible for UTIs, and their susceptibility patterns to common antibiotics in diabetic patients in the Ethiopia particularly in the study area which is important for the clinicians to choose the right empirical treatment and manage bacterial UTI in diabetic patients. It will also helpful as base line data for individuals who want to study further.

2. LITERATURE REVIEW

Diabetes mellitus is the most common endocrine disease and is associated with organ complications due to micro vascular and macro vascular disease. Urinary tract infections can be a particular problem for people with diabetes as glucose in the urine makes it a fertile culture media for bacterial growth. Susceptibility to bacterial infection increases with longer duration and greater severity of diabetes (35). High glucose content in the urine and defective host immune factors predispose to infection. The urinary tract is the principal site of infection in diabetics with increased risk of complications (18).

2.1. Anatomy of Urinary tract system and Urinary tract infection

The urinary system comprising of the various parts of the urinary tract including the renal artery and vein, kidneys, bladder, ureter, urethra and provision for urine exit. Kidneys acts as innate filters and play a vital role in removing the unwanted water soluble waste from the blood and also enables the reabsorption of essential ingredients like water, glucose and amino acids. The urinary bladder is a muscular flexible organ which accumulates the urine collected from the kidneys before they are disposed. The collected water soluble waste in the form of urine is then flushed out from the genitals by means of urethra which connects the urinary bladder and genitals (36). The urethra is a portal for the exit of urine, but also allows the entry of microbes, including pathogens, into the urinary tract (37).

Urinary tract infection is the presence of multiplying microorganisms in the tract through which urine flows from the kidneys via the bladder to the outside world. Most UTIs are caused by ascending colonization and/or infection by enteric bacteria of the perineum, the periurethral area, the urethra, the bladder and occasionally the kidney. Infection results when the bacterial virulence factors overcome the numerous host defenses (38). UTIs can be categorized anatomically. If it is localized to the bladder it is called cystitis; if there is renal involvement it is called pyelonephritis. The urethra is shorter in women (about 1.5 to 2 inches) when compared to men (8 inches); they are more prone to infections associated with the urinary tract (36, 39) .

2.2. Etiological agents

Urine is generally considered to be sterile and is believed to be germ free. Any source of possible infection occurs through urethra which initiates the incidence of the infection. The predominant pathogen responsible for UTI is *E. coli* which constitutes up to 80-85% and is followed by S*taphylococcus saprophyticus* which accounts to 5-10%. In addition, *Klebsiella*, *Proteus*, *Pseudomonas* and *Enterobacter species* are associated with UTI (36).

Various studies done worldwide have shown changing patterns in the etiology of UTIs in diabetic patients. A Prospective Study was done in Baghdad, Iraq among 134 diabetic patients to determine the causative organisms. A total of 84 (62.7%) bacteria were identified. The predominant bacterial isolates were *E. coli* (24; 28.6%) followed by *Klebsiella spp.* (17; 20%), *S. aureus* (14; 16.7%), *Proteus spp.* (13; 15.5%), and *Streptococcus fecalis* (11; 13%) (40).

A Study done in Tamilnadu, India among 189 diabetic patients showed that the overall prevalence of significant bacteriuria was 12.16%. A total of 23 bacteria were isolated. The predominant isolates were *E. coli* (16; 69.4%) followed by *K. pneumoniae* (4; 17.4%), *S .aureus* (1, 4.4%), *P. mirabilis* (1; 4.4%), and *P. aeruginosa* (1, 4.4%) (3).

A Study done in Buea and Limbe Regional Hospital, South West Cameroon showed that 102(81.6%) had significant bacteriuria with 59(47.2%) of them having asymptomatic bacteriuria and 43(34.4%) having urinary tract infections. *E. coli* (48.0%) was the most prevalent, followed by *S. aureus* (19.6%), and *P. mirabilis* (8.9%). The least prevalent uropathogen was *Pseudomonas aeruginosa* (1.0%) (41).

Similarly a Study done in Ibadan, Nigeria among 174 diabetic patients reported 37(21%) has significance bacteriuria. The most frequent causative agent of UTI is *Escherichia coli* accounting for 17(46%) of the isolate followed by *Klebsiella spp.* (11; 30%), *Proteus spp.* (2; 5%), *Staphylococcus aureus* (2; 5%), and *Pseudomonas aeruginosa* (1; 3%) (35).

In addition, prospective studies done in Mbarara Regional Referral Hospital, Uganda among 105 diabetic patients with and without symptoms of UTI indicated that the overall prevalence of UTI was 13.3%. From the 14 isolates, 12 were Gram negative while 2 were Gram positive

bacteria. *E. coli* (50.0%) was the highest uropathogens followed by *K. pneumoniae* (28.6%), *S. aureus* (14.3%), and unidentified coliform (7.1%) (4).

Moreover a study conducted in Khartoum, Sudan among 200 diabetic patients showed that the overall prevalence of UTI was 19.5%. The prevalence of bacteriuria among symptomatic and asymptomatic diabetic patients was 17.1% and 20.9%, respectively. A total of 39 bacteria were isolated. The predominant organism was *E. coli* (56.4%). Other isolates were *K. pneumoniae* (23.0%), *E. faecalis* (12.8%), and *P. mirabilis* (7.6%) (22).

In Ethiopia, a cross-sectional study done in Gondar university hospital, among 422 diabetic patients indicated that the overall prevalence of significance bacteriuria was 17.8%. A total of 82 bacteria were isolated. *E. coli* (31.7%), *Coagulase negative staphylococci* (22%), *Klebsiella spp.* (14.6%), *Enterococcus spp.* (11%), and *S. aureus* (8.5%) were the commonest bacterial uropathogens (9).

In addition a cross-sectional study in Tikur Anbessa hospital, among 413 diabetic patients showed that the overall prevalence of UTI was 10.9%. The predominant isolates were *E. coli* and *K. pneumoniae* (21).

2.3. Virulence factors

Uropathogenic bacteria have evolved a range of virulence factors that promote colonization and infection of the urinary tract. The virulence factors most commonly associated with these organisms include possession of fimbriae with adhesin tips, and production of toxins such as haemolysin and colony necrotising factor. Adhesins found on the surface of the bacterial membrane are responsible for initial attachment onto urinary tract tissues (42, 43).

Fimbriae and pili are surface glycoproteins that function as ligands for glycolipid and glycoprotein receptors on uroepithelial cells. The most common types of pili are types 1, P and S (44).

Type 1 pili are also referred to as mannose sensitive pili and they are commonly expressed in pathogenic and non-pathogenic strains of *E. coli*. During the colonization process Fim H

Adhesins bind to mannosylated receptors that are found on the host's uroepithelium. After binding to the epithelial surface the activated Fim H adhesins migrate towards deeper urothelial layers and penetrate the cell membrane (44, 45). P fimbriated pili or mannose resistant strain of *E. coli* are associated with uncomplicated pyelonephritis as the receptor for P fimbriae is the major glycolipid component present on renal cell membranes. PapG is an adhesin found at the tip of the pilus and it recognizes the α-d-galctopyranosyl-(1-4)-β-d-galctopyranoside receptor which is found on P-blood group antigens on the host's uroepithelium (46).

2.4. Pathogenesis

A UTI typically starts with periurethral contamination by uropathogens residing in the gut, followed by colonization of the urethra and subsequent migration of the pathogen to the bladder. In the bladder, the consequences of complex host–pathogen interactions ultimately determine whether uropathogens are successful in colonization or eliminated. Multiple bacterial adhesins recognize receptors on the bladder epithelium and mediate colonization. Uropathogens such as UPEC survive by invading the bladder epithelium, producing toxins and proteases to release nutrients from the host cells, and synthesizing siderophores to obtain iron. By multiplying and overcoming host immune surveillance, the uropathogens can subsequently ascend to the kidneys, again attaching via adhesins or pili to colonize the renal epithelium and then producing tissue-damaging toxins. Consequently, the uropathogens are able to cross the tubular epithelial barrier to access the blood stream, initiating bacteremia (47).

Higher glucose concentrations in urine may promote the growth of pathogenic bacteria (48). Lower urinary interleukin-6 and -8 levels were found in patients with diabetes with ASB, compared to those without diabetes with ASB. Autonomic neuropathy involving the genitourinary tract results in dysfunctional voiding and urinary retention, decreasing physical bacterial clearance through micturition, thereby facilitating bacterial growth (49).

2.5. Clinical features

Urinary tract infections in diabetic patients can be either asymptomatic or symptomatic. The presence of ASB is a predictor of symptomatic infections, in patients with DM as well as in patients without DM. The presentation of a lower UTI can be accompanied by classical symptoms as dysuria, frequency, urgency, haematuria, and/or abdominal discomfort. Acute pyelonephritis is a clinical syndrome characterized by fever and chills, flank pain, cost vertebral angle tenderness, and other general symptoms, such as nausea and vomiting. There may or may not be symptoms of lower UTI, such as dysuria (50).

Clinically, UTIs are categorized as uncomplicated or complicated. Uncomplicated UTIs typically affect individuals who are otherwise healthy and have no structural or neurological urinary tract abnormalities. These infections are differentiated into lower UTIs (cystitis) and upper UTIs (pyelonephritis) (47). Complicated UTIs are defined as UTIs associated with factors that compromise the urinary tract or host defense, including urinary obstruction, urinary retention caused by neurological disease, immunosuppression, renal failure, renal transplantation, pregnancy and the presence of foreign bodies such as calculi, indwelling catheters or other drainage devices (42). Emphysematous cystitis, pyelonephritis, renal and perinephric abscess, bacteremia, and renal papillary necrosis are more commonly seen in diabetic patients (51).

2.6. Risk factors

Diabetes mellitus doubles the risk of UTI (52). Women are more susceptible to UTI than men, and this is mainly due to short urethra, absence of prostatic secretion, pregnancy and easy contamination of the urinary tract with faecal flora (4). The increased frequency of UTIs in diabetic patients is likely due to several mechanisms including the presence of glycosuria, lower urinary cytokine concentrations, neutrophil dysfunction and increased adherence of the bacteria to uroepithelial cells. Factors that increase the risk of UTIs in diabetes include age, metabolic control, diabetic nephropathy, autonomic neuropathy and vascular complications (17, 48). History of previous UTI, previous antibiotic treatment, recent sexual behavior, type II diabetes, inadequate glycemic control, and duration of DM have strong association with significant bacteriuria in both symptomatic and asymptomatic diabetic patients (9).

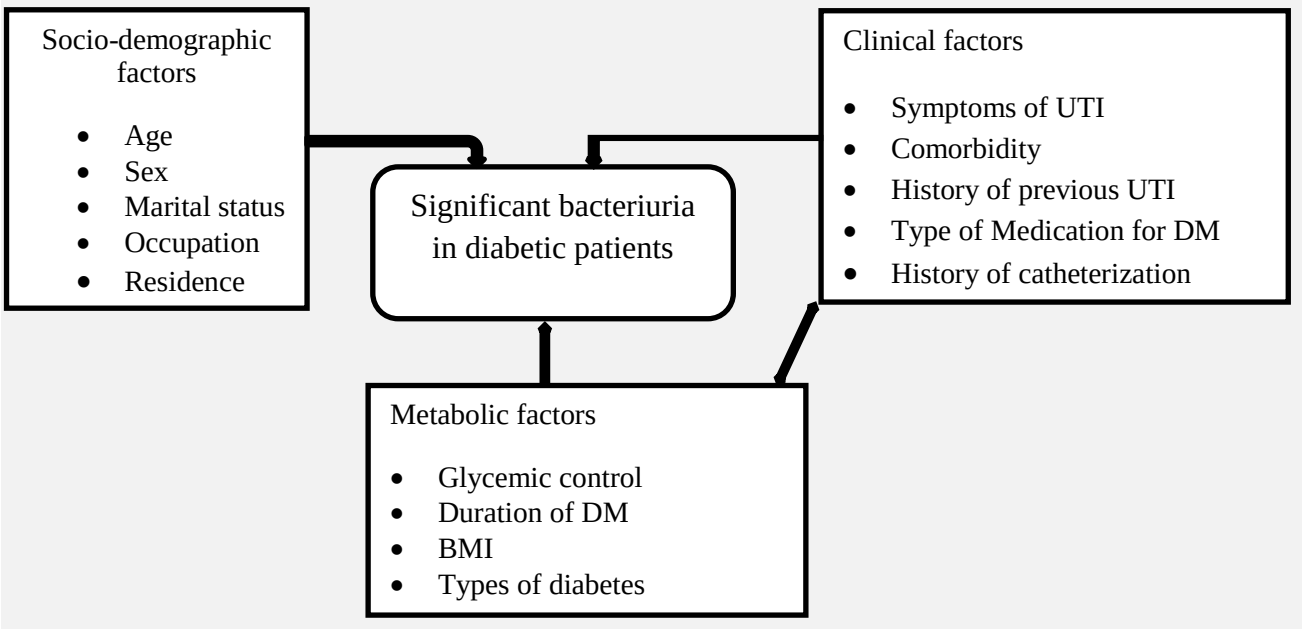

Figure 1:- Conceptual frame work on risk factors of urinary tract infection in diabetic patients

2.7. UTI in diabetic patients

Women with diabetes are more vulnerable to UTI than women without diabetes. It is a known fact that the initiation of the infection begins as asymptomatic bacteriuria which develops in to symptomatic bacteriuria as the infection progresses. Patients with diabetes are susceptible to conditions like cystopathy, nephropathy, and renal papillary necrosis, complications that incline them towards UTI (36).

