SYMPTOMATIC AND ASYMPTOMATIC BACTERIURIA AND DRUG SUSCEPTIBILITY PATTERN OF URINARY TRACT INFECTIONS AMONG DIABETIC PATIENTS ATTENDING AT TIKUR ANBESSA SPECIALIZED UNIVERSITY HOSPITAL, ADDIS ABABA, ETHIOPIA.

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List of Abbreviations

API …….Analytical profile index

ASB…….Asymptomatic bacteriuria

ATCC……...…American Type Culture Collection

CLED……Cystine Lysine Electrolyte Deficient

CLSI…….Clinical and Laboratory Standard Inistititute

DM……Diabetes Mellitus

DMIP……Department of Microbiology, Immunology and Parasitology

FBS…….……..…Fasting Blood Sugar

IRB……..……..….. Institutional Review Board

MICU……..….Medical Intensive Care Unit

MODY…….. ..Maturity Onset Diabetes of the Young

NCCLS……. National Committee for Clinical Laboratory Standards

NNIS..…….…….....National Nosocomial Infections Surveillance

OPD……...Out Patient Department

PC……..Personal Computer

RBC……...Red Blood Cell

Spp.…….. ...Species

TASUH……..…Tikur Anbessa Specialized University Hospital

SPSS…… Statistical Package for Social Science

US…….United States

UTI……. ..Urinary tract infection

WBC.....White Blood Cell

WHO.....World Health Organization

Summary

Background: The risk of developing infection in diabetic patients is higher and urinary tract is the most common site for infection. Urinary tract infection (UTI) may be symptomatic or asymptomatic. The etiology of UTI and the antibiotic resistance of uropathogens have been changing over the past years. In Ethiopia, there are some published information concerning community acquired and hospital acquired UTIs, but no study was conducted in asymptomatic bacteriuria and antimicrobial susceptibility pattern for UTIs in Ethiopian diabetic patients.

Objective: The aim of the study was to identify the prevalence of symptomatic and asymptomatic bacteriuria, to isolate the bacterial pathogens and determine drug susceptibility pattern of urinary tract infections among diabetic patients attending Diabetic referral clinics at Tikur Anbessa Specialized University Hospital, Addis Ababa.

Methods: A cross sectional survey was conducted by systematic sampling technique by taking every other diabetic patient coming to diabetic referral clinics and admitted to medical wards and medical Intensive Care Unit (ICU). A total of 413 diabetic patients were selected during the study period from June 2009 to August 2009.

Results: Nine (13.6%) of the symptomatic DM patients had bacteriuria compared with thirty six (10.4%) of asymptomatic DM patient. The overall prevalence of UTI in both groups was 45(10.9%).The bacterial pathogens isolated were predominantly gram negative and two isolates were gram positive. Of the gram negative isolates the most common organism was *Escherichia coli*, 6% and 28% followed by K*lebsiella pneumoniae*, 2% and 6% in the symptomatic and asymptomatic DM patients respectively. The other gram negative organisms found in small numbers were Pseudomonas *aeruginosa*, 1(0.2%) and *Enterobacter clocace*, 1(0.2%). The only isolate from gram positive was *Entrococcus spp*. 2 (0.5%).

The susceptibility testing results of the isolates showed that over 85% *Escherichia coli* strains were sensitive to ciprofloxacin, Amoxicillin-Clavulanic acid, ceftizadine, nitrofuranton, ceftiraxone, norfloxacin and kanamycin (85.3 – 94.1%), but there was a

higher rate of resistant to both ampicillin (67.6%), tetracycline (64.7%) and Trimethoprim-sulphamethoxazole (64.7%). *Klebsiella pneumoniae* were 100% sensitive to ciprofloxacin, ceftazidime, ceftiraxone and over 85% sensitive to gentamycin (87.5%) but showed a high rate of resistance (75%) to ampicillin, tetracyclin and Trimethoprim-sulphamethoxazole. The frequency of multiple resistances for two or more drugs among the urinary pathogens were found in thirty three (71.7%) of the isolates.

Conclusion: The prevalence of urinary tract infection in this study is high in women than men and also pyuria and glucosuria can be considered as strong association with bacteriuria. *Escherichia coli* was the most common bacterial pathogen isolated in both symptomatic and asymptomatic bacteriuria. Remarkably over (60%) of all isolates were resistances to ampicillin, tetracycline and Trimethoprim-sulphamethoxazole have been found in this study. Therefore, the investigation of bacteriuria in diabetic patients by screening for urinary tract infection is very important as it enables to treat properly and prevent the development of renal complications and eventually severe renal damage and failure.

Key words: asymptomatic bacteriuria, bacteriuria, Diabetes Mellitus, prevalence, pyuria, symptomatic bacteriuria, urinary tract infection, Ethiopia.

1. BACKGROUND

1.1 INTRODUCTION

Diabetes Mellitus (DM) is becoming a common health problem in the third world (WHO, 2003).The prevalence of DM and other non-communicable diseases is on the rise in African communities due to the ageing of the population, drastic lifestyle changes accompanying urbanization and westernization (Sobngwi et *al*., 2001). Diabetes Mellitus is classified on the basis of certain etiologies and pathogenic process that leads to hyperglycemia.

The two broad categories of DM are designated type 1 and type 2. Type 1 diabetes is the result of complete or near-total insulin deficiency due to autoimmune destruction of idiopathic β cell of the pancreas. Type 2 DM is a heterogeneous group of disorders characterized by variable degrees of insulin resistance, impaired insulin secretion, and increased hepatic glucose production. The third classification for DM includes specific genetic defects in insulin secretion or action, metabolic abnormalities that impair insulin secretion, mitochondrial abnormalities, and a host of conditions that impair glucose tolerance. Maturity onset diabetes of the young (MODY) is a subtype of DM characterized by autosomal dominant inheritance, early onset of hyperglycemia (usually <25 years), and impairment in insulin secretion. Mutations in the insulin receptor cause a group of rare disorders characterized by severe insulin resistance. DM can result from pancreatic exocrine disease when the majority of pancreatic islets (>80%) are destroyed. Hormones that antagonize insulin action can also lead to DM. Thus, DM is often a feature of endocrinopathies such as acromegaly and Cushing's disease. The fourth classification is Gestational DM (Alvin, 2005).

In Ethiopia hospital based studies showed that the prevalence of diabetes has increased from 1.9% in 1970 to 9.5% in 1999 (DeFronzo and Ferrannini, 1991; Watkins and Alemu, 2003, Teferra and Abdulkadir, 1968; Lester and Tsega, 1976; Seyoum *et al*., 1999). One study conducted in Jimma town showed that the prevalence of population based studies in Type II diabetes has 5.3 % (Yemmane *et al.*, 2007). WHO estimated the number of diabetic cases in Ethiopia to be 800,000 by the year 2000, and the number is

expected to increase to 1.8 million by 2030 (WHO, 2003). According to WHO, the prevalence of diabetes mellitus is increasing in developing countries due to population growth, aging, unhealthy diets, obesity and sedentary lifestyles (King and Aubert,1998).

