PREVALENCE AND ANTMICROBIAL SUSCEPTIBILITY PROFILE OF UROPATHOGENES AND ASSOCIATED RISK FACTORS OF URINARY TRACT INFECTION AMONG DIABETIC AND NON-DIABETIC PATIENTS ATTENDING MIZAN-TEPI UNIVERSITY TEACHING HOSPITAL, AMAN, SOUTHWEST ETHIOPIA

BY

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JIMMA, ETHIOPIA

JIMMA UNIVERSITY
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ABSTRACT

Background: *Urinary tract infection, the most common bacterial infections in urinary tract, is a major cause of morbidity particularly in patients with diabetes mellitus. Its empirical treatment is becoming difficult because of appearance of uropathogens with increasing resistance to antimicrobial agents worldwide. Local susceptibility pattern of uropathogens is, therefore, important.*

Objective: *The aim of the study was to determine the prevalence and antimicrobial susceptibility profile of uropathogenes and associated risk factors of urinary tract infection among diabetic and non-diabetic patients.*

Materials and methods*: A facility based comparative cross-sectional study was carried out involving 319 diabetic patients and 319 non-diabetic patients at Mizan Tepi University Teaching Hospital from April to July 30,2018. Structured questionnaire was used for collecting the data pertaining to socio-demographic characteristics and possible risk factors. Midstream urine was collected and cultured onto bacteriological media. All the positive urine cultures showing significant bacteriuria were further subjected to biochemical tests. Antibacterial susceptibility was determined by standard Kirby Bauer's disc diffusion method. Data were entered into Epidata version 3.1 and exported to SPSS version 20.2 for analysis. Statistically significant bacteriuria was set a P values< 0.05.*

Result*: Significant bacteriuria was detected in 48/319(15.0%) diabetic patients and in 18/319(5.6%) of non-diabetic patients. The most predominant isolate in diabetic and non-diabetic patient was E. coli at 18.8% and 27.8% prevalence. All isolates were 100% sensitive to Nitrofurantoin, Gentamycin and Ciprofloxacin and resistant to Ampicillin. Females in diabetic patients[AOR,2.001;95%CI:1.56-4.311], and females in non-diabetic patients[AOR,2.201;95%CI;1.360-4.451], fasting blood sugar greater than 126mg/dl [AOR:4.248; 95% CI;0.848-11.253], glycosuria [AOR:2.030; 95% CI;1.851-6.752] and history of urinary tract infection [AOR:1.123; 95%CI;1.001-3.701] were found to be statistically associated to significant bacteriuria.*

Conclusion and recommendation: *The prevalence of uropathogenes in diabetic patients and the resistances of most isolates to commonly used antibiotics is a major concern. Diabetic patients should be screened for urinary tract infection.*

Keywords*: Uropathogenes, Diabetes mellitus, Antimicrobial susceptibility*

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ACRONYMS

AMR	Antimicrobial Resistance
AST	Antibiotic Susceptibility Test
ATCC	American Type Culture Collection
CDC	Center for Disease Control and Prevention
CFU	Colony Forming Unit
CI	Confidence Interval
CLED	Cystein-Lactose Electrolyte Deficient
CLSI	Clinical Laboratory Standards Institute
CONs	Coagulase Negative Staphylococci
DM	Diabetes Mellitus
FBS	Fasting Blood Sugar
IRB	Instructional Review Board
MDR	Multi-Drug Resistant
MHA	Muller Hinton Agar
ML	Milliliter
MTUTH	Mizan Tepi University Teaching Hospital
MSU	Mid -Stream Urine
NCD	Non-Communicable Disease
NICE	National Institute for Health and Clinical Excellence
RBS	Random Blood Sugar
SOPS	Standard Operating Procedure
SPSS	Statistical Package for Social Science
UTI	Urinary Tract Infection
UPEC	Uropathogenic *Escherichia coli*
WHO	World Health organization

CHAPTER ONE

INTRODUCTION

1.1 Background

Urinary tract infection (UTI) is one of the most prevalent diseases with varies etiological agents annually affecting 250 million people worldwide(1). Despite great diversity of etiological agents is attributed to UTIs, bacteria are the most common causative organisms which are responsible for more than 95% of UTIs(2).

The most common bacterial species contributing to cause UTIs are gram negative, gram-positive bacterial and fungal species like *E. coli, Klebsiella* spp., *Enterobacter* spp., *Pseudomonas aeruginosa*. *Proteus mirabilis, S. saprophyticus* and *candida* species(3*).* The incidence of UTIs depends upon diverse risk factors such as diabetes mellitus (DM), advanced age, urinary tract obstructions, immunosuppression, catheterization, a difference in the infecting bacterium itself, the presence of glycosuria, lack of personal hygiene, sexual activity and neurological disorders(4). Diabetes mellitus is one of the widely known risk factor for developing UTI(5). Many studies showed that patients with DM are more vulnerable to the adverse effects of UTIs as compared to their non-diabetic counterparts(6-8).

In diabetic patients, Urinary tract is the primary site of infection which carries the risk of different complications such as emphysematous cystitis, pyelonephritis, renal or perinephric abscess, bacteremia, and renal papillary necrosis(9). The higher prevalence of UTI in diabetic patients was attributed to the differences in host immunity between diabetic and non-diabetic patients, or to a dissimilarity among infecting etiological agents(5).

Development of antibiotic resistance is a big threat to diabetic patients who are already predisposed to UTI and who in most times have dysfunctional urinary tracts prompting for instrumentation. Complications associated with diabetes are increasingly becoming of interest due to their alarming mortality rate as ranked by the World Health Organization(10).

1.2 Statement of the problem

It has been estimated globally that UTIs result in as many as 8.3 million visits to outpatient clinics, 1 million visits to emergency departments, and 100,000 hospitalizations annually(11). In developing countries urinary tract infections (UTIs) are one of the most commonly diagnosed disease among the parent seeking medical service with frequency of 180 per 10,000(12). Incidence rate of UTI was 46.9 per 1,000 person-years among diabetic patients versus 29.9 for patients without diabetes (13).

Diabetes mellitus(DM) is a complex condition leading to high blood glucose level and defined as a group of metabolic disorders characterized by increased blood glucose level resulting from defects in insulin secretion, insulin action, or both (14, 15). About 451 million people have diabetes worldwide in 2017, expected to rise 693 million by 2045, disproportionately affecting working-age people (17). An American database study during 2014 found that a UTI diagnosis was more common in subjects with diabetes compared to those without diabetes (9.4% vs 5.7%), respectively(16).

Diabetes mellitus (DM) causes several abnormalities of the host immune system. The antimicrobial and phagocytic activity of the neutrophils in diabetics shows a decreased bactericidal, reduced chemotactic activity and impaired phagocytosis(17, 18). Persistently high blood glucose levels cause generalized vascular damage affecting the heart, eyes, kidneys and nerves and resulting in various complications ranging from dysuria to organ damage and sometimes even death due to complicated UTI (19, 20).

Different bacteria, virus and fungal species can infect the urinary tract and cause infection, but the most common uropathoges are the *Enterobacteriaceae*. Gram negative *E. coli* is usually the most prevalent organism responsible for UTI and accounts for 80-85% of the total isolates(21). *Klebsiella spp.*, *Proteus*, *Pseudomonas*, *Enterococcus*, *Enterobacter spp. are also the* causative organisms of urinary tract infection. Organisms such as *Serratia* and *Pseudomonas* assume increasing importance in recurrent infections. *Proteus species* by virtue of urease production and *Klebsiella spp* through the production of extracellular slimy polysaccharides are predispose to stone formation in the kidneys and are isolated more frequently from patient with calculi(22).

Antibiotic resistance is a major global public health problem both for hospital and community-acquired infections which is currently estimated to account for more than 700,000 deaths per year worldwide. If no appropriate measures are taken it will cost approximately 10 million lives by 2050(23, 24). Antibiotic resistance is responsible for more than 2 million infections and 23,000 deaths each year in the United States(25). In Europe, more than 25,000 patients die each year from antibiotic resistant bacteria which infect about 4 million patients every year(26).

Microorganisms causing UTI vary in their susceptibility to antimicrobials from place to place and time to time. Resistant to newer and more potent antimicrobials are making the therapeutic options very limited in case of UTI(62,63). There have been several studies focusing on antibiotic susceptibility patterns of uropathogen. But the studies on the prevalence of uropathogens and their profile of antibiotic resistance in patients with and without diabetes are limited at the study area, at least as scientific publications. Thus, the screening of UTI in diabetic patients is essential and has no alternative so far. Hence, this study was performed to understand the prevalence of urinary tract infection and antibiotic sensitivity profile of isolates and associated factors in both diabetic and non -diabetic patients with clinically suspected UTI.

1.3 Significance of the study

Understanding the prevalence of urinary tract infection and their antibiogram with their main associated factors is a critical point of effort that aims to reduce the burden of urinary tract infections and drug resistance among diabetics and non-diabetics. Informative, clinical, epidemiological and operational research is of a paramount value in the reduction of urinary tract infection in the community thereby pointing ways for designing specific and effective preventive mechanisms.

The data obtained from this study can be used as a base for researchers to re- assess the ongoing situation of the problem and for those who are interested to study similar issues in other areas. Moreover, the determined antimicrobial susceptibility pattern may help the clinicians, nurses and other health professionals working on the treatment and management of UTI among diabetic and non-diabetic attendants. Understanding the specific problem of diabetics and non- diabetic individuals associated with urinary tract infection improves the well-being of them and the general public status of the community.

CHAPTER TWO

LITRATURE REVIEW

2.1 prevalence of UTI

A study conducted in India, to determine the incidence of spectrum of uropathogens and antibiotic sensitivity pattern showed that the prevalence of UTI was 34.5% v 26.7% among diabetic and non-diabetic patients, respectively(27). A study in Romanian diabetic patients, the prevalence of UTIs was 12.0%(28), in Nepal,34.5%(29), in Uganda, 22.0% among diabetic patients(30). In Sudan, the overall prevalence of UTI among diabetic was found to be 39(19.5%)(31). In a study conducted in Kenya, the prevalence of UTI was found to be 20.6%.(32). A study conducted among diabetic patients in Harar, revealed the overall prevalence of urinary tract infection was 15.4% (37/240)(33). In Debretabor town, (10.9%) v (4.7%) bacterial isolates were recovered in diabetic and non-diabetic study participants, respectively (7).

2.1 prevalence of etiologic agents of UTI

Urinary tract infection can be caused different etiologic agents. Gram negative and gram-positive bacteria were the commonest isolates. In a study conducted in Bangladesh (84.39% v 15.7%) (34), in Algeria(59% v 41%)(35), In Arbaminch(72.7% v 27.2%) in all studies conducted, gram negative bacteria were the dominant uropathogenes (36). In a study conducted in India, *Escherichia coli* was the most common isolate (45% v 63%) among diabetic and diabetic patients followed by *Klebsiella spp.(*14% v 13%) (6), similarly, in India, (23.5%)(37).in Jordan, *E. coli* was (15.5% v29.5%),among diabetic and non-diabetics,(38). In Egypt, prevalence of *E. colli* was (53.8%) followed by *Klebsiell*a spp. (17.58%) and *Candida spp*. (10.99%),(39).In Dessie, *E. coli* was the predominant uropathogenes (63.6%) followed by *Klebsiella spp*. (8.5%) (40). But in a hospital based comparative study conducted in Debretabor, the most predominant uropathogenes isolated was *S. aureus*(28.6%)(7).