High renal parenchymal glucose levels create a favorable environment for the growth and multiplication of microorganisms, which might be one of the precipitating factors of pyelonephritis and renal complications such as emphysematous pyelonephritis. Various impairments in the immune system, including humeral, cellular, and innate immunity may contribute in the pathogenesis of UTI in diabetic patients (49).

Asymptomatic bacteriuria and symptomatic urinary tract infection are more common in patients with DM. Symptomatic infection is associated with an increased severity and frequency of complications. The underlying mechanisms determining the increased risk and severity of infection are not fully described, but alterations in specific components of the host response, metabolic abnormalities, and long term complications of diabetes likely all contribute (53).

2.8. Diagnosis

The diagnosis of UTI should be suspected in any diabetic patients with symptoms consistent with UTI. The presence of leucocyte esterase, nitrite (Urinary pathogens, example *E. coli*, *Proteus species*, and *Klebsella species* are able to reduce the nitrate normally present in urine to nitrite) and microscopic haematuria are associated with UTI. Dipstick urinalysis is frequently used to test for infection and improves diagnostic precision. Microbiological analysis remains the gold standard for diagnosing UTI (54).

A urine culture should be obtained in all cases of suspected UTI in diabetic patients, prior to initiation of treatment. The only exceptions are cases of suspected acute cystitis in diabetic women who do not have long term complications of diabetes, including diabetic nephropathy, or any other complicating urologic abnormality. The preferred method of obtaining a urine culture is from voided, clean-catch, midstream urine (49). The effect of the contamination due to the commensal microbiota that colonise the periurethral meatus, the concept introduced by Kass can be applied. Kass' concept dictates that a threshold of 100,000 CFU/ml should be considered ideal for a culture to be positive in a midstream clean-catch urine sample (55).

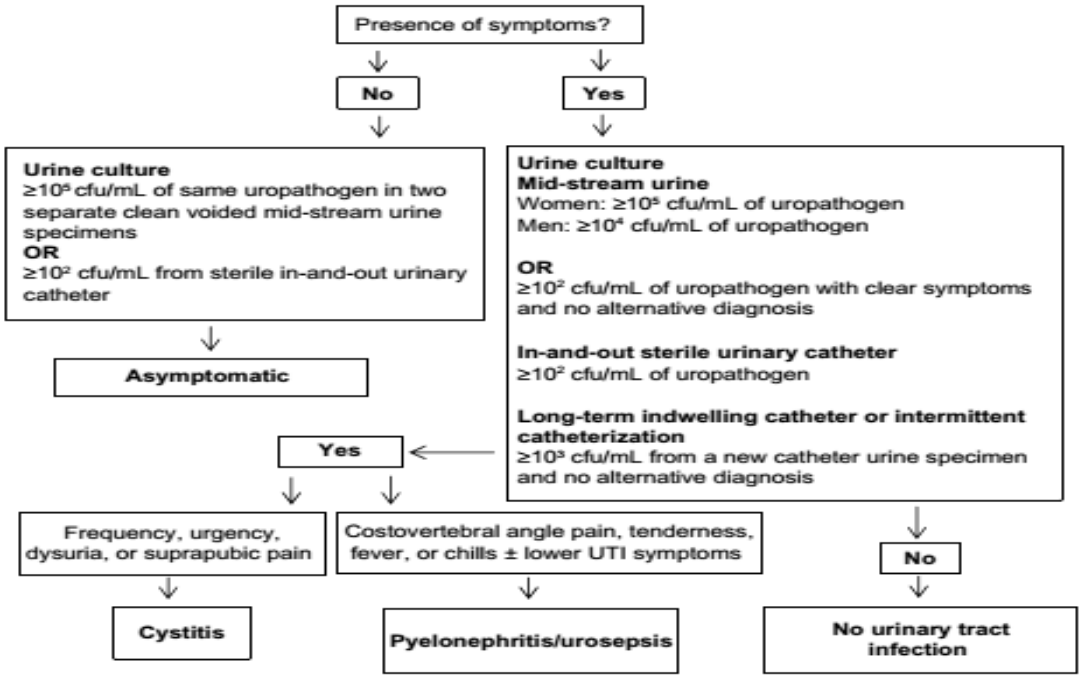

Figure 2:- Flow chart for the diagnosis of UTI in patients with diabetes mellitus (49).

The media used by most laboratories to isolate urinary pathogen is Cystine lactose electrolyte deficient (CLED) agar is because it gives consistent results and allows the growth of both Gram negative and Gram positive pathogens. The indicator in CLED agar is bromothymol blue and therefore lactose fermenting colonies appear yellow. The medium is electrolyte-deficient to prevent the swarming of Proteus species. Colony morphology of some urinary pathogens on CLED agar is appears as (56)

E. coli Yellow opaque colonies often with slightly deeper colored centre.

Klebsiella species: Large mucoid yellow or yellow-white colonies.

Proteus species: Transluscent blue-grey colonies.

P. aeruginosa: Green colonies with rough periphery.

E. faecalis: Small yellow colonies.

S. aureus: Deep yellow colonies of uniform color.

Coagulase negative staphylococci: Yellow to white colonies

2.9. Treatment

Treatment of urinary tract infection in patients with diabetes is generally similar to non-diabetic patients. Key factors to consider include whether the patient is asymptomatic or symptomatic, whether infection is localized to the bladder or kidney, and renal function (53).

The IDSA/ESCMID guidelines for the treatment of uncomplicated UTI recommended the following four agents: nitrofurantoin, fosfomycin, pivmecillinam and trimethoprim–sulfamethoxazole (SXT). For outpatients with acute pyelonephritis, the recommended therapy is oral ciprofloxacin or another fluoroquinolone, SXT, ceftriaxone, or an aminoglycoside (57, 58). β-lactam antibiotics, such as amoxicillin, cefdinir, cefaclor, or cefpodoximine, can be given when other recommended agents cannot be used. However, β-lactam antibiotics have inferior efficacy and a higher rate of resistance. Ampicillin should not be used because it displays relatively poor efficacy in the treatment of urinary tract infections and resistance rates to ampicillin are typically high (59).

2.10. Resistance pattern of UTI isolate in diabetic patients

Antibiotic resistance is an emerging and serious public health problem resulting in increased morbidity and mortality. In urinary tract infections, resistance rates against commonly prescribed antimicrobial agents are constantly rising (60). As many uropathogenic bacteria are resident in the gut, they will be exposed to oral antibiotics used for any indication. *E. coli* will frequently be resistant to oral penicillins and cephalosporins, but retains sensitivity to nitrofurantoin and quinolones. Resistance rates will vary from region to region and depend on whether the infection develops in the community or in hospital (54).

A Cross-sectional Study done in Baghdad, the *S. aureus* was found to be high resistance to ampicillin and amoxicillin (71.4% and 57%), respectively. Whereas *Streptococcus fecalis* remained susceptible to ampicillin and amoxicillin (73% and 64%), respectively. *E. coli*, *Klebsiella spp.*, and *Proteus spp.* were found to be resistance to ampicillin (54 %, 88% and 80%), respectively and to amoxicillin (58%, 82% and 80%), respectively. On the other hand trimethoprim-sulfamethoxazol was found to be effective against these microorganisms (40).

A Study done in Buea and Limbe, most of the bacterial isolates were highly sensitive to gentamicin (88.6%), imipenem (87.9%), nitrofurantoin (79.5%) and amikacin (88.3%). Some of the bacteria isolates showed resistance to ciprofloxacin (53.3%), and almost all bacterial isolates (96.3%) were found to be resistance to amoxicillin (41).

Another study done in Muhimbili, both Gram positive and negative bacteria showed high rate of resistance towards co-trimoxazole (55.6 and 50.0%, respectively) and amikacin (66.7 and 50.0%, respectively). Gram positive bacteria showed high rate of resistance towards nalidixic acid (55.6%) but no resistance to the third generation cephalosporin cefotaxime. Gram negative bacteria showed high rate of resistance to ampicillin (62.55%), penicillin (53.1%) and moderate rate of resistance to cefotaxime (18.8%) (61).

Similarly a study conducted in Ibadan showed that most isolates were sensitive to ofloxaxin, gentamicin, nitrofurantoin, nalixidic acid, and SXT while they are resistance to tetracycline, ampicillin, cefuroxime and ceftazidine (35).

In addition cross-sectional studies done in Mbarara showed that all isolates were sensitive to gentamicin 12 (100%). Majority of the Gram negative isolates were sensitive to ceftriaxone 11(91.7%), and ciprofloxacin 8 (66.7%). All Gram negative isolates showed a resistance of 100% to co-trimoxazole and 83.3% to ampicillin. *E. coli* showed 100% resistance to ampicillin and co-trimoxazole (4).

Moreover a study conducted in Khartoum showed that all isolates (100%) were susceptible to gentamicin. *E. coli* and *P. mirabilis* were 100% susceptible to cephalexin. Eight (8) out of the 9 *K. pneumoniae* isolates were susceptible to cephalexin (22).

A cross-sectional study done in Gondar, Ethiopia indicated all isolates from gram negative bacteria showed intermediate level of resistance (60-80%) to ampicillin and chloramphenicol. Low level of resistance (<60%) was observed against amoxicillin-clavulanic acid, ciprofloxacin, ceftriaxone, gentamicin and trimethoprim- sulphamethoxazole. High level of resistance (>80%) was observed against tetracycline. Gram-positive bacteria showed low level of resistance (<60%) to all antimicrobials .tested except for tetracycline (9).

3. OBJECTIVES

3.1. General Objective

- To assess the etiology , risk factors and antimicrobial susceptibility patterns of uropathogenic bacteria isolated from diabetic patients in Hawassa University Comprehensive Specialized Hospital

3.2. Specific Objectives

- To assess the distribution of bacterial uropathogens among diabetic patients.
- To assess the risk factors associated with UTIs in diabetic patients.
- To determine the antimicrobial susceptibility patterns of bacterial uropathogens to the commonly used antimicrobial agents.

4. METHODS AND MATERIALS

4.1. Study area and Period

The study was conducted at Hawassa University Comprehensive Specialized Hospital (HUCSH) in Hawassa town. Hawassa is the capital town of South Nation Nationalities and People's Regional government. It is located 275 kilometer south of Addis Ababa, and has an altitude of 1708 m above sea level with mean annual temperature and rainfall of 20.9^{O}C and 997.6 mm, respectively. Based on Central statistical agency (CSA) report in 2007 Hawassa town has about 258,808 total populations. Male and female accounts 133,123 and 125,685 respectively. HUCSH is a tertiary level teaching hospital that provides health service over 6 million inhabitants in southern Ethiopia. In the hospital, 1323 registered DM patients visits for follow up. The study was conducted from March to May 2017.

4.2. Study design

A hospital based prospective cross sectional study was conducted to assess the etiology, risk factors and antimicrobial susceptibility pattern of uropathogenic bacteria isolated from diabetic patients attending at HUCSH.