Patients with diabetes mellitus are more predisposed to infections. This predisposition is due to a combination of angiopathy, neuropathy and hyperglycemia. Neutrophil chemotaxis and adherence to vascular endothelium, phagocytosis, intracellular bactericidal activity, opsonization, complement function, lymphokine response and cell-mediated immunity are all depressed in diabetics with hyperglycemia (Llorente *et al*., 2000; McMahon and Bristian 1995; Leibovici *et al*., 1996). DM is associated with a wide variety of infections when glucose control is poor. Diabetes leads to several abnormalities of the host defense system, and higher glucose concentration in urine may serve as a culture medium for pathogenic microorganisms as well. The risk of developing infection in diabetic patients is higher and urinary tract is the most common site for infection (Pozzilli and Lesli, 1996; Carton, *et al*., 1992; Joshi, *et al*., 1999).

1.2 Literature Review

1.2.1 Epidemiology

Asymptomatic bacteriuria (ASB), symptomatic cystitis, acute pyelonephritis, emphysematous infections, papillary necrosis and candidal infections are all more common in diabetic than nondiabetic patients with increased frequency in diabetic women patients. Urinary tract infection (UTI) is a major disease burden for many patients with diabetes. ASB is several-fold more common among women and acute pyelonephritis is five to ten times more common in both sexes. Various risk factors for asymptomatic bacteriuria in women with diabetes have been suggested including age, sexual activities, microalbuminuria and duration of metabolic control and complications of diabetes (Zhanel *et al*., 1995; Geerlings et *al.*, 2000; Ronald and Ludwig, 2001).

The complications of pyelonephritis are also more common in patients with diabetes (Ronald and Ludwig, 2001; Nicolle *et al.*, 2006). It has been suggested ASB may lead to symptomatic urinary tract infection, as well as increase in the frequency of renal failure

as one of the long term adverse effects (Patterson and Anderiole, 1997). Serious complications of urinary infection, such as emphysematous cystitis, pyelonephritis, renal or perinephric abscess, bacteremia and renal papillary necrosis occur more commonly in diabetic patients (Nicolle, 2000). This infection is the most serious complication of diabetes and is well recognized as causing significant morbidity and mortality (Alvin et *al.*, 2005).

1.2.2. Etiology of Urinary Tract Infection in Diabetic Mellitus

The most common agents of UTI in diabetic patients are bacteria, viruses, fungi and tuberculosis.

a) Bacteria

Many microorganisms can infect the urinary tract in diabetic patients, but by far the most common agents are the gram-negative bacilli. *Escherichia coli* causes approximately 90% of acute UTI in diabetic patients without urologic abnormality or calculi. The other bacilli involved, i.e. *Proteus, Kellebsiella*, *Enterobacter, Serratia* and *Pseudomonas*, account for a lower proportion of uncomplicated infections (Raptis and Viberti, 2001). Gram positive cocci play a less important role in UTI in diabetic patients. *Enterobacter* and *Staphylococcus aureus* can cause bacteremic infection of the kidneys and consequential renal damage. A saprophytic novobiocin resistant coagulase negative *Staphylococcus* has been recognized as important cause of acute symptomatic UTI in young diabetic female (Patterson and Andriole, 1997).

b) Viruses

Viruses are most commonly found in urine samples without evidence of acute UTI in diabetic patients, although some adenoviruses have been implicated as a cause of cystitis (Warren et al, 1999).

c) Fungi

Fungal infection of UTI in diabetic patients is important but clinically insignificant. Diabetic patients with urinary tract *Torulopsis globrata* infection account for 20%-90% of all infections with this candida species. *Torulopsis globrata* can cause cystitis, pyelonephritis, renal or perirenal abscess, fungus born and a picture of gram negative sepsis (Sobel and Vazquez, 1996).

d) Tuberculosis

Tuberculosis is a serious co-morbidity in diabetics, in whom it is more extensive and 3-16 times more common than in non diabetics. Genital tuberculosis is diagnosed more commonly in female than in male patients. In female patients, it affects the fallopian tubes and the endometrium and may cause infertility, pelvic pain, and menstrual abnormalities. Diagnosis requires biopsy or culture of specimens obtained by dilatation and curettage. In male patients, tuberculosis preferentially affects the epididymis, producing a slightly tender mass that may drain externally through a fistulous tract; orchitis and prostatitis may also develop (Mario and Richard, 2008).

1.2.3. Pathogenesis and Routes of Infection

The urinary tract should be viewed as a single anatomic unit that is united by a continuous column of urine extending from the urethra to the kidneys. In the vast majority of UTIs, bacteria gain access to the bladder via the urethra. Ascent of bacteria from the bladder may follow and is probably the pathway for most renal parenchymal infections. The vaginal introitus and distal urethra are normally colonized by diphtheroids, streptococcal species, lactobacilli, and staphylococcal species but not by the enteric gram-negative bacilli that commonly cause UTIs. The factors that predispose to periurethral colonization with gram-negative bacilli remain poorly understood, but alteration of the normal vaginal flora by antibiotics and other genital infections appears to play an important role. Loss of the normally dominant H_2O_2-producing lactobacilli from the vaginal flora appears to facilitate colonization by *Escherichia coli* (Anthony and Edward, 2007)

Individual susceptibility to UTI is complex depending on genetic, biologic, and behavioral factors. The interaction between bacterial virulence and host defense factors can ultimately result in UTI. Each bacterial species has distinct pathogenic mechanisms that facilitate UTI. Colonization is determined by the specific bacterial adhesive characteristics, the receptor repertoire on the epithelial surface, and the surrounding fluids (Schaeffer *et al.,* 2001).

1.2.4. Clinical Features

Cystitis is usually associated with dysuria, frequency, urgency, suprapubic pain and hematuria. Lower tract symptoms are commonly present and usually predate the appearance of upper tract symptoms by several days. Pyelonephritis is classically associated with fever chills and flank pain. Nausea and vomiting may be present (Anthony and Edward, 2007).

1.2.5. Diagnosis

Diagnosis of uncomplicated UTI suggests that thorough laboratory evaluation of women with probable acute cystitis, including urinalysis, urine culture and sensitivity, are rarely necessary. Instead, such patients can be treated empirically (based on their clinical presentation) without laboratory testing. If therapy fails, laboratory testing can then be under taken. A more thorough diagnostic evaluation is indicating for women with evidence of pyelonephritis or risk factors for complicated infections and for all males with UTI. Patients in these categories should have a urinalysis, urine culture, and susceptibility testing done (Kunin, 1997). Screening for proteinuria has diagnostic value in the initial detection and confirmation of proteinuria renal disease. Highly specific immunoassays for specific proteins including albumin are also used. Proteinuria is also an important predictor of progressive kidney damage (Iseki *et al*., 2003).