A study done in Cameroon, to determine the prevalence and etiology of asymptomatic bacteriuria and antimicrobial resistance of urinary isolates in diabetics and non-diabetics, the overall prevalence of ASB was 33.2%; 38.3% and 26.1%,respectively(41), A study conducted in Tanzania, Dar es selam, to determine the prevalence and risk factors of bacteriuria in diabetic women, the prevalence of asymptomatic bacteriuria in diabetic patients was found to be 13.4% (42). In another study conducted in Addis Ababa, Tikur anbasa university Hospital, 36 (10.4%) of asymptomatic bacteriuria was found among diabetic patients(43).

2.3 Antimicrobial susceptibility of bacterial uropathogenes

A cross sectional study conducted in Nepal, to assess the spectrum of uropathogens and their antibiotic sensitivity pattern in diabetic patients showed, *E. coli* was highly resistant to ampicillin and cephalexin and sensitive to gentamicin and nitrofurantoin(29). In St.pauel's Hospital, the percentage *E. coli* resistant to ampicillin was found to be 79.2%. *K. pneumonia* showed 85.2% resistance to ampicillin. Multi drug resistance ≥ 3 classes of antibiotics was observed in 77.6% of the isolated bacterial uropathogenes(44). In Shashemene referral Hospital, 93.3% of the isolates were sensitive to gentamicin, on the contrary, none of the isolates showed sensitivity to amoxicillin (96.6%) followed by vancomycin (80%)(45). A cross-sectional study conducted at Hawassa University Referral Hospital, Gram- negative isolates showed 100% resistance against ampicillin and (82.4%) resistance to ceftriaxone, 100% sensitive to nitrofurantoin, 31 out of 33 (93.9%) bacterial isolates showed multi-drug resistance(46).

2.4 Risk factors associated to UTI

An institution based cross-sectional study conducted in Nekemte Referral Hospital to determine the prevalence of UTIs, risk factors and antimicrobial resistance pattern of the bacterial isolates from diabetic revealed that level of education, history of UTIs and glycosuria was significantly associated with UTIs(47). A study conducted by Chiţă et al, in UAE showed that, 17.7% of females and 5.2% of males developed a urinary tract infection(28). In a study was carried out at Arsho Advanced Medical laboratory, to determine the spectrum of bacterial uropathogens and their drug resistant pattern, Urinary

tract infection was the highest (43.8%) in patients of age group 25–44 followed by age groups of 45–64 (20%)(48)

2.4 Conceptual frame work

The conceptual frame work was developed after reviewing many published literatures and customized to this study that revealed these factors contributing for the observed burden of bacterial uropathogenes among diabetic and non-diabetic patients.

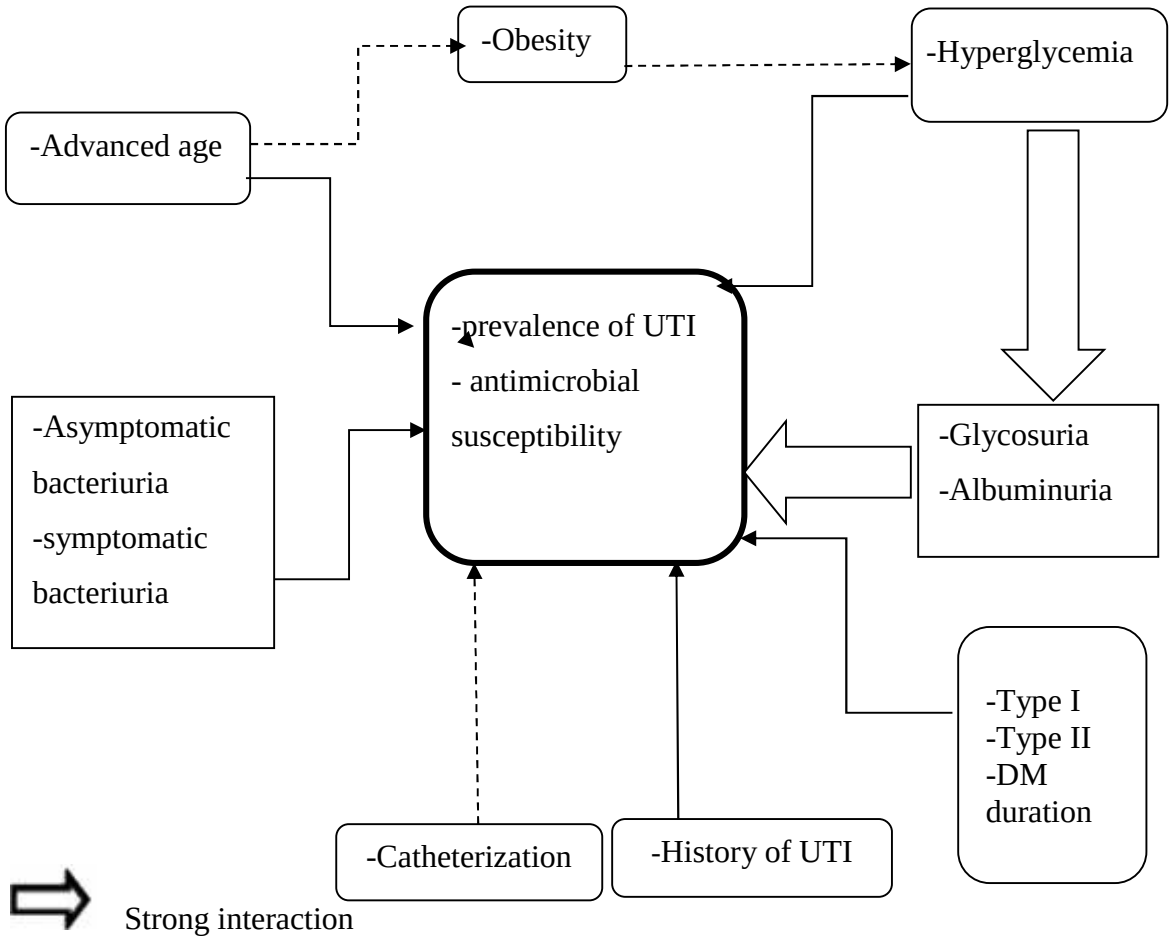

Figure 1: conceptual frame work of factors associated with significant bacteriuria among diabetes and non-diabetic patients attending MTUTH from April-July 2018. (from different reviewed literatures)

CHAPTER THREE

OBJECTIVES

3.1 General objective

- To determine the prevalence and susceptibility profile of uropathogenes and associated risk factors of UTI among diabetic and non-diabetic patients attending MTUTH from April to July, 2018.

3.2 Specific Objectives

- To isolate uropathogenes and determine their prevalence among diabetic and non-diabetic patients attending MTUTH from April to July, 2018.
- To determine the susceptibility pattern of isolated uropathogenes among diabetic and non-diabetic patients attending MTUTH from April to July, 2018.
- To assess factors associated with bacteriuria in diabetic and non-diabetic patients attending MTUTH from April to July, 2018.

CHAPTER FOUR

MATERIALS AND METHODS

4.1 Study setting

Mizan with the neighboring town of Aman forms a separate town called Mizan-Aman surrounded by Debub bench woreda. Mizan-Aman town is the largest town and administrative center in Bench-Maji Zone in the Southern Nation Nationalities People Region. Mizan- Aman has a total population of 34,080; of which 18,138 are males and 15,942 are females. This town has latitude and longitude of 7º0'N 35º35'E and an elevation of 1451 m above sea level(49). The town has one Teaching Hospital and a community Health Laboratory The Teaching Hospital is located in Mizan Aman town situated 255 km South West of Jimma town. It has a total of 136 beds and it runs multidisciplinary health care system with a total of 209 staffs, of these 155 are health professionals and the remaining are supportive staffs. The Hospital providing health care services for more than 25000 clients and over 800 known diabetic patients per year. So, this study was conducted at Mizan-Tepi University Teaching Hospital from April to July, 2018.

4.2 Study Design and Period

Institution based comparative cross-sectional study was conducted in diabetic and non-diabetic patients attending MTUTH from April to July, 2018.

4.3 Selection of Study Population

4.3.1 Source Population

All diabetic and non-diabetic patients visiting Mizan-Tepi University Teaching Hospital.

4.3.2 Study Subjects

All diabetic and non-diabetic patients suspected for UTI and sent to the microbiology laboratory.

4.4 Eligibility Criteria

4.4.1 Inclusion Criteria

A known diabetic or newly diagnosed diabetic patients with or without diabetic medication were included, non- diabetic patients with no family history of diabetes., males and females of all age, patients with signs and symptoms of Urinary tract infection as indicated by the attending clinician, any patient with asymptomatic Urinary Tract Infection suspected by the attending clinicians were included in the study.

4.4.2 Exclusion Criteria

Non-diabetic patients having FBS or RBS level ≥126 mg/dl at the time data collection., diabetic and non-diabetic pregnant women in labour and those who delivered and stayed in the hospital, any patient, pointed out by the attending clinician that were already on antibiotic treatment for any other reason were excluded from the study.

4.5 Sample Size Determination and Sampling Technique

4.5.1 Sample Size Determination

The sample size was determined using double population proportion formula

$$n = \left(Z\,{}^{\alpha}/_{2} + Z_{\beta}\right)^{2} \times \frac{P1(1-P1) + P2(1-P2)}{(P1-P2)2}$$

Where, $Z_{\alpha/2}$ is the value of Z from standard normal curve = 1.96 at α/2, α=0.05 at 95% CI. Z_{β} is the value of Z from standard normal distribution= 0.84 at β, β= 0.2 at power of 80%.

Using study conducted at Debre –Tabor as p1=10.6% and p2=4.7% (7).

$p_{1=}$ Proportion of UTI present in diabetic patients=0.106

$p_{2=}$ Proportion of UTI present in non-diabetic attendants= 0.047

Substituting these in the formula we get

$$n = (1.96 + 0.84)2\frac{0.106(1 - 0.106) + 0.047(1 - 0.047)}{(0.106 - 0.047)2}$$

$n_1=n_2$ =319 and total sample size N =638

4.5.2 Sampling Technique

All study participants were selected using consecutive sampling technique.

4.6 Variables of the Study

4.6.1 Dependent Variable

Significant bacteriuria
Antimicrobial susceptibility

4.6.2 Independent Variables

Socio–demographic characteristics like age, sex, marital status, place of residence, occupation, level of education and clinical characteristics, symptoms of UTI, catheterization, previous history of UTI, obesity, previous antibiotic treatment, fasting blood glucose level, type of diabetes, duration of diabetes, glycosuria, and albumin.

4.7 Instrument and Data Collection Procedure

4.7.1 Data Collection Tool

Structured questionnaire was used for data collection on socio-demographic characteristics, clinical information and possible risk factors through face to face interview. Laboratory investigation result of each participant was kept in the laboratory request format.

4.7.2 Blood sample collection

Capillary blood was collected form diabetic and non-diabetic patients for the diagnosis of fasting or random blood sugar by using Senso card meter (E77 Electronica).