4.3. Population

4.3.1. Source population

All diabetic patients who attend Hawassa University Comprehensive Specialized Hospital diabetes clinic.

4.3.2. Study population

All diabetic patients who attend Hawassa University Comprehensive Specialized Hospital during the study period and fulfill the inclusion criteria.

4.4. Inclusion and exclusion criteria

4.4.1. Inclusion criteria

Diabetic patients (type I or type II) who had follow up at chronic disease clinic during the study period were included in the study.

4.4.2. Exclusion criteria

Non diabetic patients and diabetic patients with known underlying renal pathology or chronic renal disease, who have taken antimicrobial agent in the last two weeks, and who were not willing to participate in the study were excluded.

4.5. Study variables

4.5.1. Dependent variable

- Significant bacteriuria

4.5.2. Independent variables

- Age
- Sex
- Marital status
- Occupation
- Residence
- Types of diabetes mellitus
- Types of medication for DM
- Duration of diabetes
- Symptoms of UTI
- History previous UTI
- History of catheterization
- Comorbidity
- Fasting blood glucose level
- Body mass index

4.6. Sampling technique and sample size determination

Convenient sampling technique was used and the sample size was calculated by using single population proportion formula by considering the following assumption

P = 17.8 (prevalence of bacterial uropathogens among diabetic patients done in Gondar) (9).

The estimated margin of error is 5% and 95% confidence interval.

$$N = \frac{(z\frac{\alpha}{2})^2 * p\,(1-p)}{d^2} = \frac{(1.96)^2 * 0.178\,(1-0.178)}{0.05^2} = 224.8$$

Where N = Minimum sample size required

$z\frac{\alpha}{2}$ = Critical value at 95% confidence interval of certainty (1.96)

P = Prevalence

d = Margin of sampling error 5%

Considering 10% for anticipated non-response rate, the final sample size was **247**

4.7. Data collection

4.7.1. Socio demographic and clinical data

Socio demographic and clinical data were collected by using structured questionnaire (Annex I) by nurses and physician respectively. Diabetic patients with symptoms of UTIs were screened by the physician.

4.7.2. Laboratory data

Ten to 20 ml of midstream urine specimen was collected from each diabetic patient in a sterile, dry, wide-necked, leak-proof container. Clean catch specimen collection was explained to the patients. Female patients were explained to wash their hands, cleanse the area around the urethral opening with clean water, dry the area with a sterile gauze pad, and collect midstream urine samples with the labia held apart. Male patients were requested to wash their hands before collecting a specimen (middle of the urine flow). The container was labeled with the date, the name and code number, and the time of collection (56).

4.8. Specimen processing and identification of bacterial uropathogens

After collection the urine samples were immediately delivered to the laboratory for processing and examination. Urine specimens were processed in the laboratory within 2 hours of collection and specimens that were not processed within 2 hours were kept refrigerated at 4-6 °C (56).

Culturing and identification of isolates

A loopful of urine was inoculated on cysteine lactose electrolyte deficient (CLED) agar, MacConkey, and Blood agar plates (Oxoid, Ltd., Basingstoke, Hampshire, England) by using a sterile calibrated wire loop with a volume of 0.001ml after the specimen was mixed. The plates were incubated aerobically at 35- 37^{o}c for 24 hour and the outcome was judged as significant/ nonsignificant growth, or contaminated (discarded). Urine culture plates showing $\geq 10^5$ colony-forming units (CFU)/ml of single bacterial species were considered as significant bacteriuria (57).

Gram reaction of the organisms, microscopic appearance and colony characteristics were the presumptive identification criteria. Indole production, citrate utilization, H_2S production, gas production, urea hydrolysis, lysine decarboxylation, lactose fermentation and motility were used for further identification of gram negative bacteria (Annex IV). Coagulase, catalase, and mannitol fermentation were used for further identification of gram positive bacteria (56).

4.9. Antimicrobial susceptibility tests

The antimicrobial susceptibility testing was performed by using the standardized Kirby Bauer disc diffusion technique according to the criteria of the Clinical and Laboratory Standards Institute (CLSI) (62). Briefly from a pure culture 3-5 select colonies of bacteria were taken by using a sterile inoculation loop and transfer to a tube containing nutrient broth and mix gently to a homogenous suspension and the turbidity of the suspension was adjusted to a McFarland standard 0.5. A sterile cotton swab was dip into the suspension and the excess suspension was removed by gentle rotation of the swab against the surface of the tube. The swab was streaked evenly over the entire surface of Mueller Hinton agar (Oxoid, Ltd., Basingstoke, and Hampshire, England).

The inoculated plates were left at room temperature to dry for 3-5 minutes. With the aid of sterile forceps the following concentration of antibiotic discs were put on the surface of Mueller-Hinton agar. The following discs with their respective concentration were used Ampicillin (AMP, 10µg), Trimethoprim-sulfamethoxazole (SXT, 1.25/23.75µg), Amoxicillin-Clavulanic acid (AMC, 30µg), Gentamycin (CN, 10µg), Ceftriaxone (CRO, 30µg), Nitrofurantoin (F, 300µg), Norfloxacin (NOR, 10µg), Nalidixic acid (NA, 30µg), Tetracycline (TE, 30µg) Ciprofloxacin (CIP, 5µg) and Penicillin (P, 10 IU). All the antimicrobials for the study were obtained from Oxoid Ltd. Bashingstore Hampaire, UK. The criteria used to select the antimicrobial agents tested were based on their availability and frequent prescriptions for the management of urinary tract infections in the study area.

The plates were incubated at 37ºC for 24 hours. Diameters of the zone of inhibition around the discs were measured to the nearest millimeter using a ruler in millimeters, and the isolates were classified as sensitive, intermediate and resistant according to the standardized table supplied by CLSI (62).

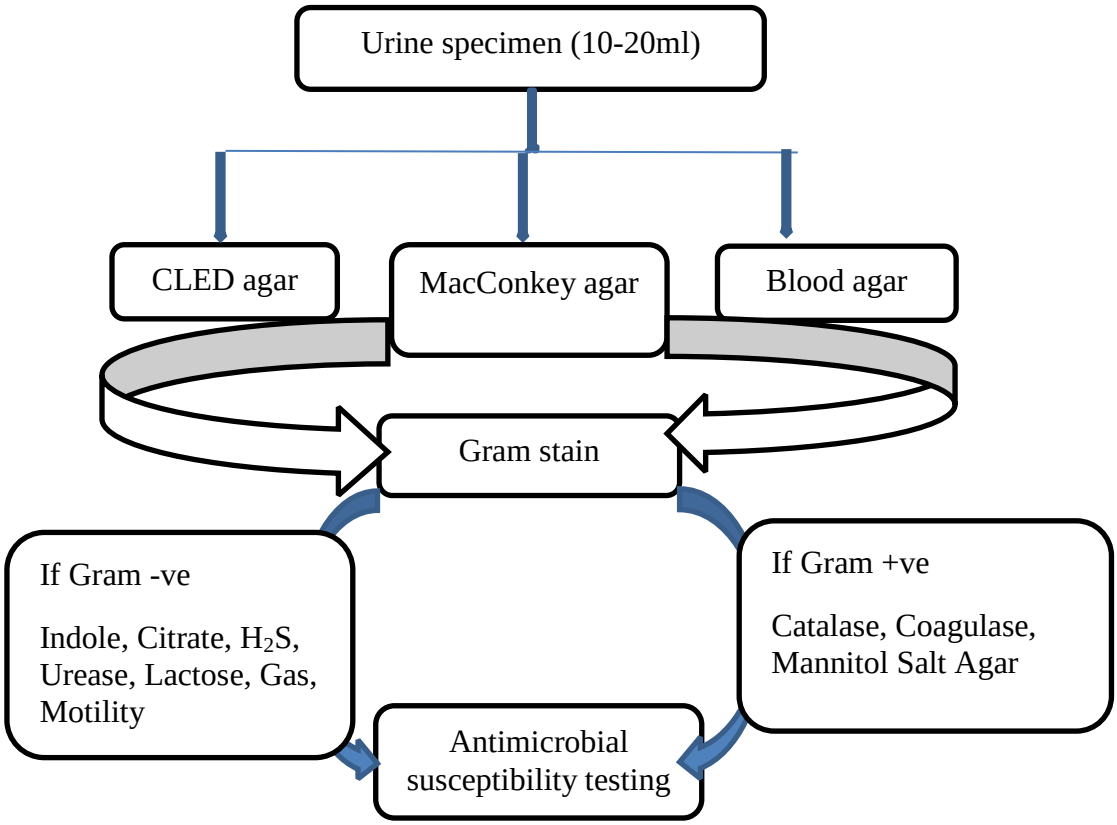

Figure 3:- Flow chart diagram of laboratory procedure

4.10. Data Quality Assurance

Training was given to data collectors on information sheet, consent form and questionnaires by primary investigator. Culture media was prepared based on the manufacturer's instruction and its sterility was checked by incubating 5% of the batch at 37^{o}C overnight and observing for any growth. Those media which showed growths were discarded the whole batch. Standard strains of *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were used to check the quality of culture and as a control for antimicrobial susceptibility testing. Moreover; Culture growth, biochemical test and antimicrobial susceptibility test results were confirmed by an experienced medical laboratory technologist working in the microbiology laboratory unit of the study area.

4.11. Data analysis

Data were cleaned, enter into a computer and statistical analysis were performed by using SPSS version 21 statistical software package. The study findings were explained in tables and figures. Both bivariate and multivariate logistic regression analysis were used to see wheather there is statistically significant association between independent and dependent variables. Probability (P) value less than 0.05 were considered statistically significant.

4.12. Ethical considerations

The research project was approved by Ethical Review Committee of Institute of Health, Jimma University. Official supportive letter was submitted and permission was obtained from Hawassa University Comprehensive Specialized Hospital administration. Written informed consent was obtained from the participant after the objectives of the study were explained to the study participants. All information obtained from the study participants were kept confidential and used only for this research purpose. The findings were communicated to the respective physicians for the management of the patients.

4.13. Dissemination of results

The findings of this study will be presented primarily on Master's thesis defense. The results will be disseminated to the public through presentation in conferences. Furthermore, a copy of the study will be given to institute of health sciences library, School of Medical Laboratory Sciences and Hawassa University Comprehensive Specialized Hospital. Finally the principal investigator and advisors will try to publish the finding of this study on known scientific Journals.

4.14. Operational definitions

Diabetes mellitus: **-** A random blood glucose concentration equal or more than 11.1mmol/l (200mg/dl) or fasting plasma glucose equal or more than 7.0mmol/l (126mg/dl) plus symptoms of diabetes mellitus.

Asymptomatic UTI: - The presence of at least 10^5 colony forming units (CFU) / ml of one or two bacterial species in clean voided midstream urine sample from an individual without any symptoms of urinary tract infection.

Symptomatic UTI: - When a patient has two or more of the following signs or symptoms: fever, urgency, frequency, dysuria, flank pain or suprapubic tenderness and a urine culture positive for 10^5 or more microorganisms per milliliter.

Significant bacteriuria: - The presence of $\geq 10^5$ colony forming units per milliliter of urine.

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

Multi drug resistance: Resistance to two or more classes of antimicrobial agents.

5. RESULTS

5.1. Socio- demographic characteristics of study subjects

A total 247 diabetic patients were investigated for UTI. Majority of the participants were male 145 (58.7%), the remaining 102 (41.3%) were female with male to female ratio of 1.42: 1. The mean age of study participants was 45.0±13.7 years (range, 18-79 years). Majority of study participants were from urban area 192 (77.7%), married 218 (88.3%) and literate 196 (79.4%). The occupational status of a study participants, 82 (33.2%) were merchant and 57 (23.1%) were house wife (Table 1).

Table 1:- Frequency of Socio-demographic variables of diabetic patients from March- May, 2017 at HUCSH, Hawassa, South Ethiopia.