Presumptive diagnosis of UTI is made by physical characteristics of the urine and by a series of chemical tests. A chemically impregnated dipstick can be used for many of these tests. Chemically impregnated reagent strips (Chemstrip Screen) provide quick determinations of pH, protein, glucose, ketones, bilirubin, hemoglobin (blood), nitrite,

leukocyte esterase, urobilinogen, and specific gravity. The dipstick is impregnated with chemicals that react with specific substances in the urine to produce color-coded visual results. The depth of color produced relates to the concentration of the substance in the urine.

The urine dipstick, however, is a relatively insensitive marker for initial increases in protein excretion, not becoming positive until protein excretion exceeds 300 to 500 mg/day (upper limit of normal less than 150 mg, with most subjects being under 100 mg). Using a specific assay for albumin is a more sensitive technique. The normal rate of albumin excretion is less than 20 mg/day (15 µg/min); persistent albumin excretion between 30 and 300 mg/day (20 to 200 µg/min) is called microalbuminuria and, in patients with diabetes (particularly type 1 diabetes), is usually indicative of diabetic nephropathy (unless there is some coexistent renal disease) (Mogensen, 1990; Eknoyan, *et al.,* 2003).

The urine sediment is examined under the microscope to identify the components of the urinary sediment.

1.2.6. Significance of the study

Urinary tract infection is the second most common infectious presentation in community practice. Worldwide, about 150 million people are diagnosed with UTI each year, costing the global economy in excess of 6 billion US dollars (Gonzalez and Schaeffer, 1999). UTIs are considered to be the commonest bacterial infections and account for a significant part of the workload in clinical microbiology laboratories (Wilson and Gaido, 2004). It also constitutes the most common infection in diabetic patient in many hospitals.

The risk of developing infection in diabetic patients is higher and urinary tract is the most common site for infection. In Ethiopia an increase prevalence of infections in diabetic patients was observed (Feleke *et al*., 2007) but no study was conducted in asymptomatic bacteriuria in DM patients in Ethiopia.

In Nigeria, a study conducted by Adeyeba *et al. (*2007), on 320 mid-stream urine samples from diabetes and healthy volunteers between the age ranges of 20-65 showed that 37 (21%) patients had significant bacteriuria. These included 23(61%) female and 14 (38.1%) male, while from 146 control healthy volunteers only 7(5%) had significant bacteriuria, which included 84(58%) males and 62(42%) females. The most frequent causative agents of UTI was *Escherichia coli* accounting for 17(46%) of the isolates followed by *Klebsiella species* 11(30%), *Candida albicans* 4(11%), Proteus 2(5%), *Staphylococcus aureus* 2(5%) and *Pseudomonas aeruginosa* 1(3%). The highest prevalence of UTI in diabetics was observed in age range of 46-55, 8(35%) in female and age 56-65 year 6(43%) in males. The antibiotic sensitivity pattern showed that most isolates were sensitive to ofloxacillin, Gentamycin, Nitrofurantoin, Nalidixic acid, Cotrimoxazole and Rocephine while they are resistant to tetracycline, ampicillin, cefuroxime and ceftazidime.

In Kenya, a study conducted by Kayima *et al*. (1996) on asymptomatic bacteriuria among diabetics attending Kenyatta national Hospital, Of 135 patients submitted midstream urine specimens for culture, 15 patients had positive cultures showing the incidence of asymptomatic UTI to be 11.1%. There were 10 female and 5 male patients with UTI. The commonest organism isolated was *Escherichia coli* at 40%. Gram-negative bacilli made up 66.7% of the isolates. Isolates were poorly sensitive to the regularly available antibiotics and sensitive to ampicillin (33%) and cotrimoxazole (33%). Nitrofurantoin inhibited growth in 93% of the isolates. Other antimicrobials with over 80% sensitivity level included: gentamicin, ceftazidime, augmentin, cefuroxime and norfloxacin. They are expensive or require parenteral administration. The incidence of asymptomatic UTI is high among diabetics and although the organisms isolated are those usually isolated in UTIs, they are not that sensitive to the commonly available antibacterial agents.

Another study conducted by Alebiosu (2003) on asymptomatic bacteriuria among Nigerian, type II diabetics (55 males and 69 females) submitted midstream urine specimens for culture. Thirty three patients had significant bacteriuria (9 males and 24 females), showing the frequency of occurrence of asymptomatic bacteriuria to be 26.6%.

The most common organism isolated was *Klebsiella pneumoniae* (42.4%). Gram-negative bacilli made up about 23 (69.7%) of the isolates. Isolates were poorly sensitive to the readily available antibiotics (ampicillin, tetracycline and cotrimoxazole), but a large number of the organisms isolated were sensitive to nitrofurantoin, gentamicin, ciprofloxacin and ofloxacin. Sensitivity to erythromycin, nalidixic acid and cefuroxime was moderate. Asymptomatic bacteriuria is, thus, more prevalent among the Nigerian diabetic population than in the non-diabetics. A changing pattern of disease is observed with *Klebsiella spp.* for the majority of asymptomatic bacteriuria among diabetics. The organisms are not sensitive to the commonly available antibacterial agents.

Only one cross sectional study was conducted in Ethiopia by Feleke *et al*. (2007) in Tikur Anbessa Specialized University Hospital among diabetic patient admitted in medical ward. A total of 114 clinical specimens (40 pus, 23 blood, and 51urine) were analyzed. The prevalence of UTIs was 14%, the type of bacteria isolated from urine specimens were *Escherichia coli* (7/51), *Klebsiella pneumoniae* (5/51), *Pseudomonas aeruginosa* (4/51), *Enterobacter spps* (2/51) and *Proteus mirablis* (1/51).

In a retrospective study conducted by Gedebou (1983) UTIs detected from in-patient and out- patient urine specimen constituted 27%. The type of bacteria isolated from urine specimens of different groups of patients are *Escherichia coli* (33%), *Klebsiella Spps* (20%), *Proteus* (17%), *Enterobacter* (6%) among the gram positive *Staphylococcus aureus* (0.5%). More than 85% of all types of urinary pathogens were resistant to at least one antibiotic. The majority (82.99%) of the in patient isolate of the most common urinary pathogens were resistant to 3 or more antimicrobial agents. In another study, which is held by Wolday and Erge (1997), UTIs is detected in 30% of urine sample. The rate was 35%for in-patients and 18% for outpatients; Gram-negative bacteria comprised 95% of all isolate. The commonest organisms being (39%) *Escherichia coli* and *Klebsiella Spps* (26%) among the gram positives, *Staphylococcus aureus* (57%) was the most common pathogen isolated. Most of the organisms were resistant to multiple drugs. This study compared to a previous report by Gedebou (Gedebou, 1983) showed a high incidence of resistance to the commonly prescribed antimicrobial agents.

In a study conducted by Habte-Gabr *et al.* (1988) on one thousand six surgical patients admitted over 10 months to a hospital in Addis, nosocomial infections were detected in 165 (16.4%) patients, wound (59%), urinary tract (26%), and respiratory tract (6%). In a study conducted by Gedebou *et al.* (1988), hospital acquired infections were detected among obstetric and gynecological patients in (17%) patients; Wound (47%), urinary tract (15%), and respiratory tract (6.8%).