4.7.3 Urine Sample Collection

Urine samples were collected from the target patients after explaining the aims and objectives of the research to them. The participants were instructed how to collect the urine sample and women in particular to clean the genitalia with clean water. About 20 ml of freshly voided Clean-catch midstream urine (MSU) samples were collected using two separate leak proof, wide mouth sterile containers.

The urine sample was examined within one hour or, when this was impracticable, it was refrigerated at 4^0c. until it could be examined, since at room temperature any bacteria present may multiply rapidly. The well- mixed urine sample was divided in to two parts, one of which was used for the quantitative cell count, urine dipstick and the other for bacteriological studies.

4.7.4 Method of quantitative cell count

A standard 10 ml. volume of the mid-stream specimen was centrifuged in a graduated tube at 3,000 revolutions per minute. for three minutes, nine and half ml. of the supernatant urine was pipetted off and tested for protein, glycosuria, leukocyte esterase and the sediment was re-suspended in the remaining ½ ml. of urine by vigorous mixing with a Pasteur pipette. A drop of the suspension was used to fill a Neubauer counting chamber and the white cells in the four area were counted microscopically and their mean was estimated as < 10 or ≥ 10 WBCs per measure area(50).

4.7.5 Bacterial Culture, Isolation and Identification

Urine samples were inoculated into Cysteine Lactose Electrolyte Deficient medium agar (Oxoid, Ltd., Basingstoke, Hampshire, England) at once by using calibrated wire inoculating loop delivering 0.001 ml. After incubation in aerobic atmosphere at 37^0c for

18-24 hours, the number of colonies was counted and multiplied by the reciprocal of the loop's volume to give the number of organisms per milliliter of urine. Colony count yielding bacterial growth of $\geq 10^5$ CFU/ml of urine was regarded as significant bacteriuria(51).

Positive cultures with significant bacteriuria were then identified at species level by their colony characteristics, gram staining reaction and the pattern of biochemical profile using standard procedures including catalase, coagulase, oxidase, sugar fermentation, hydrogen sulfide production, indole production, citrate utilization, urease and motility test.

4.7.6 Antimicrobial Susceptibility Testing

Antimicrobial testing was performed using Kirby Bauer disc diffusion method on Muller Hinton agar (MHA) (Oxoid, Ltd., Basingstoke, Hampshire, England) prepared with 4 mm thickness. Bacterial suspension was prepared using 5ml nutrient broth in a test tube by peaking up 3-5 colonies from pure cultures and adjusted to 0.5 McFarland standard which is equal to 10^8 cells/ml. A sterile cotton swab was used to distribute the bacterial suspension evenly over the entire surface of Muller Hinton agar. By using sterile forceps, the antibiotic discs were placed on the inoculated plates at least 24 mm apart from each other and 15 mm from the edge to avoid overlapping of zone of inhibition. After placing the discs, the plate was inverted upside down and incubated aerobically at 37^0c for 18-24 hours. The diameter of the zone of inhibition around each disc was measured to the nearest whole number by using ruler. Grades of susceptibility pattern was recognized as sensitive (S), and resistant(R) by comparison of zone of inhibition as indicated in the Clinical and Laboratory Standards Institute guideline(51).

Nine antimicrobial discs which have been in use for the management of urinary tract infection in the study area were used for susceptibility tests in the following concentration.Ampicillin(10µg),PenicillinG(10µg),Amoxillin/clavulanicacid(20/10µg),Gentamycin(10µg),Ceftriaxone(30µg),Cephoxitin(30µg),Ciprofloxacin(5µg),Nitrofurantoin(300µg),Trimethoprim/sulphamethoxaxole (1.25/27.75µg)All the antimicrobials used for the study were obtained from Oxoid Ltd. Bashingstore Hampaire, UK.

Work flow chart for the diagnosis of UTI

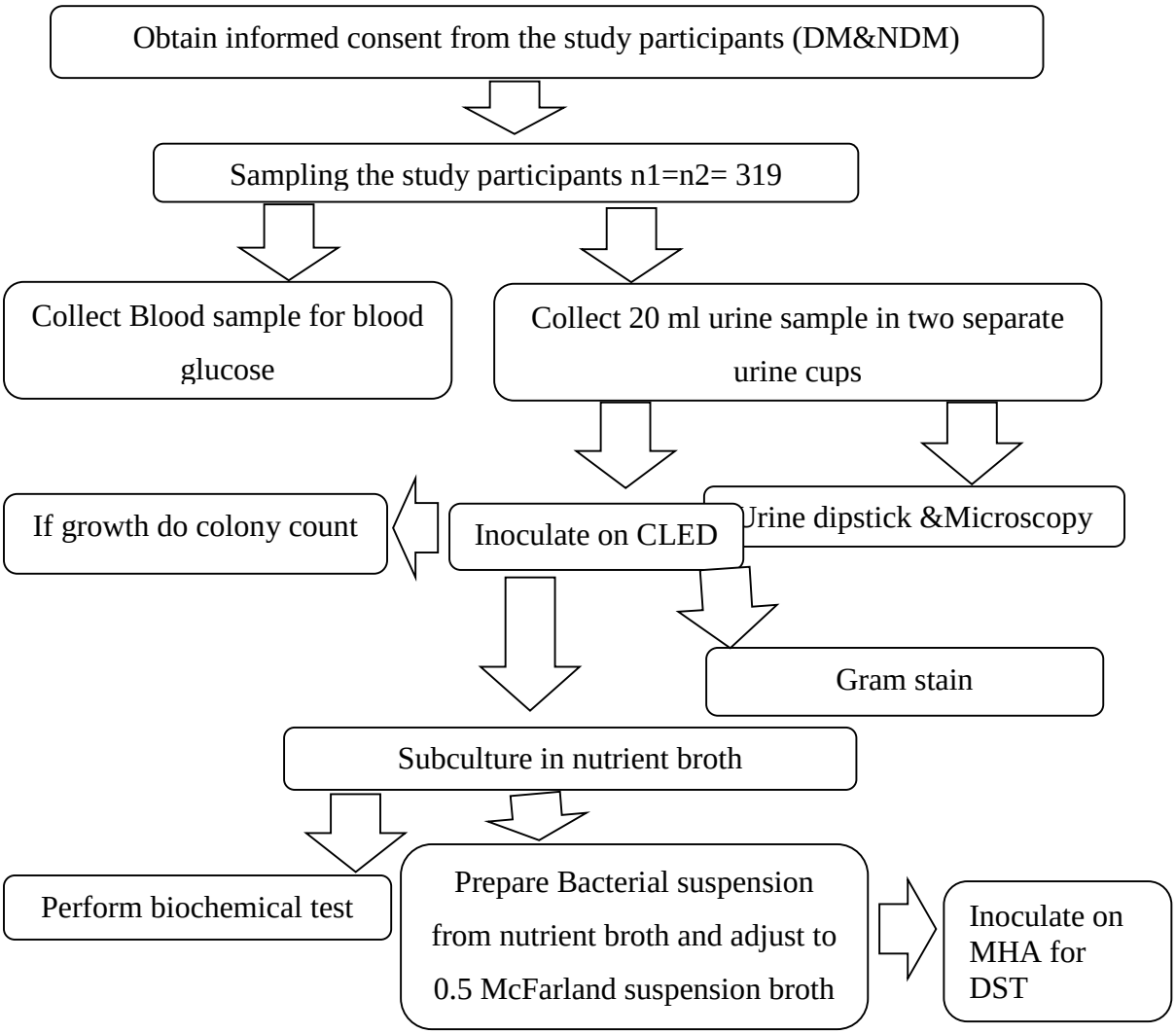

Figure 2: Work flow chart for the laboratory investigation of UTI among DM and NDM at MTUTH from April-July 2018.

4.7.7 Data quality Assurance

The questionnaire was prepared in English and translated to the local language; Amharic and translated back to English to assure its information clarity. A short one-day training was given for four data collectors on the data collection tools and data collection methods to reduce some technical and observational bias expected from the principal investigator. Just after data collection each information was checked by the principal investigator for its completeness and consistency.

During the laboratory data collection, standard operating procedures (SOPs) were strictly followed in pre-analytical, analytical and post analytical phases. Reagents and media were regularly monitored for their storage condition and expiry date according to the manufacturer's instructions. The quality of media prepared was checked by incubating one plate of each lot for sterility and standard control strains were used for performance testing. During identification of organisms for each test *Escherichia coli* (ATCC25922), *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853) were used as reference strains for culture and sensitivity testing. 0.5 McFarland standards was used to standardize the inoculum density of bacterial suspension for a susceptibility test(51).

4.7.8 Data Analysis

Data were entered and cleaned using Epi-Data version 3.1, and analyzed using SPSS version 20.2. Frequency and percentages were calculated and presented using tables and charts. Logistic regression analysis was applied to identify risk factors for urinary tract infections in diabetic and non-diabetics individuals. The independent variables were selected based on prior evidence in the literature and their effect in current analysis. Bivariate analysis was performed to find out the association of each independent variable with an outcome variable. Independent variables with a p-value of 0.25 and less during the bivariate test were then included in the multivariable logistic regression model to identify the effect of each independent variable with dependent variable and to control confounders. The prevalence estimation was made along with a 95% confidence interval (CI). The results were considered statistically significant at $P < 0.05$.

4.8 Operational Definitions

Albuminuria: The presence of ≥ +1 protein level in urine specimen in diabetic and non-diabetic patients at the time of data collection.

Asymptomatic bacteriuria: A patient without signs or symptoms of UTI as pointed out by the attending clinician at the time of data collection and then the presence bacterial count of ≥ 10^5 CFU/ml. of urine specimen.

Body mass index: <18.5 underweight, 18.5-24.9 normal, 25-29.9 over weight, >30 obese

Diabetes participant: Those known diabetes who follow their cases at the hospital and came to the diabetic clinic for checkup during the study period.

Fasting blood sugar: The result of a blood sugar taken from diabetic patient after a patient fast for at least eight hours during the study period.

Glycosuria: the presence of ≥+1glucose level in the urine specimen of diabetic and non-diabetic patients using urine dipstick at the time of data collection.

Isolates: A pure culture from urine specimen driven from a single colony that is presumed to arise from a single bacterium or fungus through microbiological procedures.

Midstream urine: Urine specimen obtained from the middle part of urine flow from diabetic and non-diabetic patients for the study.

Multi drug resistance: Bacterial isolates from diabetic and non-diabetic urine specimen that became resistant to three or more classes of antimicrobials tested in vitro on Muller Hinton agar during the study period.

Non-diabetic participant: any patient free from a disease of interest (diabetes mellitus in this case) and whose FBS/RBS <126mg/dl during data collection.

Pyuria: The average presence of more than 10 leukocytes in the urine sample per measure of four areas of Neubauer chamber during the study time.

Resistant: The capacity of uropathogenes to withstand the effect of antibiotics that are intended to kill them based on the zone of inhibition measurement in vitro diagnosis during the study time.

Susceptible: The capacity of uropathogenes to respond the effect of antibiotics that are intended to kill them based on the zone of inhibition measurement in vitro diagnosis.

Significant bacteriuria: the presence of $\geq 10^5$ colony forming units of bacteria per milliliter of urine during the study time

Symptomatic bacteriuria: a condition whereby a patient has one or more of the following signs or symptoms fever (temperature, > 38 °C), urgency, frequency, dysuria, suprapubic pain or flank pain as pointed out by the attending clinician and a urine culture positive for 10^5 CFU/ml or more uropathogenes during data collection.