Variable	Categories	Frequency	%
Age			
	18-39	84	34.0
	40-59	118	47.8
	>=60	45	18.2
Sex			
	Male	145	58.7
	Female	102	41.3
Resident			
	Urban	192	77.7
	Rural	55	22.3
Marital status			
	Married	218	88.3
	Unmarried*	29	11.7
Education			
	Illiterate	51	20.6
	Literate**	196	79.4
Occupation			
	Farmer	41	16.6
	Merchant	82	33.2
	House wife	57	23.1
	Civil servant	47	19.0
	Others***	20	8.1

*Single, Divorced, Widowed; **Primary school and above; ***Student, Daily labor

5.2. Clinical characteristics

Among 247 study participants, 198 (80.2%) patients had no symptoms of UTI and the remaining 49 (19.8%) presented with symptoms of UTI. Majority of participants were type II DM 202(81.1%). Duration of DM less than 5 years was observed in 150 (60.7%) of participants. History of previous UTI and catheterization were found in 18 (7.3%) and 7 (2.8%) of study participants, respectively. Body mass index <25kg/m^2, 25-29.9kg/m^2 and >=30kg/m^2 were found in 147(59.5%), 81(32.8%), and 19(7.7%) of study participants, respectively (Table 2).

Table 2:- Frequency of Clinical variables of diabetic patients from March to May, 2017 at HUCSH, Hawassa, South Ethiopia.

Variable	Categories	Frequency	%
Symptoms			
	Symptomatic	49	19.8
	Asymptomatic	198	80.2
Type of DM			
	Type I	45	18.2
	Type II	202	81.8
Duration of DM			
	<5 year	150	60.7
	>= year	97	39.3
FBS* (mg/dl)			
	<126	79	32.0
	>=126	168	68.0
Medication for DM			
	Tablet	150	60.7
	Insulin	74	30.0
	Both	23	9.3
Comorbidity**			
	Yes	70	28.3
	No	177	71.7
Previous UTI			
	Yes	18	7.3
	No	229	92.7
History of catheterization			
	Yes	7	2.8
	No	240	97.2
BMI * (kg/m^2)**			
	<25	147	59.5
	25-29.9	81	32.8
	>=30	19	7.7

*Fasting blood sugar; **Hypertension, Blindness ***Body mass index

5.3. Significant Bacteriuria and Bacterial Etiologies

Significant bacteriuria was observed in 26 (10.5%) of 247 diabetic patients screened for urinary tract infections. The overall prevalence of bacterial isolates of the current study was 10.5%.

Bacterial uropathogens were isolated from 26 patients among 247 diabetic patients. Out of the 26 bacteria isolated from the samples, 18(69.2%) were from female participants. Among the 26 isolate 17 (65.4%) were gram negative bacteria and 9 (34.6%) were gram positive bacteria. Six different bacteria species were isolated from study participants. The predominant bacteria isolate were *E. coli* 12 (46.2%) followed by *Coagulase negative staphylococcus* (CONs) 7(26.9%), *S.aureus* 2 (7.7%), *K. pneumoniae*. 2 (7.7%), and *K. oxytoca* 2 (7.7%). The least prevalence uropathogen was *Acinetobacter species* 1 (3.8%) as shown in Figure 4.

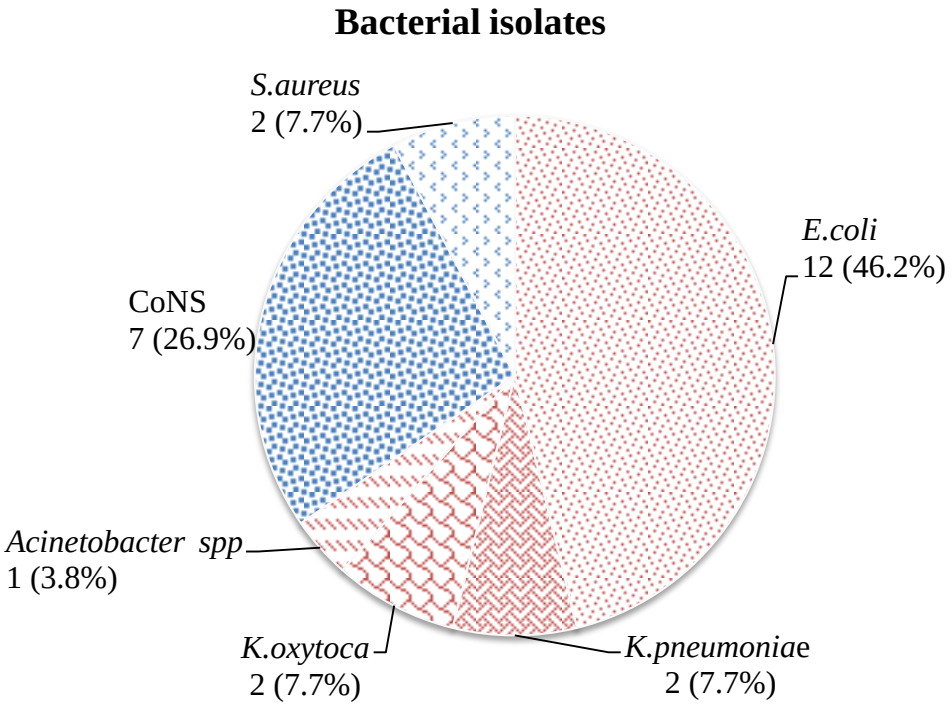

Figure 4:- Frequency of bacteria isolated from diabetic patients from March- May, 2017 at HUCSH, Hawassa, South Ethiopia.

5.4. Risk factors for significant bacteriuria

Potential risk factors for significant bacteriuria were assessed (Table 3). In bivariate logistic regression analysis age range 40-59 years , female sex, house wife occupation, symptoms of UTI, duration of DM >= 5years, insulin medication, history of previous UTI, and BMI >=30kg/m^2, were met our cutoff criteria of P < 0.25 and the candidate variables for multivariate logistic regression analysis.

Finally in multivariate logistic regression analysis to control the confounding effects of the risk variables, age range 40-59 years (AOR=0.19; 95%CI=0.05-0.65; P < 0.01), and BMI >=30kg/m^2 (AOR=14.44; 95%CI=3.55-58.77; P < 0.01) were found to have statistically significant association with significant bacteriuria. Age range of 40-59 years were 80% less likely to develop significant bacteriuria than age range of 18-39 years (AOR=0.19; 95%CI=0.05-0.65; P < 0.01). DM patients with BMI >=30 kg/m^2 (AOR=11.94; 95%CI=3.92-47.30; P < 0.01) had higher odd ratio compared with those BMI < 25 kg/m^2. However, sex, occupation, symptoms of UTI, duration of DM, medication for DM, and history of previous UTI were not found to be significantly associated with significant bacteriuria (P > 0.05) as shown in Table 3.

Table 3:- Statistical analysis of independent variables with respect to their contribution to significant bacteriuria from March- May, 2017 at HUCSH, Hawassa, South Ethiopia.

Characteristic	Significant bacteriuria	NO significant bacteriuria	COR (95%CI)	AOR (95%CI)
Age				
18-39	13 (15.5)	71 (84.5)	1	1
40-59	8 (6.8)	110 (93.2)	**0.40 (0.16-1.01)*** **	**0.19 (0.05-0.65)** **
>=60	5 (11.1)	40 (88.9)	0.68 (0.23-2.06)	0.72 (0.18-2.87)
Sex				
Male	8 (5.5)	137 (94.5)	1	1
Female	18 (17.6)	84 (82.4)	**3.67 (1.53-8.81)***	1.54 (0.35-6.83)
Occupation				
Farmer	2 (4.9)	39 (95.1)	1	1
Merchant	8 (9.8)	74 (90.2)	2.11 (0.43-10.41)	1.33 (0.21-8.42)
House wife	14 (24.6)	43 (75.4)	**6.35 (1.36-29.72)***	3.59 (0.38-34.12)
Civil servant	1 (2.1)	46 (97.9)	0.42 (0.04-4.86)	0.31 (0.02-5.03)
Others ****	1 (5.0)	19 (95.0)	1.03 (0.09-12.04)	0.42 (0.03-6.59)
Symptom				
Symptomatic	10 (20.4)	39 (79.6)	**2.92 (1.23-6.91)***	2.74 (0.92-8.13)
Asymptomatic	16 (8.1)	182 (91.9)	1	1
Duration of DM				
<5year	13 (8.7)	137 (91.3)	1	1
>=5year	13 (13.4)	84 (86.6)	1.63 (0.72-3.69)	1.30 (0.48-3.55)
Medication for DM				
Tablet	13 (8.7)	137 (91.3)	1	1
Insulin	12 (16.2)	62 (83.8)	2.04 (0.88-4.73)	1.93 (0.66-5.66)
Both	1 (4.3)	22 (95.7)	0.48 (0.60-3.85)	0.50 (0.05-4.61)
History of previous UTI				
yes	4 (22.2)	14 (77.8)	2.69 (0.81-8.88)	1.58 (0.34-7.46)
No	22 (9.6)	207 (90.4)	1	1
BMI (kg/m^2)				
<25	11 (7.5)	136 (92.5)	1	1
25-29.9	8 (9.9)	73 (90.1)	1.36 (0.52-3.52)	2.21 (0.70-6.97)
>=30	7 (36.8)	12 (63.2)	**7.21 (2.36-22.03)** **	**14.44 (3.55-58.77)** **

*P < 0.05; **P < 0.01; ***P=0.05 ****Student, Daily labor; COR= Crude odd ratio; AOR=Adjusted odd ratio; CI= Confidence interval; BMI= Body mass index; 1= Constant

5.5. Antimicrobial susceptibility testing

The result of antimicrobial susceptibility pattern of the isolate is shown in Table 4 and Table 5. The susceptibility pattern of Gram negative bacteria (n=17) against 10 antimicrobial agent presented in Table 4. Antimicrobial resistance level of Gram negative bacteria isolates ranged from 5.9% to 82.4%. From Gram negative bacteria isolates, high rate of resistant (82.4%) was observed against Ampicillin and Tetracycline. On the other hand, high rate of sensitivity (94.1%) was observed against Nitrofurantoin and Norfloxacine.

All (100%) *E. coli* isolates were sensitive to Nitrofurantoin and Norfloxacine, and 75% of the isolates (9/12) were sensitive to Ceftriaxone and Ciprofloxacin. *E. coli* isolates also showed high resistant to Tetracycline (11; 91.7%) and Ampicillin (10; 83.3%). All *Klebsella* species isolates were sensitive to Gentamicin and all of the isolates were found to be resistant to Ampicillin. Single *Acinetobacter* species isolate was sensitive to Ampicillin, Amoxicillin-Clavunic acid, Ceftriaxone, Nitrofurantoin and Norfloxacine (Table 4).

Table 4:- Antimicrobial susceptibility patterns of Gram negative bacteria isolated from diabetic patients with UTI from March- May, 2017 at HUCSH, Hawassa, South Ethiopia.

Bacteria	Total	S/R	AMP	AMC	SXT	CN	CRO	F	NOR	NAL	TE	CIP
E. coli	12	**S**	2 (16.7)	7 (58.3)	4 (33.3)	7 (58.3)	9 (75.0)	**12 (100)**	**12 (100)**	3 (25.0)	1 (8.3)	9 (75.0)
		R	**10 (83.3)**	5 (41.7)	8 (66.7)	5 (41.7)	3 (25.0)	0 (0)	0 (0)	9 (75.0)	**11 (91.7)**	3 (25.0)
K.pneumoniae.	2	**S**	0 (0)	1 (50.0)	2 (100)	**2 (100)**	1 (50.0)	1 (50.0)	2 (100)	1 (50.0)	0 (0)	2 (100)
		R	**2 (100)**	1 (50.0)	0 (0)	0 (0)	1 (50.0)	1 (50.0)	0 (0)	1 (50.0)	2 (100)	0 (0)
K. oxytoca	2	**S**	0 (0)	0 (0)	0 (0)	**2 (100)**	0 (0)	2 (100)	1 (50.0)	1 (50.0)	2 (100)	0 (0)
		R	**2 (100)**	2 (100)	2 (100)	0 (0)	2 (100)	0 (0)	1 (50.0)	1 (50.0)	0 (0)	2 (100)
Acinetobacter spp.	1	**S**	**1 (100)**	**1 (100)**	0 (0)	0 (0)	**1 (100)**	**1 (100)**	**1 (100)**	0 (0)	0 (0)	0 (0)
		R	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)	1 (100)	1 (100)
TOTAL	17	**S**	3 (17.6)	9 (52.9)	6 (35.3)	11 (64.7)	11 (64.7)	**16 (94.1)**	**16 (94.1)**	5 (29.4)	3 (17.6)	11 (64.7)
		R	**14 (82.4)**	8 (47.1)	11 (64.7)	6 (35.3)	6 (35.3)	1 (5.9)	1 (5.9)	12 (70.6)	**14 (82.4)**	6 (35.3)

S: Sensitive; R: Resistance; AMP: Ampicillin; AMC: Amoxicillin- Clavulanic acid; SXT: Trimethoprim-sulphamethoxazole; CN: Gentamicin; CRO: Ceftriaxone; F: Nitrofurantoin; NOR: Norfloxacine; NAL: Nalidixic acid; TE: Tetracycline; CIP: Ciprofloxacin

The susceptibility pattern of Gram positive bacteria (n=9) against 10 antimicrobial agent presented in Table 5. Antimicrobial resistance level of Gram positive bacteria isolates ranged from 0% to 100%. All Gram positive isolates showed resistance against Trimethoprim-sulphamethoxazole. On the other hand all of isolates were found to be sensitive to Amoxicillin-Clavulanic acid.