In the 21st century, urinary tract infections are more disquieting as antibiotic resistant pathogens spread with an alarming rate. UTIs are often treated with different broad-spectrum antibiotics. The extensive uses of antimicrobial agents have invariably resulted in the development of antibiotic resistance, which, in recent years, has become a major problem worldwide (Kumar et *al*., 2006). The resistance pattern of community acquired UTI pathogens has not been studied extensively (Goldstein, 2000). The etiology of UTI and the antibiotic resistance of uropathogenes have been changing over the past years, both in community and nosocomial infection (Stamm and Hooton, 1991).

Treatment of asymptomatic bacteriuria in patients with diabetes is often recommended to prevent the risk of symptomatic UTIs (Pozilli *et al.*, 2004). However, no published information about the resistance or sensitivity pattern of diabetic UTIs in Ethiopia is available. Knowledge of the antimicrobial susceptibility profile of uropathogens causing uncomplicated diabetic UTIs in the community, if known, should guide therapeutic decisions for the treatment of acute uncomplicated cystitis.

Thus the aim of the present study was to identify bacterial profile and antibiotic susceptibility pattern of diabetic UTIs in Tikur Anbessa Specialized University Hospital. This study is important for clinician in order to facilitate the empiric treatment and antibiotic choice for specific management of patients. Moreover, the data would also help authorities to formulate antibiotic prescription policies.

1.2.7. Hypothesis

The most prevalent pathogens isolated from patients with UTIs could be similar to those isolated in the previous study.

1.3. OBJECTIVE OF THE STUDY

1.3.1 General Objective

To identify the prevalence of symptomatic and asymptomatic bacteriuria by isolating the bacterial pathogens and determining the drug susceptibility pattern of urinary tract infections among diabetic patients attending at Tikur Anbessa Specialized University Hospital, Addis Ababa.

1.3.2 Specific Objectives

- To determine the overall prevalence of UTI in diabetic patients.
- To determine the prevalence of symptomatic diabetic UTIs and asymptomatic diabetic UTIs
- To isolate the bacterial pathogens responsible for the UTI.
- To determine drug susceptibility pattern of isolates.
- To identify risk factors associated with urinary tract infections.

II. Methodology

2.1. Study design and study period

A cross-sectional study was conducted in Tikur Anbessa Specialized University Hospital from June to August 2009.

2.2. Study area

The study was conducted at diabetic referral clinic, medical wards and medical ICU in Tikur Anbessa Specialized University Hospital, Addis Ababa, Ethiopia. Tikur Anbessa Specialized University Hospital is the largest tertiary and specialized hospital in the country serving as a referral center for patients through out the country. The hospital has 560 beds and is located at center of the City (Nefas Silk–Lafto Sub city). It receives referred patients from all parts of the country and provides local emergency service.

2.3. Source Population

The study was conducted among all diabetic patients visiting the outpatient departments of the diabetic referral clinics , patients admitted to medical wards and medical ICU.

2.4. Study Subjects

All adult diabetic patients visiting the outpatient departments of diabetic clinic and all adult diabetic patients admitted to medical wards and ICU was included in the study.

2.5. Eligibility and exclusion criteria

Inclusion criteria:

- Diabetic Patients
- Age≥18years

Exclusion criteria:

- Patient's<18 years
- Pregnant women
- Patients which have taken antibiotic recently (two weeks).
- Catheterized patient.

2.6. Definitions

According to CDC definition, symptomatic urinary tract infection (UTI) is defined when a patient has one or more of the following signs or symptoms with no other recognized cause: fever (temperature, $>38^0$C), urgency, frequency, dysuria, or suprapubic tenderness and a urine culture positive for 10^5 or more microorganisms per milliliter and no more than 2 types of microorganisms.Asymptomatic bacteriuria refers to Patients who had at least two positive urine cultures, that is the presence of urine culture positive for 10^5 or more microorganisms per milliliter with repeated isolation of the same microorganisms and no more than two species of microorganisms and patients has no fever($>38^0$C), frequency,dysuria,or suprapubic tenderness (Garner *et al*., 1996).

2.7. Study variables

Dependent variables: UTI, asymptomatic UTI, symptomatic UTI, bacteriuria.

Independent variables: age, sex, educational level, duration of diabetes, antibiotic usage,blood sugar level, type 1 DM, type 2 DM,DM with specific causes.

2.8. Sample size determination

A minimum of 413 samples included for the study based on the following formula:-

Total study subjects: $n_i = \frac{(z_{\alpha/2})^2\, p(1-p)}{d^2}$

$= 375$

10% non-response rate=38

So total sample size = 375+38=413

n_i- Sample size calculated from infinite population

Where P=prevalence of diabetic urinary tract infection 14% (Feleke, 2007).

d=marginal error of 0.035 is used to maximize sample size.

Z $\alpha_{/2}$=A standard Z score1.96 corresponding to 95% confidence level.

2.9. Sampling technique

Systematic sampling technique were employed in this study by taking every other diabetic patient coming to diabetic referral clinics and admitted to medical wards and medical ICU.

2.10. Data Collection

Data on Socio-demographic characteristics and associated risk factors were collected by pre-designed and pre-tested questionnaire by experienced Nurses attending the diabetic patients in diabetic clinic after adequate training given on how to fill the questionnaire. Recent fasting blood sugar and renal function tests were collected from the chart of the patient.

2.10.1. Sample Collection, Handling and Transport

Urine specimens: Two consecutive midstream clean–catch urine samples (30-50ml) were collected from each asymptomatic patient and one midstream clean–catch urine sample collected from each symptomatic patient (30-50ml) into a sterile screw–capped universal container after giving proper guidelines. The urine samples were immediately transported to the microbiology laboratory.