Type I diabetes: Diabetic patients as confirmed by the clinician that they don't produce insulin.

Type II diabetes: Diabetic patients as confirmed by the clinician that they do resist insulin.

Uropathogenes: Bacterial or fungal isolates from urine specimen that are responsible to cause urinary tract infection.

4.9 Ethical Considerations

The study was conducted after getting ethical clearance from Institutional Review Board (IRB) of Jimma University and official support letter from Jimma University School of Medical Laboratory Sciences. Agreement was obtained from Mizan Tepi University Teaching Hospital clinical director and Mizan Aman public health laboratory. Written and consent were obtained from each participants and assent from parents or guardians.

Data obtained in the course of the study were kept confidential and used exclusively for the purpose of the study. Any study participant has full right to withdraw from the study at any point time. All significant results were exchanged with patient's physician at regular intervals.

4.10 Data dissemination

The findings of this research is going to be submitted to the school of medical laboratory sciences, faculty of health sciences, post graduate and research coordinating office, Jimma university. It will be kept in public libraries to be used as a reference. It will also be disseminated to the SNNPs regional health bureau and Mizan Tepi source population through the concerned bodies. Beyond to this an attempt will be made to publish the findings of the study.

CHAPTER FIVE

RESULTS AND DISCUSSION

5.1 Result

5.1.1 Socio-demographic characteristics

Demographic information of diabetic and non-diabetic patients suggested that the patients included in this study were between 5-65 years of age. The mean age group for diabetic patients was (42.0 ± 11.7) and for non-diabetics it was (32.0 ± 11.5). 130diabetics and 135 non-diabetics were females. Female to male ratio was 1.4:1(Table 1).

Table 1: Socio-demographic characteristics of diabetic & non-diabetics patients, MTUTH, April-July 2018.

Variables		**Diabetic patient n=319**		**Non-diabetic patient n=319**	
		With UTI	Without UTI	With UTI	Without UTI
Age	< 18	2(4.2)	8(3.0)	1(5.6)	39(13.0)
	18-29	12(25.0)	30(11.0)	9(50.0)	71(23.6)
	30-45	17(35.4)	111(41.0)	5(27.8)	165(54.8)
	> 45	17(35.4)	122(45.0)	3(16.6)	26(8.6)
	Mean ± sd		42±11.7)		32.0±11.5)
Sex	Male	11(22.9)	119(43.9)	1(5.6)	134(44.5)
	Female	37(77.1)	152(56.1)	17(94.4)	167(55.5)
Marital status	Single	18(37.5)	68(25.1)	9(50.0)	90(29.9)
	Married	30(62.5)	203(74.9)	9(50.0)	211(70.1)
Education Read/write	Yes	42(87.5)	236(87.1)	13(72.2)	276(91.6)
	No	6(12.5)	35(12.9)	5(27.8)	25(8.4)
occupation	Employed	5(10.4)	27(10.5)	1(5.6)	23(7.6)
	Unemployed	43(89.6)	244(90.5)	17(94.4)	278(92.4)
Residence	Urban	26(54.2)	139(51.3)	9(50.0)	168(55.8)
	Rural	22(45.8)	132(48.7)	9(50.0)	133(44.2)

5.1.2 Clinical characteristics

Sixty-six (20%) diabetic patients and 40(12.5%) non-diabetic patients had a previous history of urinary tract infection at one point of their life. 91(28.5%) of diabetic patients had a fasting blood sugar greater than 126mg/dl. And 49(15.5%) of diabetic patients were

positive (≥ +1) for urinary glucose. 131(41.0%) diabetic patients had been diabetic for >5years with the mean duration of 5.1± 1.8 years. (Table 2).

Table 2: Clinical characteristics of diabetic & non-diabetic patients, MTUTH, April-July 2018.

Variables		Diabetic patients N=319		Non-diabetic patients N=319	
		With UTI	Without UTI	With UTI	Without UTI
BMI	≤18.5	4(8.3)	25(9.2)	0(0.0)	28(9.3)
	18-24.9	44(91.7)	246(90.8)	18(100)	273(90.7)
	25-29	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	≥30	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Mean±sd		19.8±1.5		20.2±1.8
UTI history	Yes	36(75.0)	30(11.1)	14(77.8)	26(8.6)
	No	12(25.0)	241(88.9)	4(22.2)	275(91.4)
UTI symptom	Present	16(33.3)	11(4.1)	2(11.1)	16(5.3)
	Absent	32(66.7)	260(95.9)	16(88.9)	285(94.7
Catheter use	Yes	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	No	48(100)	271(100)	18(100)	301(100
FBS mg/dl	≤126	4(8.3)	222(81.9)	18(100)	301(100
	>126	44(91.7)	49(48.1)	0(0.0)	0(0.0)
	Mean± sd		136.2±66		85.5±18
DM type	Type I	22(45.8)	172(63.5)	0(0.0)	0(0.0)
	Type II	26(54.2)	99(36.5)	0(0.0)	0(0.0)
DM duration	≤5 years	25(52.1)	163(60.1)	0(0.0)	0(0.0)
	>5years	23(47.9)	108(39.9)	0(0.0)	0(0.0)
	Mean±sd		5.1±1.8		
Urine glucose	Positive	37(77.1)	12(4.4)	0(0.0)	0(0.0)
	Negative	11(22.9)	259(95.6)	18(100)	301(100
Albuminuria	Positive	5(10.4)	10(3.7)	0(0.0)	10(3.3)
	Negative	43(89.6)	261(96.3)	18(100)	291(96.7
Leukocyte esterase	Positive	40(83.3)	3(1.1)	15(83.3)	4(1.3)
	Negative	8(16.7)	268(98.9)	3(16.7)	297(98.7
Pyuria	Present	36(75.0)	10(3.7)	16(88.9)	13(4.3)
	Absent	12(25.0)	261(96.3)	2(11.1)	288(95.7

5.1.3 Prevalence of Uropathogens in diabetic and Non-diabetic participant

The culture positivity rate of bacterial isolates in diabetic and non-diabetic patients was 48/319(15.0%) and 18/319 (5.6%), respectively with the total prevalence of 66(10.3%). Prevalence of significant bacteriuria in diabetic patients was higher than in non-diabetic patients ($p < 0.05$) (Table 3).

Table 3: Prevalence rate of uropathogenes in Diabetic non-diabetic patients, MTUTH, April-July 2018.

Respondent's status	Culture +ve n(%)	Culture -ve n(%)	Total	Odds ratio	P value
Diabetics	48(15.0)	271(85.0)	319	2.962(1.682-5.127)	0.001
Non-diabetics	18(5.6)	301(94.4)	319	1	
Total	66(10.3)	572(89.7)	638		

5.1.4 Prevalence of gram positive and gram-negative isolates

Out of the total 66/638 isolated uropathogenes, 34/66 (51.5%), 26/66(39.4%) and 6/66(9.1%) were gram negative, gram positive and *candida spp.*, respectively. Gram negative isolates were dominant in both diabetic 25/48(52.1%) and non-diabetes patients 9/18 (50.0%) (Figure.3).

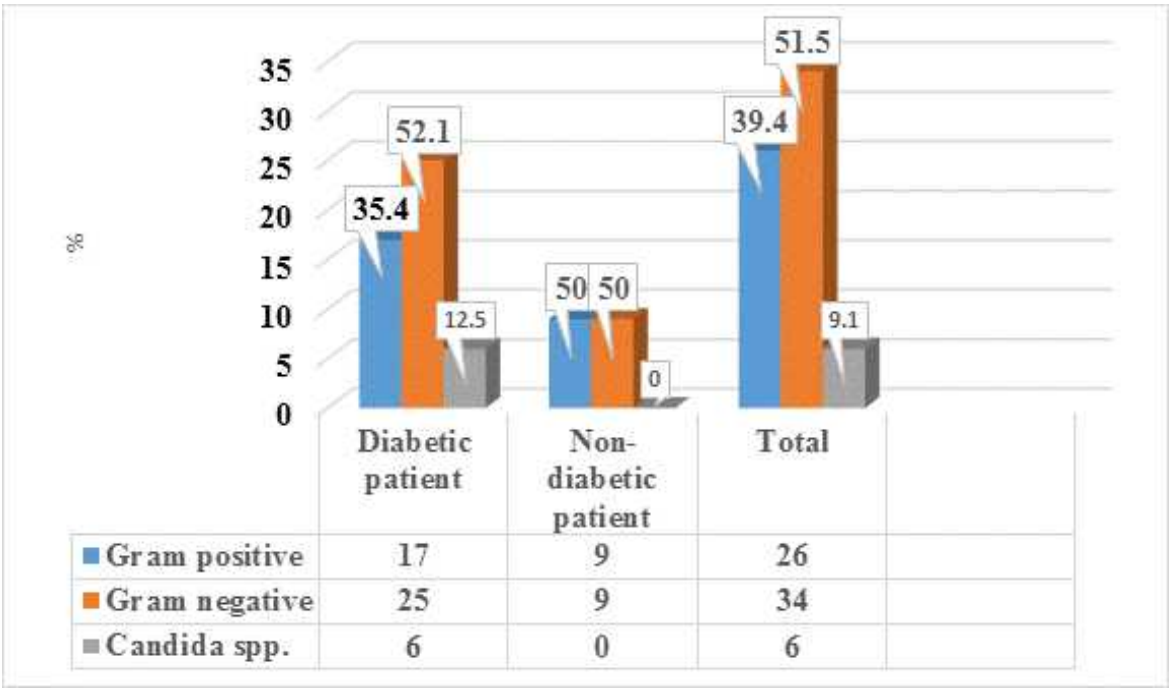

Figure 3: Prevalence rate of Gram positive and Gram- negative bacteria in Diabetic non-diabetic patients at MTUTH, April-July 2018.

5.1.5 Distribution of isolated bacterial uropathogenes

The predominant isolated uropathogenes for diabetes was *E. coli* 9/48(18.8%) followed by *S. aureus8*(16.7%), *K. peumoniae 8*(16.7%), *S. saprophyticus 7*(14.6%), *candida spp.*

6(12.6%), while in non- diabetics similarly *E. coli* 5(27.8%) was the predominant isolate followed by *S. aureus* 4(22.2%), *K. peumoniae* 3(16.7%), *and S. saprophyticus* 3(16.7%). *Candida spp*. were isolated only from patients with diabetes mellitus *(*Table 4*).*

Table 4: Total frequency of Isolated uropathogenes in diabetic and non-diabetic patients, MTUTH, April-July 2018.