Coagulase Negative Staphylococcus were the predominant gram positive isolate which showed 7(100%) sensitivity to Amoxicillin- Clavulanic acid and 6(85.7%) of the isolates were found to be sensitive to Ceftriaxone. On the other hand these bacteria were found to be resistant to Trimethoprim-sulphamethoxazole (100%) and Tetracycline 6(85.7%) as shown in Table 5.

Table 5:- Antimicrobial susceptibility patterns of Gram positive bacteria isolated from diabetic patients with UTI from March- May 2017 at HUCSH, Hawassa South Ethiopia.

Bacteria	Total	S/R	AMP	AMC	SXT	CN	CRO	F	NOR	TE	CIP	P
CONs	7	**S**	2 (28.6)	**7 (100)**	0 (0)	4 (57.1)	**6 (85.7)**	4 (57.1)	4 (57.1)	1 (14.3)	4(57.1)	3 (42.9)
		R	5 (71.4)	0 (0)	**7 (100)**	3 (42.9)	1 (14.3)	3 (42.9)	3 (42.9)	**6 (85.7)**	3(42.9)	4 (57.1)
S.aureus	2	**S**	0 (0)	**2 (100)**	0 (0)	**2 (100)**	0 (0)	**2 (100)**	1 (50.0)	0 (0)	1(50.0)	0 (0)
		R	**2 (100)**	0 (0)	**2 (100)**	0 (0)	**2 (100)**	0 (0)	1 (50.0)	**2 (100)**	1(50.0)	**2 (100)**
TOTAL	9	**S**	2 (22.2)	**9 (100)**	0 (0)	6 (66.7)	6 (66.7)	6 (66.7)	5 (55.6)	1 (11.1)	5(55.6)	3 (33.3)
		R	7 (77.8)	0 (0)	**9 (100)**	3 (33.3)	3 (33.3)	3 (33.3)	4 (44.4)	**8 (88.9)**	4(44.4)	6 (66.7)

S: Sensitive; R: Resistance; AMP: Ampicillin; AMC: Amoxicillin- Clavulanic acid; SXT: Trimethoprim-sulphamethoxazole; CN: Gentamicin; CRO: Ceftriaxone; F: Nitrofurantoin; NOR: Norfloxacine; TE: Tetracycline; CIP: Ciprofloxacin; P: Penicillin, CONs: Coagulase negative Staphylococcus

5.6. Multidrug resistance pattern of the isolates

Multidrug resistance (resistance to two or more drug) was observed in 19 (73.1%) of bacterial isolates. Of which 11 (57.9%) and 8 (42.1%) were Gram negative and Gram positive bacteria, respectively. Nine (75.0%) of the *E. coli* isolates were MDR. There was no isolate which was sensitive and resistant to all tested antibiotics (Table 6 and Table 7).

Table 6:- Multi-drug resistance pattern of Gram negative bacteria isolated from diabetic patients with UTI from March- May 2017 at HUCSH, Hawassa, South Ethiopia.

Antibiotics	*E .coli*	*K. pneumoniae*	*K. oxytoca*	*Acinetobacter* spp.	Total
SXT,TE	1				1
NA,TE		1			1
AMP,TE	2				2
AMP,AMC	2				2
AMP,NA,TE	1				1
AMP,SXT,TE	1				1
AMP,SXT,TE,CN	1				1
AMP,SXT,CRO,AMC,NA		1			1
AMP,SXT,AMC,NA,CIP,P	1				1
Total	9 (81.8%)	2 (18.2%)	0	0	11 (100%)

AMP: Ampicillin; AMC: Amoxicillin- Clavulanic acid; SXT: Trimethoprim-sulphamethoxazole; CN: Gentamicin; CRO: Ceftriaxone; F: Nitrofurantoin; NOR: Norfloxacine; NAL: Nalidixic acid; TE: Tetracycline; CIP: Ciprofloxacin

Table 7:- Multi-drug resistance pattern of Gram positive bacteria isolated from diabetic patients with UTI from March- May 2017 at HUCSH, Hawassa, South Ethiopia.

Antibiotics	CONs	*S. aureus*	Total
CIP,NOR	1		1
SXT,NOR	1		1
SXT,CIP,P	1		1
AMP,SXT,P	1		1
AMP,SXT,TE,CIP	1		1
AMP,SXT,TE,,P		1	1
AMP,SXT,TE,P,CRO	1		1
AMP,SXT,TE,NOR,CIP		1	1
Total	6 (75%)	2 (25%)	8 (100%)

AMP: Ampicillin; AMC: Amoxicillin- Clavulanic acid; SXT: Trimethoprim-sulphamethoxazole; CN: Gentamicin; CRO: Ceftriaxone; F: Nitrofurantoin; NOR: Norfloxacine; TE: Tetracycline; CIP: Ciprofloxacin; P: Penicillin; CONs: Coagulase negative Staphylococcus

6. DISCUSSION

Urinary tract infection is the commonest bacterial infectious disease with a high rate of morbidity and financial cost. The risk of developing infection in diabetes is higher due to abnormalities in the host defence and high glucose in urine (3). The aim of this study was to assess the etiology, risk factors and antimicrobial susceptibility pattern of uropathogenic bacteria isolated from diabetic patients in Hawassa University Comprehensive Specialized Hospital.

In the present study the overall prevalence of significant bacteriuria in diabetic patients was 10.5%. This is similar to the findings reported previously in Addis Ababa (10.9%) (21), Debre Tabor (10.9%) (63) and Romania (10.7%) (64), but lower than a study done in Gondar (17.8%) (9) and other studies done in Sudan (19.5%) (22), Nepal (21%) (65), Iraq (35.3%) (5), and Pakistan (51%) (66). This variation in prevalence might be due to the difference in sample size, geographical location, standard personal hygiene, and variation in the screening test used.

In our study the most frequently isolated bacterial uropathogens were *E. coli* (12; 46.2%), *Coagulase negative staphylococcus* (7; 26.9%), *K. pneumoniae* (2; 7.7%), *K. oxytoca* (2; 7.7%), *S. aureus* (2; 7.7%), and *Acinetobacter spp.* (1; 3.8%). The predominant bacteria isolate in our study was *E .coli*. This is similar with previous study finding in Ethiopia and other countries in Sudan (56.4%), Nigeria (46%), and Cameroon (48%) (9, 22, 35, 41). *E .coli* considered the most predominant uropthogen due to a number of virulence factors specific for colonization and invasion of the urinary epithelium, such as the P-fimbriae and S fimbriae adhesions or it could be due to the presence of unique structure in Gram negative bacteria which help for attachment to the uro-epithelial cells and prevent bacteria from urinary lavage, allowing for multiplication and tissue invasion (67, 68).

The second most common isolate was *Coagulase negative staphylococcus* (CONs) (26.9%). This is in agreement with a study done in Gondar (22.0%) (9), but contradict with a study done in Addis Ababa (28%) (21) and other countries in Sudan (23%), Uganda (28.6%), India (20%), Iraq(15.1%), and Nepal (21.6%) (3-6, 22), where *K .pneumoniae* was the second commonest isolate. The high isolation rate of CONs in this study could be change in pattern of infection in diabetic patients (3).

Urinary tract infection appears to be multifactorial in subjects with diabetes and various diabetes-related risk factors. In the present study BMI >=30 kg/m^2 was about fourteen times more likely to develop significant bacteriuria (AOR=14.44; 95%CI=3.55-58.77; **P < 0.01**). This is in agreement with a study done in Saudi Arabia (69) and Spain (70). But a study in Iran to determine the association between BMI and UTI in adult, there was no significant association (71). The increase incidence of UTI in obese patients could be due to their inability to exert sufficient pressure to empty the bladder. May also be because of poor control of diabetes (69).

In our study age range with 40- 59 years were 80% less likely to develop significant bacteriuria compared with age range of 18-39 years (AOR=0.19; 95%CI=0.05-0.65; **P < 0.01**). A study in Gondar also showed 30.7% of bacteriuria within 20- 35 age range, but not statistically significant (9). Other studies in Sudan (22), and Saudi Arabia (69) reported that no significant association between age and significant bacteriuria. The variation may be due to the difference in distribution and categories of age. Although it might be the age range of 18-39 years were more sexually active than with their counterparts.

From our study significant bacteriuria was high among female diabetic patients (17.6%) than male diabetic patients (5.5%). This is in agreement with many other studies done in Gondar (21.2%), Debre Tabor (16.3%), Uganda (16.4%), Romania (15.3%) (4, 9, 63, 64) were high prevalence of bacteriuria in female than male, but contradict with a study done in Sudan high prevalence in male (21.2%) than female (14%) (22). The high prevalence of bacteriuria in female due to a result of short urethra, close proximity the urethra to the anus, decrease of normal vagina flora, less acidic pH of vaginal surface, lack of prostate secretion, and poor hygienic condition may access entry of bacteria in to bladder and may cause infection (3, 9).

The frequency of UTI in this study is also high their occupation is house wife (14; 53.8%). There was no statistical significant association between significant bacteriuria and occupation (**P>0.05**). Similarly a study in Iran to determine the impact of demographic factors reported that high prevalence UTI among married patients 50(33.3%) and unemployed individual 30 (26.3%) (72). This might be due to exposure to sexual action.

The presence of Asymptomatic bacteriuria is a predictor of symptomatic infections in patients with Diabetes mellitus as well as in patients without Diabetes mellitus (50, 70). In our study there was no statistical significant association between significant bacteriuria and symptom of UTI (**P >0.05**). In the present study significant bacteriuria was detected in 20.4% of symptomatic diabetic patients. This is in agreement with report from Sudan (17.1%) (22), Debre Tabor (19.0%) (63), but higher than studies from Addis Ababa (13.6%) (21) and lower than studies from Gondar (51.4%) (9), and Cameroon (34.4%) (41). This variation in prevalence might be due to the difference in sample size and geographical location.

In our study the frequency of UTI was higher among duration of DM greater than 5 years (13.4%) compared to those duration of DM less than 5 years (8.7%), although not statistically significant (**P > 0.05**). This is in agreement with a study done in Gondar (9), Sudan (22), Saudi Arabia (69) and Iran (72). But a study done in Romania longer duration is a significant risk factor associated with UTI (73). Duration of diabetes had been described as risk factor for complicated UTI, probably because of concurrent neuropathy (69).

In this study type of medication for DM was not statistically significant association with significant bacteriuria (**P > 0.05**). The frequency of UTI among those using insulin for medication was high (12; 16.2%) than others. This is in agreement with a study done in Iran reported that high frequency of UTI (14; 46.7%) among insulin user. Also the report showed no significant association with UTI (72). The use of insulin may be a marker of disease severity(74).

In the present study history of previous UTI was not statistically significant associated with significant bacteriuria (**P > 0.05**). This study in agreement with a study done in Sudan (22). But a study done in Gondar a significant association between history of UTI and significant bacteriuria was observed (9). This might be due to ineffective treatment or presence of resistance strains from those who had previous history of UTI.