2.11. Laboratory Procedures

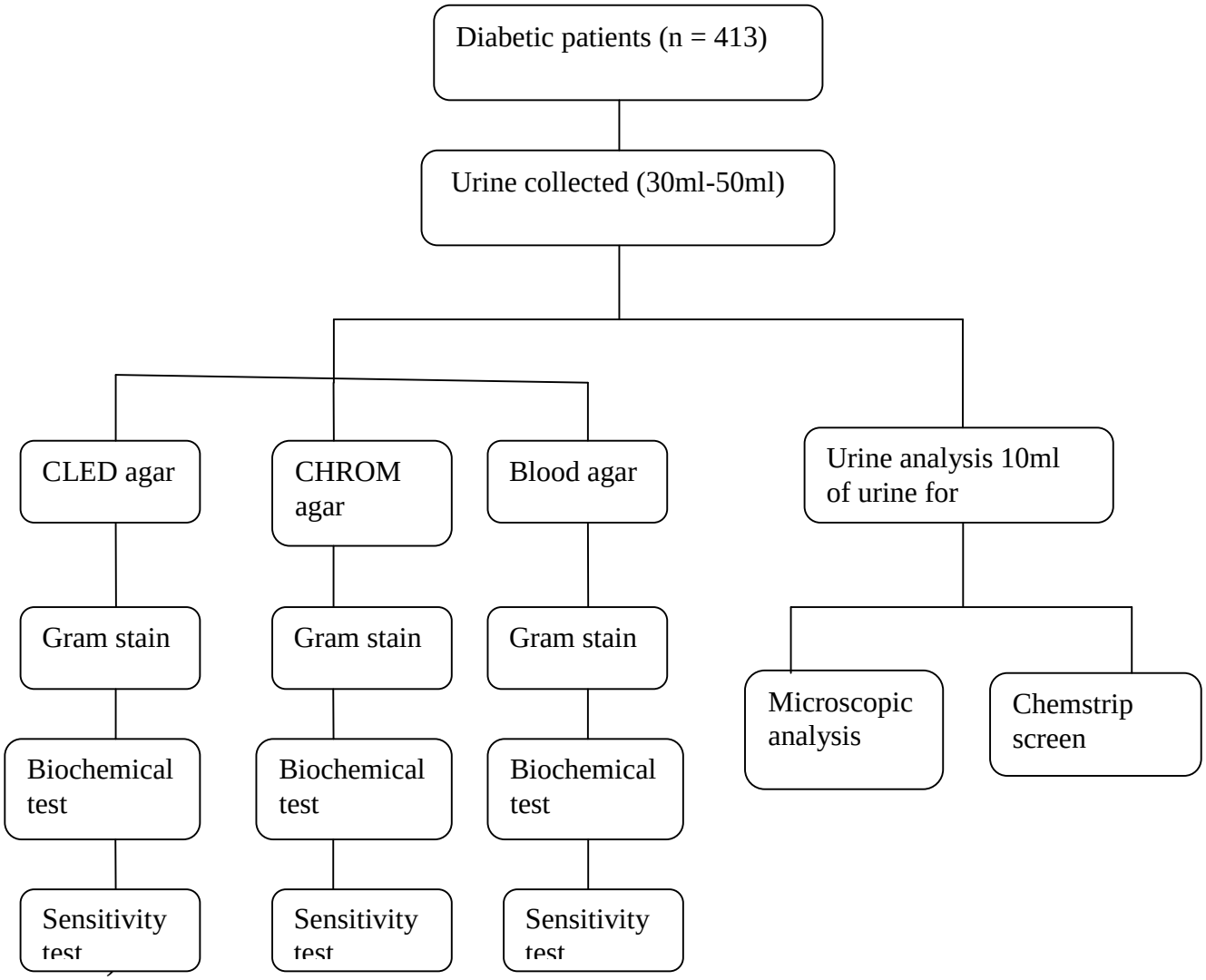

Figure 1. Study Flow Chart for DM patients attending diabetic referral clinics, medical wards and MICU of TASUH, Addis Ababa, Ethiopia (June to August 2009).

2.11.1. Urinanalysis

Albuminuria, Glucosuria, and ketonuria were determined using a dip-slide method (Linear Chemicals SL, Barcelona, Spain). Ten mL of each well-mixed urine sample was centrifuged at 500-1000 gram for 5 minutes. The supernatant was discarded and a drop of the deposit was examined microscopically at high magnification for pus cells, red blood cells, epithelial cells, casts, crystals, and yeast cells (Annex V).

2.11.2. Culture and Identification

A loop-full calibrated loop designed to deliver 0.01 milliliter of well mixed un-centrifuged urine was streaked on to the surface of blood agar (Oxoid Ltd, Basingstoke, Hampshire, UK), Cystine Lactose Electrolyte Deficient (CLED) medium (M6: Plasmatec Laboratories,United Kingdom) and CHROMagar (CHROMagar Company, Paris, France) prepared as per the manufacturer instruction and incubated aerobically at 37^0C. After 24 hours of incubation, the plates were examined for the presence of colonies. The number of colony forming units (CFUs) was multiplied by 100 to determine the number of microorganisms per milliliter in the original specimen (Annex IV).

The use of blood agar is recommended in addition to Cystine Lactose Electrolyte Deficient (CLED) medium (M6: Plasmatec Laboratories, United Kingdom) because it assists in the rapid identification of pathogens which grow poorly on CLED medium. CHROMagar (CHROMagar Company, Paris, France) is used for rapid presumptive identification of bacteria based on unique colors' of colony on this media.

All positive urine cultures were identified by their characteristic appearance on their respective media, gram-staining reaction (Annex III) and confirmed by the pattern of biochemical reactions using the standard method. Members of the family enterobacteriaceae and non- enterobacteriaceae were identified by indole production, H_2S production, citrate utilization, motility test, urease test, oxidase, carbohydrate utilization tests and other tests using API 20E identification kits (Biomerieux, France).

2.11.3. Antimicrobial susceptibility testing

The antimicrobial susceptibility testing of the isolate was performed by the disk diffusion technique as modified by the Clinical and Laboratory Standard Inistititute (CLSI formerly NCCLS, 2007) (Annex VI).

From a pure culture 3-5 selected colonies of bacteria were taken and transferred to a tube containing 5 ml sterile normal saline and mixed gently a homogenous suspension was formed and incubated at 37^{o}C until the turbidity of the suspension become adjusted to a McFarland 0.5.

A sterile cotton swab was used and the excess suspension was removed by gentle pressing and rotation of the swab against the inside wall surface of the tube. The swab was then used to distribute the bacteria evenly over the entire surface of Mueller Hinton agar (Oxoid Ltd, Basingstoke, Hampshire, UK). The inoculated plates were left at room temperature to dry for 3-5 minutes and a set of 14 antibiotic discs.

Fourteen antibiotic disks (Oxoid Ltd, Basingstoke, Hampshire, UK) were then delivered on the surface of the plate. The drugs for disc diffusion testing were in the following concentrations: Ampicillin (AMP) 10µg, Amoxicillin-Clavulanic acid (AMC) 20µg, Ceftazidime (CAZ) 30µg, Ceftriaxone (CRO) 30µg, Ciprofloxacin (CIP) 5µg, Trimethoprim-sulphamethoxazole (SXT) 25µg, Erythromycin (E) 15µg, Gentamicin (CN) 10µg, Methicillin (MET) 5µg, Nalidixic acid (NA) 30µg,Nitrofurantoin (F) 300µg, Norfloxacin (NOR) 10 µg, Tetracycline (TE) 30µg and Vancomycin (VA) 30µg.

The plates were then incubated in aerobic atmosphere at 37^{o}C for 24-48 hours. Diameters of the zone of inhibition around the disc were measured to the nearest millimeter using a graduated caliper in millimeters, and the isolates were classified as sensitive, intermediate, and resistant according to the standardized table supplied by the CLSI (CLSI, 2007). High, intermediate and low level of resistance is defined when the percentage of resistance is >80%, 60-80% and < 60%, respectively.

2.11.4. Reference strains

Escherichia coli (ATCC-25922), *Pseudomonas aeruginosa* (ATCC-27853) and *Staphylococcus aureus* (ATCC-25923) were used as reference strains for quality control throughout the study .All the strains were obtained from EHNRI.