Isolated bacteria	Diabetic mellitus status						Total frequency
	Diabetic patient			Non-diabetic patient			
	M	F	T	M	F	T	
E. coli	3	6	9(18.8)	0	5	5(27.8)	14(21.2)
K. pneumoniae	2	6	8(16.7)	0	3	3(16.7)	11(16.7)
Citrobacter spp	0	2	2(4.2)	0	2	2(11.1)	4(6.1)
K. ozoni	0	2	2(4.2)	0	0	0	2(3.0)
P. mirabilis	0	1	1(2.1)	0	0	0	1(1.5)
P. aeroginosa	0	1	1(2.1)	0	0	0	1(1.5)
M. morgani	0	1	1(2.1)	0	0	0	1(1.5)
Seratia spp.	0	1	1(2.1)	0	0	0	1(1.5)
S. aureus	2	6	8(16.7)	1	3	4(22.2)	12(18.2)
S.saprophyticus	1	6	7(14.6)	0	3	3(16.7)	10(15.2)
S. epidermidis	0	2	2(4.2)	0	1	1(5.6)	3(4.5)
Candida spp.	3	3	6(12.5)	0	0	0	6(9.1)
Total	**11 (22.9)**	**37 (77.1)**	**48 (100)**	**1 (5.6)**	**17 (94.4)**	**18 (100)**	**66 (100)**

5.1.6 Asymptomatic and symptomatic bacteriuria

Out of 48 culture positive diabetic patients, 17(35.4%) and 31(64.6%) were asymptomatic and symptomatic bacteriuria, respectively. Out of 48 culture positive non-diabetic patients, the prevalence of asymptomatic bacteriuria was 12(66.6%) and the rest 6(33.3%) were symptomatic bacteriuria (Table 5).

Table 5: Prevalence of symptomatic and asymptomatic bacteriuria in diabetic and non-diabetics, MTUTH, April-July 2018

Patient status	Clinical Symptom	Sex	UTI	N (%)
Diabetic	Symptomatic	M	yes	7(58.3)
			No	5(41.7)
		F	yes	24(85.7)
			No	4(14.3)
	Asymptomatic	M	yes	4(3.4)
			No	114(96.6)
		F	yes	13(8.1)
			No	148(91.9)
Non-diabetic	Symptomatic	M	yes	1(12.5)
			No	7(87.5)
		F	yes	5(62.5)
			No	3(37.5
	Asymptomatic	M	yes	0
			No	127(100)
		F	yes	12(6.8)
			No	164(93.2)

5.1.7 Age and gender wise prevalence of uropathogenes

In diabetic patients, the highest frequency of uropathogenes 10 (20.8%), 12(25.0%) and 14(29.2%) was seen in the age group between 18-19, 30-45 and greater than 45, respectively. While in non-diabetic patients, the highest frequency of uropathogenes 19(28.8%),16(24.2%) and17(285.8%) were found in the age group between 18-29, 30-45 and greater than 45 years, respectively (Table 6).

Table 6: Prevalence of uropathogenes according to age and gender in Diabetic and non-diabetic patients at MTUTH, April-July,2018.

Age	Gender	UTI positive diabetic patients	UTI positive non-diabetic patients	Total
		n (%)	n (%)	n (%)
<18 years	Male	1(2.1)	0	1(1.5)
	Female	1(2.1)	1(5.5)	2(3.0)
18-29 years	Male	2(4.2)	0	2(3.0)
	Female	10(20.8)	9(50)	19(28.8)
30-45 years	Male	5(10.4)	0	5(7.6)
	Female	12(25)	4(22.2)	16(24.2)
>45 years	Male	3(6.1)	0	3(4.5)
	Female	14(29.2)	3(16.7)	17(25.8)
total		48	18	66(100)

5.1.9 Antibiotic susceptibility pattern

Antimicrobial susceptibility testing was performed for 11 bacterial isolates identified from both diabetic and non-diabetic patients' urines sample. All gram negative and gram-positive bacterial isolates were high resistanct to ampicillin and susceptible to Gentamycin, Ciprofloxacin, trimethoprim/sulphamethoxazole and nitrofurantoin. *E. coli* was 100% resistant to ampicillin, 74.2% to penicillin G, 50% to amoxicillin and 50% to cephoxitin (Table 8

Table 7: Resistance pattern of individual bacterial isolates to different antibiotics, MTUTH, April-July,2018.

Bacterial isolates	R	Antimicrobials								
		AMP	AMX	CN	CRO	CIP	SXT	N	CEF	PG
E. coli	#	14	7	0	5	0	0	0	7	10
N=14	%R	(100)	(50)	(0.0)	(35.7)	(0.0)	(0.0)	(0.0)	(50)	(74.2)
K. pneumoniae	#	11	6	0	4	0	0	0	7	0
N=11	%R	(100)	(54.5)	(0.0)	(36.4)	(0.0)	(0.0)	(0.0)	63.6)	(0.0)
K. ozoni	#	2	2	0	2	0	0	0	1	2
N=2	%R	(100)	(100)	(0.0)	(100)	(0.0)	(0.0)	(0.0)	(50)	(100)
P. mirabilis	#	1	0	0	1	0	0	0	1	1
N=1	%R	(100)	(0.0)	(0.0)	(100)	(0.0)	(0.0)	(0.0)	(100)	(100)
P.aeroginosa	#	1	1	0	1	0	1	0	0	0
N=1	%R	(100)	(100)	(0.0)	(100)	(0.0)	(100)	(0.0)	(0.0)	(0.0)
M. morgani	#	1	1	0	1	0	0	0	1	1
N=1	%R	(100)	(100)	(0.0)	(100)	(0.0)	(0.0)	(0.0)	(100)	(100)
Citrobacter	#	4	4	0	4	0	0	0	3	4
N=4	%R	(100)	(100)	(0.0)	(100)	(0.0)	(0.0)	(0.0)	(75)	(100)
Seratia spp.	#	1	1	0	1	0	0	0	1	1
N=1	%R	(100)	(100)	()	(100)	(0.0)	(0.0)	(0.0)	(100)	(100)
S. aureus	#	10	0	0	0	0	0	0	1	1
N=12	%R	(83.3)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(8.3)	(8.3)
S.saprophyticus	#	10	0	0	0	0	0	0	0	1
N=10	%R	(100)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(10)
S. epidermidis	#	3	0	0	0	0	0	0	1	0
N=3	%R	(100)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(33.3)	(0.0)
Total	#	58	22	0	19	0	1	0	23	21
	%R	(96.7)	(36.7)	(0.0)	(31.7)	(0.0)	(1.7)	(0.0)	(38.3)	(35.0

R=Resistance,AMP=Ampicillin;AMX=Amoxacillin/clavulunicacid;CN=Getamicine;CRO=Ceftriaxone;CIP=Ciprofloxacin;SXT=Trimethoprim/Sulfamethoxazole;N=Nitrofurantoin;CEF=Cephoxitin; PG=Penicillin G, CONs=coagulase negative staphylococci

5.1.10 Multi drug resistance

Twenty-nine (56.7%) out of 60 gram-negative bacteria isolates showed resistance to antibiotics ≥ three antimicrobial agents (Table9).

Table 8: Multiple antimicrobial resistance pattern of bacterial isolates from urine of diabetic and non-diabetic patients at MTUTH March to June,2018.

Bacterial isolates	Antibiotic used	N (%)	Total MDR ≥3antibiotic classes
E. coli **n=14**	AMP, AMX, PG	4(13.8)	
	AMP, AMX, PG, CEF	3(10.3)	
	AMP, AMX, PG, CRO, CEF	3(10.3)	10(66.7)
K. pneumoniae **n=11**	AMP, AMX, PG	5(17.2)	9(75.0)
	AMP, AMX, PG, CEF, CRO	4(13.8)	
K. ozone **n=2**	AMP, AMX, PG, CEF, CRO	1(3.4)	2(100)
	AMP, AMX, PG, CEF, CRO	1(3.4)	
P. mirabilis **n=1**	AMP, PG, CEF, CRO	1(3.4)	1(100)
P. aeruginosa n**=1**	AMP, AMX, CRO, SXT	1(3.4)	1(100)
M. morgani **n=1**	AMP, AMX, PG, CRO, CEF	1(3.4)	1(100)
Citrobacter **n=4**	AMP, AMX, PG, CRO, CEF	2(6.9)	4(100)
	AMP, AMX, PG, CRO	2(6.9)	
Seratia spp. **n=1**	AMP, AMX, PG, CRO, CEF	1(3.4)	1(100)
S. aureus **n=12**	-	0	0
S. saprophyticus **n=10**	-	0	0
S. epidermidis **n=3**	-	0	0
Total 60		**29**	**29(56.7%)**

AMP.Ampicillin,AMX.Amoxacillin/clavulanicacid,CN.Gentamicin,CRO.Ceftriaxione,CIP.Ciprofloxacil,SXT.Trimethoprim/sulphamethoxazole,N.nitrofurantoin,CEF.Cephoxitin,PG.Penicillin G

5.1.11 Factors Associated with Significant Bacteriuria in DM and non-DM patients

Bivariate logistic regression analysis showed that variables for diabetic patients such as sex, marital status, previous history of UTI, FBS, type of diabetes, glycosuria and albuminuria, and variables for non-diabetic patients such as education, gender and marital status were showed association with UTI at a p value less than 0.25 and all these were considered as candidates for multivariate logistic regression analysis to identify the confounders (Table 9&10).

In multivariate logistic regression analysis significant association of female [AOR, 2.001;95% CI;1.560-4.311] v [AOR, 2.201;95% CI;1.360-4.451] with bacteriuria was seen in both diabetic and non-diabetic patients, respectively. Fasting blood glucose level >126mg/dl [AOR, 4.248; 95% CI;0.848-11.253] was significantly associated with bacteriuria. Diabetic patients with positive urine glucose had higher odds to develop

bacteriuria [AOR, 2.03; 95% CI;1.852-6.752]. Previous history of UTI also had significant association [AOR, 1.123; 95% CI;1.001-3.701] with bacteriuria in diabetic patients (Table 9 & 10).

Table 9: Risk factors associated with urinary tract infection among diabetic patients at MTUTH, April-July 2018.

Variables		Significant bacteriuria		Bivariate	Multivariate	P value
		Yes	No	COR(95%CI	AOR(95%CI	
Sex	Female	37(77.1)	152(56.1)	2.6(1.3-5.4)	2.0(1.6-4.3)	0.036 *
	Male	11(22.9)	119(43.9)	1	1	
Marital status	Single	18(37.5)	68(25.1)	1.8(0.9-3.4)	0.3(0.4-1.3)	0.139
	Married	30(62.5)	203(74.9)	1	1	
UTI history	Yes	36(75)	30(11.1)	24(11-55.3)	1.2(1.1-3.7)	0.020 *
	No	12(25.0)	241(88.9)	1	1	
DM type	II	26(55.2)	99(36.5)	2.1(1.1-3.8)	2.6(0.8-9.2)	0.135
	I	22(45.8)	172(63.5	1	1	
FBS(mg/dl)	>126	44(91.7)	49(18.1)	49(17-145)	4.2(2.8-11.2)	0.009 *
	≤126	5(8.3)	222(81.9	1	1	
Glycosuria	Present	37(77.1)	12(4.4)	72.5(29-176)	2.0(1.9-6.8)	0.001 *
	Absent	11(22.9)	259(93.6)	1	1	
Albuminuria	Present	20(41.7)	11(4.1)	16(7.3-38.8)	0.1(0.2-1.3)	0.170
	Absent	28(58.3)	260(95.9)	1	1	

CRO=Crude odds ratio; AOR=Adjusted odds ratio; CI=Confidence Inter3val * p value P<0.05

Table 10: Risk factors associated with urinary tract infection among non-diabetic patients at MTUTH, April-July 2018.