Antibiotic resistance among the commonly isolated uropathogens to the commonly used antibiotics is emerging and this makes clinicians to have limited choices of drugs for the treatment of urinary tract infection (4). In the present study gram negative bacteria showed high rate (16; 94.1%) of sensitivity to Nitrofurantoin and Norfloxacine. This is in agreement with

study done in Sudan and Iraq (10, 22). Also the isolates showed high rate (82.4%) of resistant to Ampicillin and Tetracycline. Similar result reported in Gondar, Uganda and India (3, 4, 9). The high sensitivity pattern of Nitrofurantoin and Norfloxacine might be due to its unavailability and is not frequently prescribed in the study area.

In our study *E. coli* showed 12(100%) sensitivity to Nitrofurantoin and Norfloxacine while high resistance to Ampicillin (10; 83.8%) and Tetracycline (11; 91.7%). A study done in Addis Ababa showed that high rate of sensitivity (>85%) to Nitrofrantoin, Norfloxacine, Ciprofloxacin, Gentamicin, Ceftriaxone, Ceftazidine and Amoxacillin-Clavunic acid (21). A study done in Gondar reported high rate of resistance for Ampicillin (16; 61.5%) and Tetracycline (21; 80.8%) (9). The high level of resistance may be due to easy availability of the antibiotic. In this study *Klebsella spp.* Showed high rate of sensitivity to Gentamicin (4; 100%), Nitrofurantoin (3; 75%) and Norfloxacine (3; 75%). Similar finding reported from Debre Tabor (63), Sudan (22), and Uganda (4). Also the isolates showed high resistance to Ampicillin (100%). Similar finding were reported from Gondar (9).

Gram positive bacteria showed high rate of sensitivity (100%) to Amoxacillin-Clavunic acid and (66.7%) to Gentamicin, Ceftriaxone and Nitrofurantoin. However, high rate of resistance observed in Trimethoprim-sulphamethoxazole (88.9%), and Penicillin (66.7%). Similar finding reported from Sudan, it showed 100% sensitivity to Amoxacillin-Clavunic acid and Nitrofurantoin (22). Study in Gondar also report high rate of sensitivity to Amoxacillin-Clavunic acid (91.4%), Gentamicine (77.1%), and Ceftriaxone (80.0%) (9). But in contrary to our study a study in Gondar reported low rate of resistance to Trimethoprim-sulphamethoxazole (57.1%), Penicillin (51.4%) and Ampicillin (17.1%) (9).

Multidrug resistance of the isolate was observed in 73.1% of the bacteria uropathogen. This is comparable with the study from Addis Ababa (71.7%) (21), but relatively higher than a study done in Gondar (59.8%) (9), Debre Tabor (56.7%) (63), and Sudan (28.2%) (22). Nine (75%) of *E.coli* isolate were MDR. This is comparable with study done in Gondar (61.5%) (9), but higher than a study done in Debre Tabor (50%) (63) and Sudan (22.7%) (22). Reason for multidrug resistance of the isolate might be inappropriate and incorrect administration of antimicrobial agents as empirical treatment.

LIMITATION OF THE STUDY

1. This study done only for those who visited the diabetic clinic during the study period and may not represent the general population.
2. This study identify only bacteria etiology, other etiology may contribute for UTI.
3. The study design did not include control group.

7. CONCLUSSION AND RECOMMENDATION

The current study showed that the overall prevalence of significant bacteriuria in diabetic patients was 10.5%. The predominant isolated uropathogens were *E. coli* (46.2%) followed by *Coagulase negative staphylococcus* (26.9%). Significant bacteriuria was significantly associated with BMI. Gram negative bacteria showed high rate of sensitivity to Nitrofurantoin and Norfloxacine. Gram positive bacteria show high rate sensitivity to Amoxacillin-Clavunic acid. Multi-drug resistance has been shown in 73.1% of bacterial isolates.

Based on the findings of the present study the following recommendations are made:

- Diabetic patients should not be neglected to screen bacterial uropathogens.
- Diabetic patients should control their BMI to decrease the risk of UTI.
- Nitrofurantoin, Norfloxacine and Amoxacillin-Clavunic acid can be used as a drug of choice for immediate empiric treatment.
- Regular monitoring of antimicrobial susceptibility pattern is very essential to establish reliable information about resistance pattern of urinary pathogens for optimal empirical therapy of diabetic patients with UTI.
- A more comprehensive survey should be carried out, in order to isolate other causes of UTI, such as fungi and parasites.

REFERENCES

1. Prakash D, Saxena R. Distribution and Antimicrobial Susceptibility Pattern of Bacterial Pathogens Causing Urinary Tract Infection in Urban Community of Meerut City, India. *ISRN Microbiology*. 2013;2013:1-13.

2. Pargavi B, Mekala T, Selvi A, Moorthy K. Prevalence of Urinary Tract Infection among Diabetics patients in Vandavasi, Tamil Nadu, India. *International Journal of Biological Technology*. 2011;2(2):42-5.

3. Senthamarai S, Sivasankari S, Anitha C, Vasumathi A, Venugopal V, Thenmozhivalli PR, *et al.* A study on clinical presentation, bacterial profile and its antibiotic sensitivity pattern in urinary tract infections among diabetic patients attending tertiary care hospital, Tamilnadu. *Int J App Res*. 2016;2(3):157-9.

4. Ampaire L, Butoto A, Orikiriza P, Muhwezi O. Bacterial and Drug Susceptibility Profiles of Urinary Tract Infection in Diabetes Mellitus Patients at Mbarara Regional Referral Hospital, Uganda *BMRJ*. 2015;9(4):1-5.

5. Ahmed A. Prevalence of urinary tract infection in diabetic patients and identification of the causal microorganisms. *Zanco J Med Sci*. 2013;17(1):363-9.

6. Yadav K, Prakash S. Antimicrobial Resistance Pattern of Uropathogens Causing Urinary Tract Infection (UTI) Among Diabetics *Biomed Res Int*. 2016;1:07-15.

7. Sharma V, Gupta V, Mittal M. Prevalence Of Uropathogens In Diabetic Patients And Their Antimicrobial Susceptibility Pattern. *NJLM*. 2012;1(1):26-8.

8. Beyene G, Tsegaye W. Bacterial uropathogens in Urinary tract infection and antibiotic susceptibility pattern in Jimma University specialized hospital, South west Ethiopia *Ethiop J Health Sci* 2011 21(2):141-6.

9. Yismaw G, Asrat D, Woldeamanuel Y, G.Unakal C. Urinary Tract Infection: Bacterial etiologies, drugresistance profile and associated risk factors in diabetic patients attending Gondar University Hospital, Gondar, Ethiopia *European Journal of Experimental Biology*. 2012;2(4):889-98.

10. Al-Qaseer A, Abdul-wahab B, Abbas O. Bacteriological finding of urinary tract infection in diabetic patients. *Int J Adv Res*. 2014;2(10):274-9.

11. Almazini M. Incidence and sensitivity of bacterial uropathogens among diabetic patients *Euro J Exp Bio*. 2016;6(3):8-12.

12. American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. *Diabetes Car*. 2014;37(1):S81-S9.

13. Acharya D, Bogati B, Shrestha G, Gyawali P. Diabetes mellitus and Urinary Tract Infection Spectrum of Uropathogens and their Antibiotic Sensitivity Pattern. *JMMIHS*. 2015;1 (4):24-8.

14. Kumar V, Hasan S. Prevalence of uropathogens in diabetic patients types 1 and 2, and their corresponding resistance pattern *Ind J Bas App Med Res*. 2015;5(1):85-96.

15. Jha P, Baral R, Khanal B. Prevalence of Uropathogens in Diabetic Patients and Their Susceptibility Pattern at a Tertiary Care Center in Nepal-A Retrospective Study. *IJBLS*. 2014;3(2):9-34.

16. Sibi G, Devi A, Fozia K, Patil B. Prevalence, Microbiologic Profile of Urinary Tract Infection and its Treatment with Trimethoprim in Diabetic patients. *Res J Microbiol*. 2011:1-9.

17. Abdulla M, Jenner F, Alungal J. Urinary tract infection in type 2 diabetic patients: risk factors and antimicrobial pattern. *Int J Res Med Sci* 2015;3(10):2576-9.

18. Gambhir S, Dalal B, Modak M, Lahiri K. Bacteriological Profile and Antibiotic Susceptibility Pattern of Urinary Tract Infection in Diabetic Patients. *IJSR*. 2016;5(2):298-301.

19. Sewify M, Nair S, Warsame S, Murad M, Alhubail A, Behbehani K, *et al.* Prevalence of Urinary Tract Infection and Antimicrobial Susceptibility among Diabetic Patients with Controlled and Uncontrolled Glycemia in Kuwait. *J Diabetes Res*. 2016:1-7.

20. Ronalda A, Ludwig E, Baldo A. Urinary tract infections in adults with diabetes. *Int J Antimicrob Agent*. 2001;17:287-92.

21. Yeshitela B, Gebre-Selassie S, Feleke Y. Asymptomatic bacteriuria and symptomatic urinary tract infections in patients with diabetes mellitus in Tikur Anbessa Specialized University Hospital, Addis Ababa, Ethiopia. *Ethiop Med J*. 2012;50(3):239-49.

22. Hamdan H, Kubbara E, Adam A, Hassan O, Suliman S, Adam I. Urinary tract infections and antimicrobial sensitivity among diabetic patients at Khartoum, Sudan. *Annal Clini Microbiol Antimicrob*. 2015;14(26):1-6.

23. Thiraviam M, Yadesaand D, Adugna T. Antibiotic resistant pattern of urinary tract infection causing Escherichia coli isolated from diabetic mellitus and non-diabetic mellitus patients with special reference to Rifampicin resistance *Int J Curr Microbiol AppSci* 2014;3(3):668-74.

24. Saleem M, Daniel B. Prevalence of Urinary Tract Infection among Patients with Diabetes in Bangalore City. *Int J Emerg Sci*. 2011;1(2):133-42.

25. Samuel OO, Mathew AO, Mopelola DA, Tosin O, Grace A, Anthony BO. Asymptomatic Urinary Tract Infection In Diabetic Patients in Ago Iwoye, Ogun State, Nigeria. *J Am Sci* 2014;10(4s):72-8.

26. Guariguata L, Whiting D, Hambleton I, Beagley J, Linnenkamp U, Shaw J. Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes res clin pract* 2014;103:137-49.

27. Adly N, Ragab Y, Hashem A, Ahmady A. Effect of diabetes on occurrence of urinary tract infection and asymptomatic bacteriuria among diabetic and ketacidosis patients in Egypt. *Int Res J Microbiol*. 2015;6(3):027-36.

28. Pastakia S, Pekny C, Manyara S, Fischer L. Diabetes in sub-Saharan Africa – from policy to practice to progress: targeting the existing gaps for future care for diabetes. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*. 2017;10:247-63.

29. International Diabetes Federations. Diabetes Atlas. 5th edition. 2012 update.

30. Sachithananthan V, Loha E, Gose M. Prevalence of Diabetes Mellitus, Hypertension and Lipodystrophy in HAART Receiving HIV Patients in Southern Ethiopia. *ISRR*. 2013;1(1):1-11.

31. Yemane T, Belachew T, Asaminew B, Befekadu O. Type II diabetes mellitus in Jimma Town, South West Ethiopia. *Eth J Health Sci*. 2007;17(2):107-14.

32. Aswani S, Chandrashekar U, Shivashankara K, Pruthvi B. Clinical profile of urinary tract infections in diabetics and non-diabetics. *AMJ*. 2014;7(1):29-34.

33. Prakash R, Pal S, Sharma N, Adekhandi S, Juyal D, Gaurav V. Diversity of uropathogens and their resistogram in diabetic and non-diabetic patients in sub Himalayan region of Uttarakhand, India: A case control study. *Inte J Med Pub Health* 2014;4(1):115-9.

34. Peteros J, Fernandez L, Oncenes C, Opena E. Difference on the Incidence of Urinary Tract Infection between Diabetic and Non-diabetic Women Admitted in Metro Cebu, Philippines. *Mindanao J SciTech*. 2014;12:171-8.