2.12. Data entry and analysis

Clinical data were collected using questionnaire. Laboratory results were registered in a laboratory logbook. Double data entry and statistical analysis were performed using software packages SPSS version 17 to assess differences between variables based on the findings. Prevalence rate was calculated for the sum of the numbers of positive cases of examined subjects. X^2 test was done along with P-value to see the presence of associations. P-value < 0.05 was considered significant. Crude odds ratio was done to determine whether any association exists with a confidence interval of 95%

2.13. Ethical Considerations

The proposal was evaluated and ethically cleared by the Department of Microbiology, Immunology and Parasitology (DMIP) and by the Institutional Review Board (IRB) of Faculty of Medicine, Addis Ababa University. Permission was obtained from Department of Internal Medicine and Endocrinology and Metabolism Units to conduct the study in diabetic center. All the information contained with in the questionnaire was kept confidential. Written informed consent was obtained from all individuals enrolled in the study. Positive culture results forwarded to the treating physician to adjust their treatment based on culture and sensitivity results.

III. RESULTS

3.1 Study subjects

A total of 413 DM patients participated in the study during June to August 2009. Of those 413 DM patients 181 were males and 232 were females showing an overall male to female ratio of 1:1.71. Their ages ranged from 18 to 83 years with a mean of 48.12±14.2 (Figure 2).

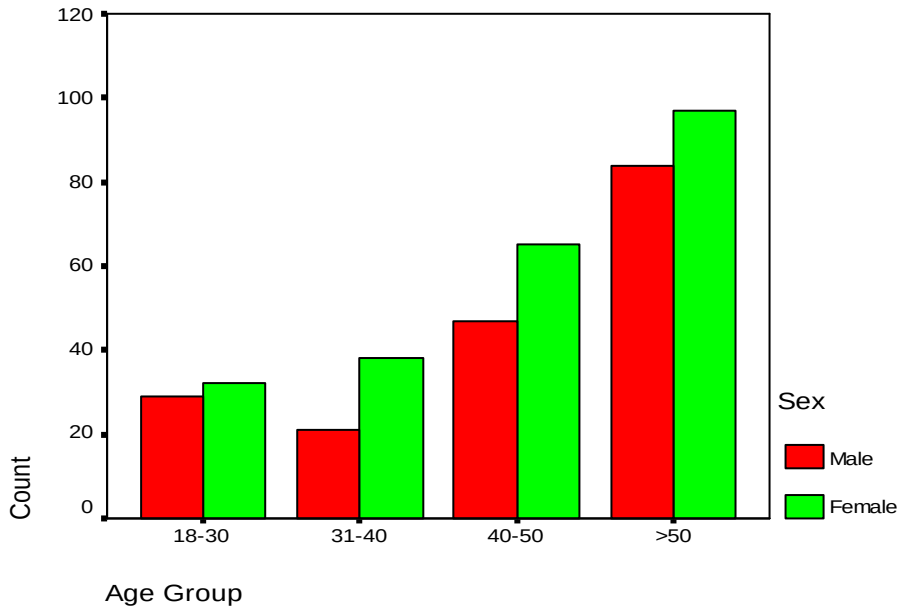

Figure 2. Age and sex distribution of 413 DM patients investigated for bactriuria at Tikur Anbessa Specialized University Hospitals (TASUH) (June to August 2009).

The mean fasting blood glucose level (FBS) in the population studied were 167.9 mg/dl (range 41 mg/dl to 460 mg/dl).Of the 413 patients, 107 (25.9%) had type 1 and 306 (74.1%) type 2 DM. The distribution of DM among rural and urban patients was 49 (11.9%) and 364 (88.1%) respectively. The majority of the patients came from urban. Among 413 patients 402 were from OPD and the remaining 11 were from inpatient department. Regarding their educational status, 74.3% of the study subjects were literates having their education from elementary school to the university, while 25.7% were illiterates.

Among the different symptoms of UTIs flank pain was the most common symptom which accounted 42(10.2%), followed by burning during micturition 36(8.7%). Urinary frequency and urgency accounted 33(8%) (Table 1).

Table 1 Frequency of Signs and Symptoms for UTIs among DM patients attending diabetic referral clinics medical wards and MICU of TASUH, Addis Ababa, Ethiopia (June to August 2009).

Symptom	**Frequency**	**%**
Flank pain	42	10.2
Burning during micturition	36	8.7
Urgency	33	8
Frequency of urine	33	8
Fever	17	4.1
Dysuria	8	1.9

3.2 Urine analysis findings

The urine analysis findings in the 413 DM patients are summarized in (Figure 3). Of the 413 patients, 265 (64.2%) had glucoseuria, 58(14%) had albuminuria, 10(2.4%) had ketoneuria and 8(1.9%) had granular casts. From the urine microscopic examinations, 85(20.6%) had leucocytes, 17(4.1%) had erythrocytes and 7(1.7%) had yeast cell.

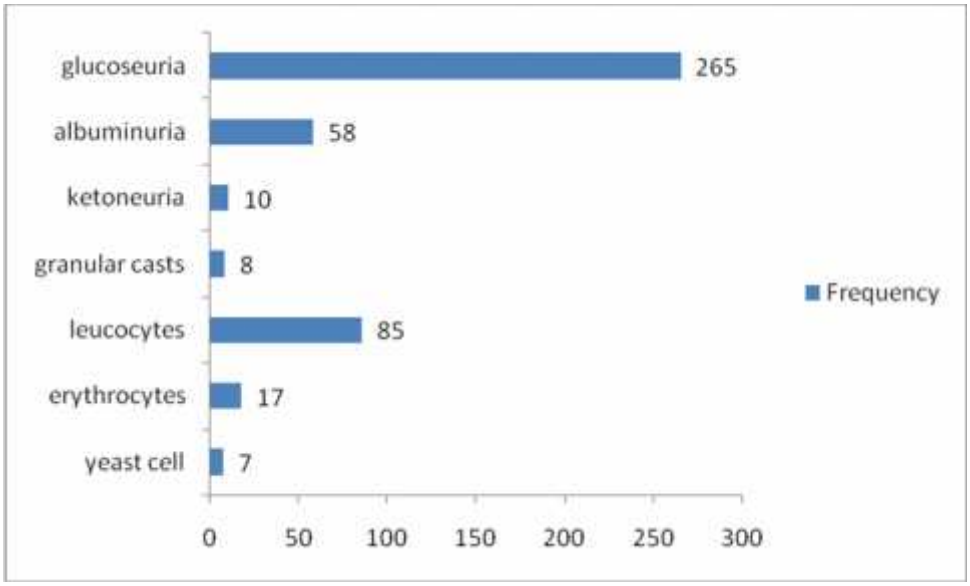

Figure 3. Urinalysis findings study for DM patients attending diabetic referral clinics, medical wards and MICU of TASUH (June to August 2009).

3.3 Etiologic Agents

Nine (13.6%) of the symptomatic DM patients had bacteriuria compared with thirty six (10.4%) of asymptomatic DM patients. The over all prevalence of UTIs in both groups was 45(10.9%) as shown in (Table 2).