Variables		UTI		Bivariate	Multivariate	P value
		Positive	Negative	COR(95%CI	AOR(95%CI	
Sex	Female	17(94.4)	167(55.5)	3.6(1.8-10.8)	2.2(1.4-4.5)	0.014*
	Male	1(5.6)	134(44.5)	1	1	
Education	Unable to read/write	5(27.5)	25(8.3)	4.2(1.4-12.8)	0.3(0.4-1.9)	0.227
	Able to Read/write	13(72.5)	276(91.7)	1	1	
Marital status	Single	9(50.0)	90(29.9)	2.3(0.9-6.1)	0.4(0.6-1.1)	0.106
	Married	9(50.0)	211(70.1)	1	1	

CRO=Crude odds ratio; AOR=Adjusted odds ratio; CI=Confidence interval * p value <0.05

5.2 Discussion

According to our results (table 3), bacteriological investigation of the 48 diabetic patients and 18 non-diabetic patients showed a prevalence of 15.0% and 5.6% of urinary tract infections, respectively. This prevalence may be considered as very important when compared with those reported by previous studies conducted in Romania (12.0%) (28), Uganda (22.0%%) (30), Kenya (20.6%) (32), Sudan (19.5%) (31), Harar (15.4%) (33) among diabetic patients; and in Debre Tabor (10.9% v4.7%) (7) among diabetic and non-diabetic patients, respectively. However, studies reported in Nepal (34.5%v26.7%)(29) and India (34.55% v26.7%)(27) showed higher culture positivity rate among diabetic and non-diabetic diabetic patients, respectively. Irrespective of differences in the prevalence among varies studies, increased occurrence of significant bacteriuria among diabetic patients might be due to decreased antibacterial activity due to defects in neutrophil function, enough availability of protein, ,increased adherence to uroepithelial cells and presence of glycosuria which favors bacterial growth(52).

According to our study, the results show a predominance of Gram-negative bacteria (51.2%) compared to Gram positive bacteria (39.4%) with the remaining (9.1%) fungal species (Fig 3). Similar findings were reported from previous studies in Bangladesh (84.3%)(34), Algeria (59.1%)(35), Arbaminch (72.7%)(36), in which all studies isolated gram negative bacteria as the dominant causative agent of UTIs in both diabetic and non-diabetic patients.

Our results reveal that the first Gram-negative bacteria responsible for urinary tract infections is *E. coli (18.8%) v (27.8%)* was found to be the most predominant organism in diabetic and non-diabetic patients, respectively Irrespective of risk factors associated with it, *E. coli* was found as the most predominant causative agent of urinary tract infection which is in harmony with the data obtained by various studies done in India (14% v 13%)(6), Jordan (15.5% v29.5%)(38) among diabetic and non-diabetic patients and Egypt (53.8%)(39), Dessie(63.6%)(40) among diabetic patients. *E coli* is a bacterium of the digestive tract, it can spread (especially in women for anatomical reasons) down to the anus and then back in the urinary tract by multiplying and causing a urinary tract infection(53). *E. coli* has the ability to colonize the urogenital mucosa with virulence

factors like adhesins, pili, fimbriae, which can bind to the glycoconjugate receptor of the epithelial cells of human urinary tract so that it can initiate infection itself(54). Nowadays, *E coli* is the most common organism causing UTIs in individuals with diabetes(55).

Moreover, we report here that the dominant bacterial genus of urinary tract infections in Gram-positive bacteria was *S. aureus* (22.2%.). *S. aureus* was found to be the common uropathogen in diabetic patients (28.9%) in Debretabor, Ethiopia (7). Patients with diabetes are more likely than those without diabetes to be infected with *Staphylococcus aureus* and gram-negative rods(56).

When diabetic and non-diabetic patients were stratified according to their age, our results showed that the 18-29 years group and greater than 45 years group females had the highest prevalence at 19(28.8%) and 17(25.8%) of UTIs, respectively (Table.6). Relatively comparable data was found in a study done at Arsho where urinary tract infection was the highest (43.8%) in patients of age group 25–44 followed by age groups of 45–64(48). This may be due to a decrease in urinary flow, incomplete bladder emptying after urination, prolapse (descent) of the bladder and vagina in women or to the prostate's aging in Men (57). It is well known that in elderly men, the bactericidal activity of prostatic fluid is reduced which promotes bacterial growth. On the other hand, after menopause, the decrease in estrogen impregnation results in a reduction in the number of lactobacilli and an increase in pH responsible vaginal colonization by *Escherichia coli* and other *Enterobacteriaceae (57, 58)* Furthermore, the female urethra is shorter and exposes women to more urinary infections due to gastrointestinal colonization

This study showed that most isolates from diabetic and non-diabetic patients including *E. coli* were sensitive to Nitrofurantoin, Gentamicin and trimethoprim/sulpfamethoxazole. Ampicillin. showed the least sensitivity towards *E. coli* followed by penicillin G and Amoxacillin/clavulunic acid; which was consistent with reports of different studies conducted in different areas in which *E. coli* was resistant to Ampicillin and Amoxacillin/clavulunic acid (44, 59). High percentage of *E. coli* isolates was resistant to this compounds was due to the production of ß-lactamase which breaks the antibiotics structure (a four atom ring known as beta lactam) resulting in deactivating the molecules's antimicrobial property(60).

According to our study, 29(56.7%) of the total isolates were resistant to three or more than three types of commonly used drugs. 10(66.7) of *E. coli*, isolates were resistant to three or more antibiotics. A similar study conducted in Nepal, reported (78.6 %) of total isolates were MDR (52), but less in Debretabor 1(12.5)(7)isolates of were MDR. Formation of biofilms inside the bladder causes recurrent infections and also increases the chance of MDR strain causing UTI. Irrational use of antibiotics, over-the-counter sale of antibiotics, and some new drug formulations which may be of poor quality; thus producing antimicrobial-resistant strains(61, 62).

Our study revealed that, diabetic females [AOR;2.201;95%CI:1.640-4.520] had two times more likely to have UTI than males. Similarly, non-diabetic females [AOR;2.201; 95% CI:1.360-4.451] had 2.2 times more likely to have UTI than males. Which was similar to other studies conducted in UAE (28). Prevalence in women is due to decrease of normal vaginal flora (*Lactobacilli*), less acidic pH of vaginal surface, short & wide urethra, proximity of urethra to anus and poor hygienic conditions(57, 58).

In this study, Significant bacteriuria was strongly associated to higher fasting blood glucose level (>126mg/dl) in diabetic patients [AOR=4.148; 95% CI=0.843-11.203, p=0.020] than non-diabetic patients with FBS ≤ 126 mg/dl. The presence of glucose in the urine showed statistically significant association to the prevalence of uropathogenes [AOR=2.010; 95% CI=1.861-6.772. p=0.001]. Patients with previous history of UTI showed statistically significant association to UTI [AOR=1.122;95%CI=1.048-3.730, p=0.040] than patients with no previous history of UTI. This findings agreed to a previous study conducted in, Nekemte(47) and Hawassa university(46). The prevalence of urinary tract infection among hyperglycemic diabetic patients may be due to impaired granulocyte function (4), and glycosuria favors bacterial growth (9).

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The results of this study demonstrated the prevalence urinary tract infection, the susceptibility pattern of isolates to commonly used antibiotics and risk factors for UTI in diabetic and non-diabetic patients.

The present study reports UTIs are more common in patients with diabetes mellitus than non-diabetic patients showing gram negative bacterial isolates were predominant being *E. coli* the most common uropathogene in both diabetic and non-diabetic patients.

The antibiotic sensitivity test shows most gram negative and gram-positive bacterial isolates were found to be resistant to ampicillin, amoxicillin/clavulanic acid, penicillin G and sensitive to Gentamycin, ciprofloxacin, nitrofurantoin and sulphamethoxazole / trimethoprim. Most bacterial uropathogene isolates were resistant to two to three antimicrobials.

Significant bacteriuria was significantly associated with higher blood glucose level (FBS >126 mg/dl) a previous history of UTI and glycosuria ($P<0.05$) in this study.

6.2 Recommendation

Though there are many papers focused on prevalence of UTI, their antimicrobial susceptibility profile and its risk factors in diabetes and non-diabetes, there are still many problems that need to be studied. Management strategies remain to be pursued in many major problems especially against diabetes.

- ✓ The current study recommends, regular control of blood glucose level, detection of glucose in the urine and urine culture should be made in all diabetic patients

- ✓ Appropriate use of prescribed antibiotics is mandatory to prevent antimicrobial resistance against uropathogenes causing urinary tract infection in both diabetic and non-diabetic patients.
- ✓ Empirical treatment for UTIs may not be effective, therefore treatment should be based on the results of culture and sensitivity tests. But in the absence of laboratory facilities for drug sensitivity tests, it is recommended to use nitrofurantoin, gentamycin, ciprofloxacin and sulphamethoxazole/trimethoprim for treatment of UTIs, but not penicillin and ampicillin since most isolates showed 100% resistance.
- ✓ More studies should be carried out in this area, with regard to more classes of antibiotics so that diverse antibiogram can be developed and help to motivate other community life to improve future which is of relevance to many millions of diabetics and non-diabetics in relation to urinary tract infections.

6.3 Limitation of the study

Extended beta lactamase producing bacteria and methicillin resistant bacteria were not determined in this study because of lack of reagents during the study time. Moreover, a limited class of antibiotics that were available during the study period were included for antimicrobial test in the study. Thus, it may not include all antibiotics used in clinical practice.

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APPENDICES

ANNEX I: PARTICIPANT INFORMATION SHEET: ENGLISH VERSION

1. English version

Name of the organization: Mizan Tepi University Teaching Hospital

Title of the research "Prevalence and antimicrobial susceptibility profile of uropathogenes and associated risk factors among diabetic and non-diabetics attending MTUTH".

Name of researcher: Mulugeta Mengistu (MSc candidate)

Introduction

You are kindly invited to participate in a study to be conducted by MSc student at MTUT Hospital. It is aimed at determining the prevalence of urinary tract infection, antimicrobial susceptibility profile of isolates and associate factors among diabetic and non-diabetic attendants of Mizan Tepi University Teaching Hospital.

Purpose of the study

The main objective of this study is to determine the Prevalence of urinary tract infection, antimicrobial Susceptibility Profile of isolates and associated risk factors of UTI in Diabetic Patients and Non-Diabetic Patients Attending the Diabetic Clinic of Mizan-Tepi University Teaching Hospital, Aman, South- West Ethiopia''. Participation in this study is exclusively voluntary. If you are not interested to participate or if you once decided to participate and want to withdraw at any time, there will be no consequence on your duty. If you decide to participate, you have to sign on the consent form and may be given a copy of this information sheet.

Expected from you as a participant?

As a participant if this study you are expected to give answers for some questions about clinical and socio-demographic conditions and agree to give urine and blood samples. You need to know that the result might be discussed with appropriate individuals who can

give you appropriate consult if the result is significant. But your any of identifier will not be disclosed rather than identification code will be used in such conditions.

Time you will spend to participate in this study

You will spend about 10-15 minutes until you provide the specimen together with your response to the questionnaire and the consent you signed.

Risks you will face in participating in this study

There is no risk associated with the specimen collection since you give urine sample as natural way and these specimens would follow the routine procedures for the laboratory procedures. Bus you may feel a little pain in figure prick during capillary blood collection but it brings no problem upon you.

Confidentiality

All information that you give and the results will be used for this study only. Only limited number of professionals will have access to the information.

Benefit from the participation

As this study is MSc student research, there will be no payment for the participants and you will not be asked to pay for the laboratory examination. In case your result is clinically significant, it will help you for further diagnosis and treatment.