35. Adeyeba O, Adesiji Y, Omosigho P. Bacterial Urinary Tract Infections in patients with Diabetes Mellitus. *Int J Trop Med*. 2007;2(3):89-92.

36. Vasudevan R. Urinary Tract Infection: An Overview of the Infection and the Associated Risk Factors. *J Microbiol Exp.* 2014;1(2):1-15.

37. Foxman B. The epidemiology of urinary tract infection. *Nat Rev Urol* 2010;7:653-60.

38. Ponnusamy P, Nagappan R. Extended Spectrum Beta -Lactamase, Biofilm-producing Uropathogenic Pathogens and Their Antibiotic Susceptibility Patterns from UrinaryTract Infection. *Intl J Microbiol Res*. 2013;4(2):101-18.

39. Kumar S, Dave A, Wolf B, Lerma E. Urinary tract infections. *Disease-a-Month* 2015;61:45-59.

40. Khalil Z. Uropathogenic Infections Among Patients with Diabetes in Baghdad City. *Iraqi Journal of Science.* 2016;57(1B):360-6.

41. Njunda A, Assob N, Nsagha S, Nde F, Kamga F, Nkume A, *et al.* Uropathogens from diabetic patients with asymptomatic bacteriuria and Urinary tract infections. *Scientific Journal of Microbiology* 2012;1(6):141-6.

42. Hooton T. Uncomplicated urinary tract infection. *New Engl J Med.* 2012;366:1028–37.

43. Nielubowicz G, Mobley H. Host–pathogen interactions in urinary tract infection. *Nat Rev Urol* 2010;7:430-41.

44. Kau A, Hunstad D, Hultgren S. Interaction of uropathogenic Escherichia coli with host uroepithelium. *Curr Opin Microbiol.* 2005;8(1):54-9.

45. Bien J, Sokolova O, Bozko P. Role of Uropathogenic Escherichia coli Virulence Factors in Development of Urinary Tract Infection and Kidney Damage. *Inte J Neph*. 2012:1-15.

46. Mulvey M. Adhesion and entry of uropathogenic Escherichia coli. *Cell Microbiol*. 2002;4(5):252-71.

47. Flores-Mireles A, Walker J, Caparon M, Hultgren S. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nat Rev Microbiol* 2015;13(5):269-84.

48. Mnif M, Kamoun M, Kacem F, Bouaziz Z, Charfi N, Mnif F, *et al.* Complicated urinary tract infections associated with diabetes mellitus: Pathogenesis, diagnosis and management. *Indian J Endocr Metab*. 2013;17(3):442-5.

49. Nitzan O, Elias M, Chazan B, Saliba W. Urinary tract infections in patients with type2 diabetes mellitus: review of prevalence, diagnosis, and management. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy* 2015;8:129-36.

50. Hoepelman A, Meiland R, Geerlings S. Pathogenesis and management of bacterial urinary tract infections in adult patients with diabetes mellitus. *International Journal of Antimicrobial Agents* 2003;22:S35-S43.

51. Sharma B, Bansal R, Gupta B. Asymptomatic bacteriuria in diabetics. *JIACM* 2011;13(1):55-9.

52. Kapur A. Diabetes and chronic infections. *Journal of the Ceylon College of Physicians*. 2015;46:34-8.

53. Fünfstück R, Nicolle L, Hanefeld M, Naber K. Urinary tract infection in patients with diabetes mellitus. *Clinical Nephrology*. 2012;77(1):40-8.

54. Sheerin N. Urinary tract infection. *Medicine* 2011;39(7):384-9.

55. Mandal J. Pathogenesis and Laboratory Diagnosis of Childhood Urinary Tract Infection. *EMJ Urol* 2016;4(1):101-7.

56. Cheesbrough M. District Laboratory Practice in Tropical Countries. New York: Cambridge University Press; 2006.

57. Pezzlo M. Laboratory Diagnosis of Urinary Tract Infections: Guidelines, Challenges, and Innovations. *CMN*. 2014;36(12):87-93.

58. Zowawi H, Harris P, Roberts M, Tambyah P, Schembri M, Pezzani M, *et al*. The emerging threat of multidrug-resistant Gram-negative bacteria in urology. *Nat Rev Urol* 2015;12:570-84.

59. Barber A, Norton J, Spivak A, Mulvey M. Urinary Tract Infections: Current and Emerging Management Strategies. *CID*. 2013;57(5):719-24.

60. Schmiemann G, Gágyor I, Hummers-Pradier E, Bleidorn J. Resistance profiles of urinary tract infections in general practice - an observational study. *BMC Urology*. 2012;12(33):1-5.

61. Lyamuya E, Moyo S, Komba E, Haule M. Prevalence, antimicrobial resistance and associated risk factors for bacteriuria in diabetic women in Dar es Salaam, Tanzania. *African Journal of Microbiology Research* 2011;5(6):683-9.

62. CLSI. Performance standards for antimicrobial disk susceptibility testing; twenty sixth CLSI supplements M100S Wayne, PA: Clinical and Laboratory Standard Institute; 2016.

63. Worku S, Derbie A, Sinishaw M, Adem Y, Biadglegne F. Prevalence of Bacteriuria and Antimicrobial Susceptibility Patterns among Diabetic and Nondiabetic Patients Attending at Debre Tabor Hospital, Northwest Ethiopia. *International Journal of Microbiology*. 2017:1-8.

64. Chita T, Licker M, Sima A, Vlad A, Timar B, Sabo P, *et al*. Prevalence of urinary tract infections in diabetic patients. *Rom J Diabetes Nutr Metab Dis*. 2013;20(2):099-105.

65. Simkhada R. Urinary tract infection and antibiotic sensitivity pattern among diabetics. *Nepal Med Coll J* 2013;15(1):1-4.

66. Ijaz M, Ali S, Khan S, Hassan M, Bangash I. Urinary tract infection in diabetic patients; Causative bacteria and antibiotic sensitivity. *J Med Sci* 2014;22(3):110-4.

67. Alemu A, Moges F, Shiferaw Y, Tafess K, Kassu A, Anagaw B, *et al.* Bacterial profile and drug susceptibility pattern of urinary tract infection in pregnant women at University of Gondar Teaching Hospital, Northwest Ethiopia. *BMC Research Notes*. 2012;5(197):1-7.

68. Nongrum S, Thaledi S, Singh V, Narang V, Mehta S, Garg R, *et al.* Association of Uropathogens with Asymptomatic Urinary Tract Infection in Diabetes Mellitus Patients. *Int J Curr Microbiol App Sci* 2016;5(10):355-61.

69. Al-Rubeaan K, Moharram O, Al-Naqeb D, Hassan A, RaWullah M. Prevalence of urinary tract infection and risk factors among Saudi patients with diabetes. *World J Urol* 2013;31:573-8.

70. Ribera M, Pascual R, Orozco D, Pérez Barba C, Pedrera V, Gil V. Incidence and risk factors associated with urinary tract infection in diabetic patients with and without asymptomatic bacteriuria. *Eur J Clin Microbiol Infect Dis*. 2006;25:389-93.

71. Nassaji M, Ghorbani R, Tamadon M, Bitaraf M. Association Between Body Mass Index and Urinary Tract Infection in Adult Patients. *Nephro Urol Mon* 2015;7(1):1-4.

72. Raoofi A, Ghavami M, Shahhamzeh M, Ghasemi M, Hedartabar R, Salehi L. The Impact of Demographic Factors and Blood Sugar Control on the Incidence of Urinary Tract Infections in Khorramabad in 2013. *Iran Red Crescent Med J*. 2016;18(5):1-6.

73. Chita T, Timar B, Muntean D, Badiţoiu L, Horhat F, Hogea E, *et al.* Urinary tract infections in Romanian patients with diabetes: prevalence, etiology, and risk factors. *Therapeutics and Clinical Risk Management*. 2017;13(1-7).

74. Boyko E, Fihn S, Scholes D, Abraham L, Monsey B. Risk of Urinary Tract Infection and Asymptomatic Bacteriuria among Diabetic and Nondiabetic Postmenopausal Women *Am J Epidemiol*. 2005;161(6):557-64.

Annex I. Questionnaire

Questionnaire: administered to investigate the bacteriological causative agent, its associated factors and antimicrobial susceptibility pattern among diabetic patients attending Hawassa University Comprehensive Specialized Hospital

Patient ID code_____

S.no	Questions	Response of categories	Remark
Socio demographic characteristics			
101	Age in year's	_____	
102	Sex	1. Male 2. Female	
103	Place of Residence	1. Urban 2. Rural	
104	Marital status	1. Married 3. Divorced 2. Single 4. Widowed	
105	Educational level	1. No school 3. High school 2. Primary 4. Higher education	
106	Occupation	1. Farmer 4. Civil servant 2. Merchant 5. Others 3. House wife	
Clinical data			
201	Symptoms (≥ 2)	1. Yes 2. No	202-208
202	Dysuria	1. Yes 2. No	
203	Increased Frequency	1. Yes 2. No	
204	Urgency	1. Yes 2. No	
205	Hematuria	1. Yes 2. No	
206	Fever and chills	1. Yes 2. No	
207	Flank pain	1. Yes 2. No	
208	Suprapubic pain	1. Yes 2. No	
209	Type of DM	1. Type I 2. Type II	
210	Duration of diabetes mellitus	_____	
211	Last fasting blood glucose level checkup	_____	
212	Type of medication for DM that the patient takes	1. Tablet 2. Insulin 3. Both	
213	Other clinical condition (co-morbidity)	_____	

214	History of previous UTI	1. Yes 2. No	
215	History of catheterization	1. Yes 2. No	
216	Height in meter	_____	
217	Weight in kg	_____	
218	BMI (kg/m^2)	_____	

Laboratory Data

1. Serial No (code) _____
2. Date of specimen collection: _____Time:_____

3. Cultures and Identification

   Significant bacteriuria: 1. Yes 0. No

   Name of the bacteria isolated _____

4. Antimicrobial susceptibility testing S I R
   - Ampicillin ……. ……. ……
   - Co-trimoxazole ……. …… ……...
   - Amoxicillin- Clavulanic acid ……. ……. ……
   - Gentamycin …….. …… ……
   - Ceftriaxone …….. ……..... ……..
   - Nitrofurantoin …….. …… …….
   - Norfloxacin …….. …… ……..
   - Nalidixic acid ……... …… …….
   - Tetracycline …….. ……. ……..
   - Ciprofloxacin …….. …… …….
   - Penicillin …… ……. ……..

Comment……...……

… ……..

Annex II: Information Sheet and Consent Form

1. Information Sheet

Title of the Research Project: Urinary tract infection: Bacterial etiologies, antimicrobial susceptibility profile and associated risk factors in diabetic patients attending in Hawassa University Comprehensive Specialized Hospital Southern Ethiopia, Hawassa.

Name of Principal Investigator: Aley Mohammed (BSc)

Name of the advisors: Dr. Getenet Beyene (PhD, Associate professor)

Mr. Lule Teshager (MSc)

Mr. Deresse Daka (MSc, Assistance professor)

Name of the Organization: Jimma University, Institute of Health, School of Medical Laboratory Sciences

Name of sponsor: Jimma University

Information sheet and consent form prepared for Diabetic patients attending Hawassa Comprehensive specialized Hospital who is going to participate in Research Project.

Purpose of the study:

The purpose of this research will be to determine Bacterial uropathogens, antimicrobial susceptibility profile and associated risk factors in diabetic patients attending in Hawassa University Comprehensive Specialized Hospital.

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

Procedures

We are asking you and others to participate voluntarily in this study, which would require your response to an interview, to be physically examined and to give urine sample for laboratory

examination. You will be given instruction how to collect the urine samples in clean/sterile container by health workers.

Risks associated with the study

There is no anticipated risk by participating in the study.

Benefits and Compensation:-

There will be no special benefits to you except if there is any positive finding in laboratory examination the result will be reported to your physician for appropriate treatment and management.

Confidentiality:-

Any information that is collected about you will be kept private and in a secured place.