Table 2. Prevalence of UTIs among symptomatic and asymptomatic DM patients attending diabetic referral clinics ,medical wards and MICU of TASUH, Addis Ababa, Ethiopia (June to August 2009).

Study Groups	**Significant Bacteriuria (n = 413)**		
	Yes	**No**	**Total**
	Number (%)	**Number (%)**	**Number (%)**
Asymptomatic	36(10.4)	311(89.6)	347(100)
Symptomatic	9(13.6)	57(86.4)	66(100)
Total	45(10.9)	368(89.1)	413(100)

P=0.44Significant bacteriuria: asymptomatic Vs symptomatic, Chi square test (Fisher's exact).

Age was a significant risk factor for DM patient (P<0.05). Among patients with bacteriuria, 6 (3.32%) were males and 39 (16.81%) were females. Among DM patient with out bacteriuria 175(96.68%) were males and 193 (83.19%) were females. Significant relationship was found between bacteruria and gender (P<0.05).

Bacteriuria was prevalent among the DM patients in different years of onset. This study showed that there was no significant association between bacteriuria and the duration of diabetes (P>0.05). Among the 45 patients who had bacteriuria, 22 patients had DM <10 years duration, 18 patients had for 10-20 years of diabetic history, and only 5 had bacteriuria of more than 20 years duration.

Of the 45 patients who had positive urine culture 37(10.42%), had no microalbuminuria. Among 8 (13.79%) DM patients with micro-albuminuria also had bacteriuria (Table 3). No significant relationship was found between bacteriuria and micro-albuminuria (P>0.05).

Thirty seven (11.28%) of the 365 DM patient with normal value of urea (<40mg/dl) had bacteriuria compared to 8/48 (16.67%) of DM patients with abnormal value (>40mg/dl) (P >0.05). Among 307 DM patients with normal value of creatinine (male 0.9 – 1.2 mg/dl, Female 0.5 – 0.9 mg/dl), 31(10.01%) had bacteriuria compare to 14/106 (13.2%) of DM patients with abnormal value of creatinine (P >0.05). This study showed that no evidence of a significant relation among bacteriuria and renal function (Table 3).

Table 3.Variables of the study for DM patients with and without bacteriuria attending diabetic referral clinics, medical wards and MICU of TASUH (June to August 2009).

Variable	**Significant Bacteriuria**		
	Present n = 45 (%)	Absent n = 368 (%)	**P value**
Age(Years)	52±15.1	47±14.1	0.04
Sex			
Male	6(3.3)	175(96.7)	0.001
Female	39(16.8)	193(83.2)	
Types of DM			
Type I	7(6.6)	99(93.6)	0.1
Type II	38(12.4)	269(87.6)	
DM Duration(Years)			
<10	22(15.4)	216(84.6)	0.39
10 – 20	18(7.9)	111(92.1)	
>20	5(20)	41(80)	
Albuminuria			
Positive	8(13.8)	50(86.2)	0.45
Negative	37(10.4)	318(88.6)	
Ketoneuria			
Positive	44(10.9)	359(89.1)	0.93
Negative	1(10)	9(90)	
Blood Glucose control			
Poor	28(9.6)	263(90.4)	0.19
Good	17(17.9)	105(86.1)	
Urea			
Normal	37(11.3)	328(88.7)	0.17
Abnormal	8(16.7)	40(83.3)	
Createinine			
Normal	31(10.1)	276(89.9)	0.38
Abnormal	14(13.2)	92(86.8)	

No significant association between DM patients with poor glycemic control (< 90 or >130) and good glycemic control (90–130 mg/dL) (P>0.05) (Table 3).

Thirty four (18.37%) of the hundred eighty five patients with glucoseuria had bacteriuria compared to 11/228 (4.82%) of DM patients with out glucoseuria. The difference was statistically significant, X2 = 1.44, (P = <0.001). The odd of developing bacteriuria was almost seventeen times in patients with glucoseuria compared with DM with out glucoseuria OR= 16.9 (95% CI =16.48-17.36) (Table 4).

Table 4 Prevalence of bacteriuria in relation to glucoseuria among DM patients attending diabetic referral clinics medical wards and MICU of TASUH (June to August 2009).

Glucoseuria	**Number**	**Presence of bactriuria**	**%**
Positive	185	34	18.37
Negative	228	11	4.82
Total	**413**	**45**	**10.9**

X^2=1.44; P=0.00(Fisher's exact).Odds ratio=16.9(95%CI=16.48-17.36)

3.4 Simultaneous detection of bacteriuria and pyuria

Forty one (48.23%) of DM patients with bacteriuria had pyuria. Forty four (51.77%) of participants with bacteriuria showed no evidence of pyuria. Using urine culture as gold standard, the sensitivity, specificity, positive predictive value and negative predictive values of pyuria in detection of urinary tract infection were 71.1%, 82.8%,35.5%,and 94.4%, respectively (Table 5).

Table 5. Comparison of significant pyuria for the diagnosis of bacteriuria among DM patients attending diabetic referral clinics medical wards and MICU of TASUH (June to August 2009)

Significant Pyuria	**Significant Bacteriuria**		
	Yes	**No**	**Total**
	Number (%)	**Number (%)**	**Number (%)**
Yes	32(48.23)	63(51.77)	90(100)
No	13(1.22)	305(98.78)	323(100)
Total	45(10.9)	368(89.1)	413(100)

P=0.00, Significant pyuria with and without significant bacteriuria: Sensitivity 71.1%; specificity, 82.8%; positive predictive value, 35.5% and negative predictive value, 94.4%.

3.5 Bacterial etiologies of UTI

Bacterial etiologies from 413 urine specimens of DM patients had 46 urinary pathogens in 45 DM patients were isolated showing an isolation rate of 10.9%. The number of isolates was greater than the number of specimens, because in one specimen two types of pathogenic bacteria were isolated. Among these, 10/46(21.74%) were from symptomatic patients and 36/46(78.26%) were from asymptomatic patients (P >0.05).

As shown in table 6, forty four gram-negative and two gram-positive bacteria were isolated from diabetic patients. Of the gram negative isolates, the most common organism was *Escherichia coli* (34) followed by *Klebsiella pneumoniae* (8) in both the groups. Two isolates of *Pseudomonas aeruginosa* and *Enterobacter clocace* were also observed. The only gram positive isolate was *Entrococcus spp*. 2 (0.5%).

Table 6. Distribution of bacterial etiologic agents of urinary tract infections among symptomatic and asymptomatic DM patients attending diabetic referral clinics , medical wards and MICU of Tikur Anbessa Specialized University Hospital (TASUH), (June to August 2009).