Your rights as a participant

You have full right to withdraw from the study at any time and for this you will face no problem.

Contact Address: .Mulugeta Mengistu (MSc candidate) Cell phone: +251-913-44-73-44 [email:mullermengistu11@gmail.com]
Jimma university, Institute of Health, School of Medical Laboratory Sciences.

ANNEX II: PARTICIPANT INFORMATION SHEET: AMHARIC VERSION

ለጥናቱ ተሳታፊ መረጃ የአማርኛ ግልባጭ

የድርጅቱ ስም፡-ጅማ ዩኒቨርሲቲ የህክምና ማእከል የሜዲካል ላቦራቶሪ ሳይንስ ትምህርት የሜዲካል ማይክሮባዮሎጂ ክፍል

የጥናቱ ርዕስ:- በስኩዋር ህመምተኞች በሆኑና ባልሆኑት ላይ ያለዉን የሽንት ባንባ ኢንፌክሽን አምጪ ተዋስያንን መጠንና ስርጭት ማወቅ እንዲሁም ለጸረ ህዋስ መድሃኒቶች ያላቸዉን ምላሽ መለየት እንዲሁመ ተዛማች መንስኤዎችን መለየት፡፡

የተመራማሪዉ ስም፡- ሙሉጌታ መንግስቱ

የጥናቱ አላማ፡-የዚህ ጠናት አላማ "በስኩዋር ህመምተኞች በሆኑና ባልሆኑት ላይ ያለዉን የሽንት ቡዋንባ ኢንፌክሽን አምጪ ተዋስያንን መጠንና ስርጭት ማወቅ እንዲሁም ለጸረ ህዋስ መድሃኒቶች ያላቸዉን ምላሽ መለየት እና ተዛማች መንስኤዎቸን ማወቅ" ነዉ፡፡

ጥናቱ የሚያስገኘው ጥቅም:- በጥናቱ በመሳተፍዎ ምንም አይነት ክፍያ አይጠየቁም ወይም አርሶ የሚያገኙት ገንዘብ የለም ነገር ግን በጥናቱ ወቅት የምርመራ ዉጤቱ ህክምና የሚያስፈልገዉ ከሆነ ተጨማሪ ምርመራ እና ህክምና እንዲያገኙ ይረዳዎታል፡፡

ስጋትና ጉዳት፡- ለጥናቱ ናሙና በሚሰጡበት ወቅት ምንም አይነት ጉዳት አያስከትልቦትም፡፡

ሚስጢራዊነት፡- የሚሰጡት ማንኛዉም መረጃ ምስጥራዊነቱ የተጠበቀ ነዉ፡፡ከዚህ ጥናት ጋር ተያያዝነት ባላቸዉ በማናቸዉም ነገሮች ላይ የእርሶ ማንነት በስም አይጻፍም ዉጤቱም ከጥናቱ አላማ እና ከእርሶ ፈቃድ ዉጭ ለሌላ ተላልፎ አይሰጥም፡፡

ከጥናቱ ስለማቃረጥ፡-በዚህ ጥናት ላይ የሚሳተፉት በመጀመሪያ ፈቃደኛ የሆኑ ብቻ ናቸዉ፡፡ለጥናቱም ተስማምተዉ ከተጀመረ በሓላ በማንኛዉም ሰአት የማቋረጥ ሙሉ መብት አሎት፡፡ከጥናቱ በማቋረጥዎ በእርሶ ላይ የሚያመጣዉ ጉዳትም ሆነ ተጽእኖ የለም፡፡

ስለተባበሩኝ እጅግ አመሰግናለሁ፡፡ ለተጨማሪ መረጃ በሚከተለዉ አድራሻ ማግኘት ይችላሉ፡፡

ሙሉጌታ መንግስቱ ስ.ቁ 0913447344 (email፡ mullermengistu11@gmail.com)

ANNEX III: CONSENT FORM FOR PARTICIPANTS: ENGLISH VERSIONS

1. Consent Form for ages older than 18 years 'old

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

It is therefore with full understanding of the situation that I agreed to give the informed consent voluntarily to the researcher to give my specimen for the mentioned study.

Participant's Signature: ----------Date ----------

2. **Assent form for the age 12-17 years' old**

The objective and the application of the study were briefly explained to me. I am also informed that all information contained within the laboratory request is to be kept confidential. Moreover, I have been well informed of my right to refuse information, decline to cooperate and drop out of the study if I want and none of my actions will have any bearing at all on my overall health care.

It is therefore with full understanding of the situation that I agreed to give the assent form voluntarily to the researcher to give my specimen for the mentioned study and agreed to use the sample for further study in my signature.

Participant's Signature: ---------- Date ----------

Guardians signature/fingerprint----------Date ----------

3. Parental/Guardian Consent Form (for ages less than 11 years old)

I was informed take whatever time I need to discuss the study with my family and friends, or anyone else I wish to. The decision to let my child join, or not to join, is up to

me, and will take him/her about 10 minutes, it is not painful and my child can stop participating at any time and will not lose any benefits as thereof.

As parent or legal guardian, I assure in my signature to become my child a participant in the research study described in this form.

Guardian's Signature/fingerprint: ---------- Date ----------

Investigator's name---------- Signature: ---------- Date ----------

ANNEX IV: CONSENT FORM FOR PARTICIPANTS: AMHARIC VERSIONS

1. የስምምነት ማረጋገጫ ቅጽ ከ18 አመት እድሜ በላይ ለሆኑ

እኔ ፊርማዬ በስተመጨረሻው ላይ የሚገኘው ግለሰብ የዚህ ጥናት አላማ ተገልፆልኛል፡፡ በተጨማሪም እኔ የምሰጠው መረጃም ሆነ ናሙና ለዚህ ጥናት ብቻ እንደሚዉልና በሚስጥር እንደሚያዝ ተገልፆልኛል፡፡ በዚህ ጥናት ለመሳተፍ ስምና ሌላ አድራሻ መግለፅ እንደማያስፈልገኝ ተረድቻለሁ፡፡ ከዚህ በተጨማሪም በጥናቱ ላለመሳተፍ መወሰን ወይንም በፈለግኩት ጊዜ ማቋረጥ እንደምችልና ሳቋርጥም ለማቋረጥ የፈለግኩበትን ምክንያት ለማስረዳት እንደማልገደድ እንዲሁም በጥናቱ ለመሳተፍ ፈቃደኛ አለመሆኔ ወይም በጥናቱ ሂደት ላይ ተሳታፊ ከሆንኩ በኋላ አቋርጬ መውጣቴ በእኔ ላይ የሚደርሰው አንዳችም ተፅእኖ እንደሌለ ተረድቻለሁ፡፡ ሆኖም እኔ በዚህ ጥናት ላይ ተሳታፊ ለመሆን ስስማማ በሚገኘው ጠቃሚ መረጃ የሽንት ቱቦ ኢንፌክሽን በስኳር ህመምተኞችም ሆነ ባልሆኑት አስታማሚዎች ላይ እያመጣ ያለውን ጫና ለመቀነስ የሚረዳ መሆኑን ተስፋ ለማድረግ ነው፡

እኔም ይህን ከተረዳሁ በኋላ ለተመራማሪዉ ናሙና ለመስጠት ፈቃደኝነቴን እገላጻለሁ

ፊርማ---------- ቀን፡----------

1. የታዳጊዎች የፈቃደኝነት ማረጋገጫ ከ 12-17 ለሆኑ

የጥናቱ አላማ በግልጽ ተነግሮኛል፡፡በተጨማሪም ከላቦራቶሪ የሚወጣዉ ዉጤት ለጥናቱ አላማ ብቻ እንደሚዉል ተገልጾልኛል፡፡ ከጥናቱም በፈለኩት ጊዜ መዉጣት አንደምችልን በመዉጣቴም ምንም አይነት ጉዳት እንደማይደርስብኝ ተገልጾልኛል፡፡ ይህን ከተረዳሁ በሁዋላ ለአጥኚዉ ናሙና ለመስጠት ፈቃደኛ ነኝ፡፡

የወላጅ/አሳዳጊ ፊርማ----------ቀን---------- የተሳታፊ ፊርማ----------

2. የወላጅ ወይም ያሳዳጊ ፈቃደኝነት ቅፅ / ከ11 አመት እድሜ በታች ላሉ ታዳጊዎች ብቻ/

በዚህ ጥናት ዉስጥ የእርሶ ልጅ ስለተመረጠ እባክዎ ስለ ልጅዎ በዚህ ጥናት የመሳተፍ ፈቃደኛነትዎን ያሳውቁን ዘንድ እና እርስዎ ፈቃደኛ ከሆኑ ከልጅዎ ናሙና እንድንወስድ ይሽዉም 10 ደቂቃ በላይ እንደማይወስድ ህመምም የሌለዉና እንዲሁም በተፈለገዉ ጊዜ ከጥናቱ መዉጣት እንደሚችል እንገልጻን፡፡

የወላጅ/ አሳዳጊ ፊርማ ----------ቀን----------

ANNEX V: QUESTIONNAIRE ENGLISH AND AMHARIC VERSION

A. ENGLISH VERSION

Questionnaire and lab report format for the “Prevalence and antimicrobial susceptibility profileof uropathogenes and associated risk factors of UTI among diabetic and non-diabetics attending MTUTH.”

Serial no (code no) ---------- Date----------

Name of health facility ----------

Category of respondents **1. Diabetic patient** **2. Non- diabetic**

Socio demographic character			
s.no	variable		Remark
01	Sex	1. Male 2. Female	
02	Age	…….	In years
03	Ethnicity	1. Bench 2. Meei 3. Dizi 4. Others	
04	Religion	1. Protestant 2. Orthodox 3. Muslim 4. other	
05	Marital status	1.single 2. married 3. Divorced 4. Widowed	

06	Educational level	1.Able to read/write 1.Unable to read/write	
07	Occupational status	1.Employed 2.unemployed	
08	Place of residence	1.Urban 2,Rural	
09	Body mass index	1.weght**……** 2.Heght**……...**	
Clinical history			
10	Type of diabetes	1.Type I 2.Type II	Diabetics only
11	Duration of diabetes	**……..**	Diabetics only
12	Previous history of UTI	1.Yes 2.No	
13	History of antibiotic usage	1.Yes 2.No	
14	Clinical symptoms of UTI	1.yes A. fever B. Urgency C. Dysuria D. Flank pain 2.No	
15	History of catheterization	1.Yes 2.No	
16	Family history of diabetes	1.Yes 2.No	For non-diabetics
Laboratory result			
	Blood glucose level	…….	mg/dl
	Urine chemical test		
	Urine glucose level	1.Positive 2.Negative	
	Leukocyte esterase	1.positive 2.Negative	
	Presence of Nitrite	1.positive 2.Negative	
	Albuminuria	1.positive 2.Negative	
	Urine microscopy		
	pyuria	1.present…WBC/field 2.Absent	
	Culture		
	Growth on CLED	1.Yes……CFU/ml 2,No	

	Gram stain	1.Gram positive	
		2.Gram negative	
		3.yeast	
	Biochemical tests	+	-
	coagulase		
	catalase		
	Oxidase test		
	Lactose fermentation		
	Indole production		
	Urea hydrolysis		
	Mannitol		
	Hydrogen sulfide		
	Gas production		
	Glucose fermentation		
	Citrate utilization		
	Motility		
	Lysine		
	Bacterial isolate	**……**	