Voluntary participation and withdrawal:-

Your participation in this study is voluntary. You may decide not to participate or you may leave the study at any time. Your decision will not result in any penalty or loss of benefits to which you are entitled. Your decision will not put at risk at any present or future medical care or other benefits to which you otherwise entitled. You should ask the study investigators listed below any questions you may have about this research study. You may ask questions in the future if you do not understand something that is being done. Use the following address for any question.

Mr. Aley Mohammed, Phone No +251 911911529, Email: maleyhamza@gmail.com

Dr. Getnet Beyene, Phone No +251 911644093, Email: rgetenet@yahoo.com

Mr. Lule Teshager Phone No +251 922783320, Email: lule_teshager2007@yahoo.com

Mr. Deresse Daka Phone No +251 911968912, Email: drsdk200@gmail.com

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

2. Consent Form

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

Signature:-_____ Date:-_____

Annex III: Amharic Version of Study Information and Consent Form

1. መረጃ ለጥናቱ ተሳታፊዎች

የፕሮጀክቱ ርዕስ፡ ለስኳር ህመም ከሚታከሙት ዉስጥ የሽንት ትቦ ኢንፌክሽን አምጪ ረቂቅ ተህዋሳትን መለየትና በሽታ አምጪ ተህዋሲያኑ ሊያክም የሚችል መድሃኒት መምረጥ

የተመራማሪዉ ስም፡ አቶ አልይ መሀመድ

የ አማካሪዎች ስም፡ ዶ/ር ጌትነት በየነ

አቶ ሉሌ ተሻገር

አቶ ደረሰ ዳካ

የድርጅቱ ስም ፡ ጅማ ዩኒቨርሲቲ በጤና ሳይንስ እንስቲቱት የህክምና ላቦራቶሪ ትምህርት ክፍል

የእስፖንሰር ስም ፡ ጅማ ዩኒቨርሲቲ

የጥናቱ ዓላማ:

የዚህ ጥናት አላማ የስኳር ህመምተኞች ብዙውን ጊዜ ለህመም የሚዳረጉበት የሆነው የሽንት ትቦ ኢንፌክሽን አምጪ ረቂቅ ተህዋሳትን በመለየት በሽታ አምጪ ተህዋሲያኑ ሊያክም የሚችል መድሃኒት መምረጥ በዚህም የስኳር ህመምተኞች አንዱ የጤና እክል የሆነውን የዚህን ኢንፌክሽን በአግባቡ መቆጣጠር እንዲቻል ማድረግ ነው፡፡

መመሪያ

እርስዎ በጥናቱ ለመሳተፍ ከፈቀዱ በጤና ሃኪሞች አጠቃላይ ምርመራ ይደረግልዎታል፡፡ ከዚያም የተወሰኑ ጥያቄዎችን በመጠየቅና መጠነኛ የሆነ የውሃ ሽንት ናሙና እንድትሰጡኝ እንጠይቃችኋለን፡፡ የውሃ ሽንቱ ለዚሁ ጥናት በተዘጋጀ እቃ ውስጥ አድርጋችሁ በጥንቃቄ የምታመጡበትን መንገድ እነግራችኋለሁ፡፡

በጥናቱ ሳቢያ ሊከሰቱ የሚችሉ ጉዳቶች

የሚወሰደው ናሙና ሽንት ብቻና እራሰዎ ያለ ምንም ተጨማሪ መሳርያ የሚሰጥ ስለሆነ የሚያመጣዉ ችግር የለም

የሚሰጥዎት ጥቅም

የተለየ የገንዘብ ጥቅም የለዉም ነገር ግን በሽታ አምጪ ህዋሳት በላቦራቶሪ መኖራቸው ከተረጋገጠ በኋላ ተገቢውን መድሃኒት እንዲወስዱ ውጤቱ ለህኪምዎ ተልኮ መዴኒኑቱን በህኪምዎ ትዕዛዝ ይሰጥዎታል፡፡

ሚስጥራዊነት

የእርስዎ የግል መረጃ በሙሉ ሚስጥራዊነቱ የተጠበቀ ይሆናል፡፡

በፈቃደኝነት ላይ የተመሰረተ ተሳትፎ

በዚህ ጥናት ላይ ለመሳተፉ በእርሶ ሙሉ ፈቃደኝነት ላይ የተመስርተ ይሆናል። በዚህ ጥናት እየተሳተፉ ባሉበት ድንገት ማቋረጥ ቢፈልጉ የማቋረጥ መብትዎ የተጠበቀ ነው፡፡ ለምን ማቋረጥ እንደፈለጉ ምክንያት እንዲያቀርቡም ሆነ ጥናቱ እንዲቀጥሉ አይገደዱም፡፡ በጥናቱ መሳተፍ ባለመፈለግዎ በእርስዎ ላይም ሆነ በሚያገኙት አገልግሎት ላይ የሚያመጣው ምንም አይነት ችግር አይኖርም፡፡ የእርስዎ በጥናቱ መሳተፍ ግን ለሚደረገው ጥናት ትልቅ እገዛ እንደሚሆን ሳልጠቁምዎት አላልፍም፡፡ ስለጥናቱ ለሚኖሮት ማንኛውም አይነት ጥያቄ የሚከተሉትን አድራሻዎች መጠቀም ይችላሉ::

አቶ አልይ *መሀመድ* ስ. ቁ +251 911911529, ኢሜል: maleyhamza@gmail.com

ዶ/ር ጌትነት በየነ ስ. ቁ +251 911644093, ኢሜል: rgetenet@yahoo.com

አቶ ሉሌ ተሻገር ስ. ቁ +251 -922783320, ኢሜል: lule_teshager2007@yahoo.com

አቶ ደረሰ ዳካ ስ. ቁ +251 911968912, ኢሜል: drsdk200@gmail.com

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

2. የስምምነት ማረጋገጫ ፎርም

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

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

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

ፊርማ፡_____ ቀን፡ _____

Annex IV: General Laboratory Procedure

I. Laboratory procedure for collection and culturing of urine sample

1. Give the patient a sterile, dry, wide-necked, leak proof container and request a 10–20 ml of clean catch midstream specimen.

- **Female patients:** Wash the hands, cleanse the area around the urethral opening with clean water, dry the area with a sterile gauze pad, and collect the urine with the labia held apart.
- **Male patients:** Wash the hands before collecting a specimen (middle of the urine flow).

2. Label the container with the date, the code number of the patient, and the time of collection. As soon as possible, deliver the specimen with a request form to the laboratory.
3. Bring the culture media to room temperature.
4. Gently swirl the container to mix the sample.
5. Tip it to a slant and with a 0.001ml inoculating loop touch the surface so that the urine is sucked up into the loop. Never dip the loop into the urine.
6. Deposit 0.001ml of the urine on each CLED, MacConkey, and blood agar plate and streak half the plate by making a straight line down the center, followed by close passes at right angles through the original, and ending with oblique streaks crossing the two previous passes.
7. Incubate the plate aerobically at 35-37 ^{0}C for 18-24 hours.
8. If there is no growth after 24-48hr of incubation discard the plates and issue final report.
9. If there is growth after 24-48hr of incubation, count the number of colonies of each morph type present. Each colony counted represents 1000CFU in the original sample.
10. If the number of colony is more than 100 perform definitive biochemical identification and sensitivity test.

II. Laboratory procedure for Gram staining technique

The Gram stain, used to distinguish between Gram-positive and Gram-negative cells, is the most important and widely used microbiological differential stain. In addition to Gram reaction, this stain also allows determination of cell morphology, size, and arrangement.

1. Labeling the slides clearly with the date and patient's code number.

2. Making of smears by spread evenly covering an area about 15-20mm diameter on a slide.
3. Drying of smears after making smears, the slide should be left in a safe place to air-dry, protected from flies and dust.
4. Fix the dried smear by using heat or chemicals.
5. Cover the fixed smear with crystal violet stain for 30-60 seconds.
6. Rapidly wash off the stain with clean water.
7. Tip off all the water, and cover the smear with lugol's iodine for 30-60 seconds.
8. Wash off the iodine with clean water.
9. Decolorize rapidly (few seconds) with acetone alcohol. Wash immediately with clean water.
10. Cover the smear with neutral red or safranine stain for 2 minutes.
11. Wash off the stain with clean water.
12. Wipe the back of the slide clean, and place in a draining rack for the smear to air-dry.
13. Examine the smear microscopically, first with the 40 X objective to check the staining and to see the distribution of materials and then with the oil-immersion objective to look for bacteria and cells.

Result

1. Gram positive bacteria ----------dark purple
2. Gram-negative bacteria ----------pale to dark red

III. Laboratory procedure for Biochemical testing

Identification of gram positive bacteria was based on their gram reaction, catalase and coagulase tests results.

Catalase test

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

Procedure

1. Pour 2–3 ml of the hydrogen peroxide solution into a test tube.
2. Using a sterile wooden stick take the test organism and immerse into the hydrogen peroxide solution.
3. Look for immediate bubbling.
4. Interpretation:
   Active bubbling …….Positive catalase test
   No bubbles ……Negative catalase test

Coagulase test

This test is used to differentiate *Staphylococcus aureus* which produces the enzyme coagulase from other *Staphylococcus spp*.

Procedure

1. Place a drop of physiological saline on two separate slides
2. Emulsify the test organism in each of the drop to make thick suspension
3. Add one drop of plasma to one of the suspensions and mix gently. Look for clumping of the organism within 10 seconds
4. Interpretation
   Clumping within 10 seconds …… *S. aureus*
   No clumping within 10 seconds …….other *Staphylococcus species*

Identification of gram Negative bacteria was based on their test result with a series of biochemical tests.

Procedure

1. A suspension of the test organism was prepared with nutrient broth. 3-4 colony of test organism in 5 ml nutrient broth.
2. A loop full of the bacterial suspension was inoculated in to triple sugar iron agar, citrate agar, urea agar, SIM, and lysine decarboxylase agar medium.
3. Media was incubated at 35-37 ^{O}C for 18-24 hours.

4. Media was looked for colour change (turbidity for motility) of the medium
5. The test organism was identified by considering the result of these biochemical tests.

Table 8: Biochemical tests for identification of Gram negative bacteria

Species	Lactose	Indole	Urea	Manitol	H_2S	Gas	Citrate	Motility	LDC
E. coli	+	+	-	+	-	+	-	+/-	+
K. pneumoniae	+	-	+	+	-	+	+	-	+
K. oxytoca	+	+	+	+	-	+	+	-	+
Acinetobacter spp.	-	-	v	-	-	-	+/-	-	-

IV. Laboratory procedure for Antimicrobial sensitivity testing

Antimicrobial susceptibility tests measure the ability of an antimicrobial agent to inhibit bacterial growth in vitro.

Procedure

1. A suspension of the test organism was prepared by emulsifying several colonies of the organism in small volume of nutrient broth.
2. Match the turbidity of the suspension against the turbidity standard which has a similar appearance to an overnight broth culture.
3. With a sterile swab take sample from the suspension (squeeze the swab against the side of the test tube to remove the excess fluid).
4. The inoculum was spread evenly over the Muller-Hinton agar plate with the swab.
5. The antimicrobial disc was placed using sterile forceps/needle on the inoculated plate.
6. The plate was incubated aerobically at 35-37^{O}C for 18-24 hours.
7. The test was read after checking that the bacterial growth is neither heavy nor light. The radius of the inhibition zone was measured.
8. The reaction of the test organism was interpreted to each antibiotics used as sensitive, intermediate, or resistance according to the standardized table supplied by CLSI.

Annex V: Declaration sheet

I, the undersigned, declare that this thesis is my own original work and it has not been presented in other universities, colleges or other institutions for similar degree or other purpose. In addition to that all sources of materials used for the thesis have been dully acknowledged.

Name of the principal investigator

1. Aley Mohammed (BSc)

Signature _____ Date_____

This thesis has been submitted for examination with my approval as university advisor

Name of the advisors

1. Dr. Getnet Beyene (PhD, Associate professor)

Signature _____ Date_____

2. Mr. Lule Teshager (MSc, PhD Candidate)

Signature _____ Date_____

This thesis has been submitted with my approval as an examiner.

Name of examiner:

1. Mr. Mulatu Gashaw (MSc)

Signature: _____ Date: _____-