DST test report

Identified bacteria	Zone of inhibition in millimeter for								
	AMX	AMP	CN	CRO	CIP	CEF	SXT	N	PG

B. ቃለ-መጠይቅ በአማርኛ

“በስኩዋር ህመምተኞች በሆኑና ባልሆኑት ላይ ያለዉን የሽንት ቡዋንባ ኢንፌክሽን አምጪ ተዋስያንን መጠንና ስርጭት ማወቅ እንዲሁም ለጸረ ህዋስ መድሃኒቶች ያላቸዉን ምላሽ መለየት እና ተዛማች መንስኤዎቹን ማወቅ”በሚል ርዕስ የተዘጋጀ መጠይቅ

ኮድ ---------- ቀን----------

የተቋዋሙ----------

የተሳታፊዉ የጤና ሁኔታ **1.** የስኩዋር ህመምተኛ የሆነ **2.** የስኩዋር ሂመምተኛ ያልሆነ ማህበራዊ እና ስነ-ህዝብ			
ተ.ቁ	መለያዎች		አስተያየት
01	ጸታ	1. ወንድ 2. ሴት	
02	ዕድሜ	…….	በአመት

03	ብሄር	1. ቤንች 2. ሜኢ 3. ዲዚ 4. ሌላ	
04	ሃይማኖት	1. ፕሮቴስታንት 2. ኦርቲዶክስ 3. ሙስሊም 4. ሌላ	
05	የጋብቻ ሁኔታ	1.ያላገባ/ች 2. ያገባ /ች 3. የተለያዩ 4. ባል/ሚስት የሞተባቸዉ	
06	የትምህርት ደረጃ	1.ማንበብ/መጻፍ የሚችል	
		1. .ማንበብ/መጻፍ የማይችል	
07	የስራ ሁኔታ	1.የመንግስት/በሌላ ተቃም የተቀጠረ 2.ያልተቀጠረ	
08	የመኖሪያ ስፍራ	1.ከተማ 2,ገጠር	
09	የሰዉነት መጠን ኢንዴክስ	1.ክብደት……	
		2.ቁመት……..	
ክሊኒካዊ ታሪክ			
10	ስኩዋር ህመም አይነት	1.አይነት አንድ 2.አይነት ሁለት	ለስኩዋር ህመምተኞችብቻ
11	ስኩዋር ህመም ቆይታ	……..	ለስኩዋር ህመምተኞችብቻ
12	የሽንት ቡዋንባ ኢንፌክሽን ቅድመ ታሪክ	1.አለ 2.የለም	
13	የጸረ-ተዋስያን መድሃኒት አጠቃቀም ቅድመ ታሪክ	1.አለ 2.የለም	
14	የሽንት ቡዋንባ ኢንፌክሽን ምልክት	1.አለ ሀ. ትኩሳት ለ.የሽንት ቶሎ ቶሎ መምጣት ስሜት ሐ.ለመሽናት መቸገር መ.የጎን ህመም ስሜት 2. የለም	
15	የሽንት ማሶገጃ ካቴተር ተጠቅሞ ስለማወቅ	1. አዎ 2.አይደለም	
16	በቤተሰብ ዉስጥ የስኩዋር ህመምተኛ ነበር/አለ	1.አለ 2.የለም	የስኩዋር ህመምተኛ ላልሆኑ

ANNEX VI: LABORATORY PROCEDURES

Urine dipstick (Comber-TestUX Strips-Roche)

Nitrite

Principle-Diazonium salt +tetrahydobenzoquinoline = pink azo dye

The nitrite has 92% 100% sensitivity for UTI but only a 35% to 85% specificity.

Leukocyte esterase test:

Principle: Indolecarboxylic acid ester = indoxyl+ acid in acid medium

Indoxyl+diazonium salt=violetazole dye

It detects esterase enzyme released in urine from granules of leukocytes, positive in pyuria.

It has 75% to 96% sensitivity and 94% to 98% specificity for detecting pyuria

Glucose test:

Principle: Glucose O_2 =D glucose-o-lactone +H_2O_2 catalyzed by peroxidase

H_2O_2 + chromogen=oxidized chromogen(colored) +H_2O.

Gram Stain

The test detects the type of microorganisms isolated based on its staining reaction.

Procedure

1) A dried smear was made and fixed.

2) The fixed smear was covered with crystal violet for 30 seconds.

3) The stain was rapidly washed off with water.

4) The water was tipped off and the smear was covered with Lugol's iodine for 60 seconds.

5) The iodine was washed off with clean water.

6) The smear was decolorized rapidly for few seconds with acetone water.

It was washed immediately with clean water.

7) The smear was covered with neutral red for few minutes.

8) The stain was washed off with clean water

9) The back of the slide was wiped clean and placed in a draining rack for the smear to air-dry. Gram positive bacteria appeared purple while Gram negative appeared pale to dark red (Cheesbrough, 2010).

Biochemical Tests

Biochemical tests Including Catalase test, Coagulase test, Oxidase test, Indole test, Citrate as elucidated by Cheesbrough (2010) were carried out on the colonies to ascertain organisms isolated

Catalase test

This test detects the presence of Catalase an enzyme that catalyses the release of oxygen from hydrogen peroxide.

Procedure

1) 2ml of hydrogen peroxide solution was poured into test tubes for each isolate.

2) Several colonies of the test organisms were removed using a sterile wooden stick and immersed into the hydrogen peroxide solution in the test tube.

3) Immediate bubbling was looked for.

- Active bubbling indicates positive catalase test.
- No bubbles indicate negative catalase test**.**

Coagulase test

This test detects the presence of coagulase enzyme.

Procedure for Coagulase test

1) A drop of water was placed on the end of two separate grease-free slides for each isolate.

2) A colony of the test organism was emulsified in each of the drops to make suspensions.

3) A loopful of plasma was added to one of the suspensions. It was mixed
gently and clumping of the organism was looked for within 10 seconds.

- Clumping within 10 seconds indicates that the organism is *Staphylococcus aureus* growth.
- No clumping within 10 seconds indicates that there is no bound Coagulase.

Oxidase test

This test detects the production of oxidase enzyme by some microorganisms.

Procedure

1) A piece of filter paper was placed in a clean petri dish and 3 drops of freshly prepared oxidase reagent was added.

2) Using a piece of stick, a colony of the test organism was removed and smeared on the filter paper.

- Development of blue-purple color within a few seconds indicates a positive oxidase test.
- No blue-purple color indicates a negative oxidase test.

Indole test

The test detects the production of indole in tryptophan containing medium by some bacteria when Kovac's reagent is added to it.

Procedure

1) The test organism was inoculated in a bijou bottle containing 3 ml of sterile tryptone water.

2) The bijou was inoculated at 37oC for up to 48 hours.

3) 0.5ml of Kovac's reagent was added to the bijou bottle. It was shaken gently.

- A red color in the surface layer within 10 minutes indicates a positive indole test.
- No red surface layer indicates a negative indole test.

Citrate test

This test detects the utilization of citrate

Procedure

1) The test organism was inoculated into sterile peptone water broth and incubated for few hours.

2) A sterile straight wire was then used to inoculate Simmons citrate agar with the broth culture.

3) It was incubated at 37oC for 48 hours.

- Development of a blue color growth indicates a positive citrate test.

ANTIBIOTIC SUSCEPTIBILITY TEST PROCEDURE, CLSI 2017

A. Preparation of Turbidity Standard Equivalent to McFarland 0.5

1. First, 1 % v/v solution of sulphuric acid by adding 1ml of concentrated sulphuric acid to 99 ml of water was prepared.
2. Then 1 % w/v solution of barium chloride was prepared by dissolving 0.5 g of dehydrate barium chloride ($BaCL_{12}$. $2H_2O$) in 50 ml of distilled water.
3. Finally, 0.6 ml of the barium chloride solution was added to 99.4 ml of the sulphuric acid solution, and mixed well.
4. A small volume of turbid solution was transfer to a capped tube of the same type as used for preparing the test and control inocula.
5. Escherichia coli ATCC25922 was used to test the performance of the method and grown the nutrient agar

B. Inoculation of test organism on to Muller-Hinton agar

1. Using a sterile wire loop, 3-5 well-isolated colonies were touched and emulsified in 3-4 ml of sterile nutrient broth.
2. Turbidity of the suspension was matched to the turbidity standard by mixing the standard immediately before use and turbidities was compared to be easier to view against sheet of paper
3. Using a sterile swab, the suspension was inoculated on to plate of Muller-Hinton agar. Excess fluid was removed by pressing and rotating the swab against the side of the tube above the level of the suspension and streaked the swab evenly over the surface of the medium in three directions, rotating the plate.
4. With the Petri dish top in place, the agar was allowed for 3-5 minutes to dry.
5. Using sterile forceps the appropriate antimicrobial discs were evenly distributed on the inoculated plate by lightly pressed down to the agar.
6. Within 30 minutes of applying the discs, the plate was inverted and incubated at 35 0C for 16-18 hours and then 24 hours.
7. After overnight incubation, the diameter of each zone of inhibition was measured in mm using a ruler on the underside of the plate.

ANNEX VII: PERFORMANCE STANDARDS CHART FOR ANTIMICROBIAL SUSCEPTIBILITY TESTING FOR UROPATHOGENES, CLSC 2017.

Antimicrobial agent	Disc content	Zone diameter interpretative criteria (nearest whole mm)		
		S	I	R
Amoxicillin/clavulunate(AMC)	20/10µg	≥18	14-17	≤13
Ampicillin (AMP)	10 µg	≥17	14-16	≤13
Ceftriaxone (CRO)	30 µg	≥23	20-22	≤19
Cephoxitin(CEF	30 µg	≥18	15-17	≤14
Ciprofloxacin(CIP)	5 µg	≥21	16-20	≤15
Gentamicin(CN)	10 µg	≥15	13-14	≤12
Nitrofurantoin (N)	300 µg	≥17	15-16	≤14
Penicillin G(PG)	10 IU µg	≥29	-	≤28
Trimethoprim-sulfamethzaxole (SXT)	1.25/23.75 µg	≥16	11-15	≤10

ANNEX VIII: DECLARATION SHEET

I the undersigned, declare that this thesis entitled' Prevalence and antimicrobial susceptibility profile of uropathogenes and associated risk factors of urinary tract infection among diabetic and non-diabetic patients attending Mizan Tepi University Teaching Hospital" is my original work in partial fulfilment of the requirements for the degree of master of sciences in medical microbiology and all sources of materials used for this thesis have been acknowledged and referenced in accordance with the requirement.

Name of Principal Investigator	**Signature**	**Date**
Mulugeta Mengistu Worku	_____	_____

ANNEX IX: APPROVAL SHEET OF THESIS

JIMMA UNIVERSITY

As internal examiner, I hereby certify that I have read and evaluated this thesis prepared, by Mulugeta Mengistu Worku entitled **"Prevalence and antimicrobial susceptibility profile of uropathogenes of and associated risk factors urinary tract infection among diabetic and non-diabetic patients attending Mizan Tepi University Teaching Hospital, South west Ethiopia"** I recommend that it can be submitted as fulfilling of the thesis requirement.

Approval of the Internal Examiner	**Signature**	**Date**
DR. Getnet Beyene (PhD)		
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