Organisms	**Symptomatic**	**Asymptomatic**	**Total**
	No.	**No.**	**No.**
Escherichia coli	6	28	34
Klebsiella pneumoniae	2	6	8
Pseudomonas aeruginosa	-	1	1
Enterobacter clocace	1	-	1
Entrococcus spp	1	1	2
Total	**10**	**36**	**46**

3.6 Antimicrobial susceptibility testing

The susceptibility testing results of the isolates showed that over 85% *Escherichia coli* strains were sensitive to all the antibiotics tested but there was a higher rate of resistance to both ampicillin, tetracycline and Trimethoprim-sulphamethoxazole (64.7%-67.6%) of the isolates. A similar pattern was observed, among the K*lebsiella pneumoniae* isolates, that was sensitive to all the major antibiotics tested and also showed resistance to ampicillin, tetracycline and Trimethoprim-sulphamethoxazole among 75% of the isolates tested .

In general over 80% gram negative isolated were found effective to ciprofloxacin, Amoxicillin-Clavulanic acid, ceftazidime, ceftraxone and norfloxacillin. A large number of isolates (>64%) were resistant to ampicillin, tetracycline and Trimethoprim-sulphamethoxazole (Table 7).

Table 7. Antimicrobial Susceptibility Patterns of Bacteria Isolated from Urinary Tract Infections from DM patients who were attending diabetic referral clinics , medical wards and MICU of TASUH (June to August 2009).

Organisms		**Antimicrobial agents**													
		AMP	**AMC**	**CN**	**CAZ**	**E**	**MET**	**NA**	**F**	**TTC**	**SXT**	**CRO**	**NOR**	**CIP**	**VAN**
Escherichia coli (n=34)	S*	11	30	27	29	ND	ND	23	32	10	12	30	29	29	ND
	I*	-	1	-	2	ND	ND	1	-	2	-	2	-	-	ND
	R*	23	3	7	3	ND	ND	10	2	22	22	2	5	5	ND
Klebsiella pmeumoniae (n=8)	S	2	6	7	8	ND	ND	4	2	2	2	8	6	8	ND
	I	-	1	-	-	ND	ND	1	-	-	-	-	1	-	ND
	R	6	1	1	-	ND	ND	3	6	6	6	-	1	-	ND
Pseudomonas aeruginosa (n=1)	S	1	1	1	-	ND	ND	1	1	-	-	1	1	1	ND
	I	-	-	-	1	ND	ND	-	-	-	-	-	-	-	ND
	R	-	-	-	-	ND	ND	-	-	1	1	-	-	-	ND
Enterobacter cloacae (n=1)	S	-	-	-	-	ND	ND	-	-	-	-	1	-	-	ND
	I	-	-	-	-	ND	ND	1	1	-	-	-	1	1	ND
	R	1	1	1	1	ND	ND	-	-	1	1	-	-	1	ND
Entrococcus spp.(n=2)	S	1	1	-	1	2	1	1	2	-	2	1	1	1	2
	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	R	1	1	2	1	-	1	1	-	2	-	1	1	1	-
Total (n=46) **	S	32.6%	82.6 %	76.1 %	82.6 %	100 %	50 %	65.2 %	80.4 %	26.1 %	35 %	89.1%	80.4%	84.8 %	100 %
	I	-	4.4%	2.2 %	6.5 %	-	-	4.4 %	2.2 %	4.4 %	-	4.4 %	4.4 %	2.2 %	-
	R	67.4%	13%	21.7 %	10.9 %	-	50 %	30.4 %	17.4 %	69.5 %	65.2%	6.5 %	15.2%	13 %	-

ND=Not Done *S= Sensitive *I=Intermediate *R=Resistant ** Expressed in percent.

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

The frequency of multiple antimicrobial resistances to two or more drugs of the urinary pathogens were found in thirty three (71.7%) of the isolates. While 13% of bacteria were resistant to one antibiotic, only 15.2% were found to be sensitive to all antibiotics tested (Table 8).

Table 8. The frequency of multiple antimicrobial resistance of bacteriuria from DM patients attending diabetic referral clinics, medical wards and MICU of TASUH, Addis Ababa, Ethiopia (June to August 2009)

	Antibiogram pattern									
Organisms	No. of Isolates	R0 No. (%)	R1 No. (%)	R2 N0. (%)	R3 No. (%)	R4 No. (%)	R5 N0. (%)	R6 No. (%)	R7 No. (%)	R8 No. (%)
E. coli	34	5 (14.7)	6 (17.6)	6 (17.6)	7 (20.6)	2 (5.9)	3 (8.8)	1 (2.9)	2 (5.9)	2 (5.9)
K.pneumoniae	8	2 (25)	-	-	2 (25)	3 (37.5)	-	-	1 (12.5)	-
P.aeruginosa	1	-	-	-	1 (100)	-	-	-	-	-
E.clocace	1	-	-	-	-	-	-	1 (100)	-	-
Entrococucs spp.	2	-	-	-	-	1 (50)	-	-	-	1 (50)
	46	**7 (15.2)**	**6 (13)**	**6 (13)**	**10 (21.7)**	**6 (13)**	**3 (6.5)**	**2 (4.3)**	**(6.5)**	**3 (6.5)**

R0= No Resistance to antibiotic
R1=Resistance to one antibiotic
R2=Resistance to two antibiotic
R3=Resistance to three antibiotic
R4=Resistance to four antibiotic
R5=Resistance to five antibiotic
R6=Resistance to six antibiotic
R7=Resistance to seven antibiotic
R8=Resistance to eight antibiotic

IV. Discussion

Diabetic patients are at high risk of developing UTIs, so that special attention must be given to prevent complications. Successful management of patients with bacterial urinary tract infection depends on the identification and types of organisms that cause a disease and the selection of an effective antibiotic against the organism (Water *et al*, 1979).

The results of this study have shown that the overall prevalence of bacteriuria was (10.9%). Significant bacteriuria was detected in 9 (13.6%) of the symptomatic and 36 (10.4%) of asymptomatic DM patients (P>0.05) (Table 2).The prevalence of urinary tract infection (UTIs) agree with the previous study conducted in Ethiopia (14%) (Feleke *et al.,* 2007) among symptomatic bacteriuria and among asymptomatic bacteriuria conducted in Kenya (11.1%) (Kayima *et al.,* 1996), and in Iran (10.9%) (Mohammad *et al.,* 2006). In contrast, higher prevalence bacteriuria have been reported in Nigeria (26.6%) by Alebiosu *et al*. (2003), (21%), by Adeyba *et al*. (2007), in Iran (20%) by Zamanzad and Moezzi (2006) and in Netherlands (26.6%) by Greelings *et al.* (2000). The higher figures recorded in the above studies could be explained in that the studies based on the diagnosis of asymptomatic bacteriuria with one urine culture, as against two urine cultures in the present study; the later decreased the observed prevalence of bacteriuria.

Various risk factors for bacteriuria among diabetic patients such as age, sex, duration of diabetics, types of diabetes, nephropathy, glucoseuria and albuminuria were investigated. This study showed that age was a risk factor for bacteriuria in DM patients. The average age in diabetic patients with UTI was higher than patients without UTI. This finding is in line with that of other studies conducted in Nigeria (Odetoyin *et al*., 2008), in Iran (Zamanzad and Moezzi, 2006), in India (Janifer *et al.,* 2009) and in Netherlands (Greelings *et al,* 2000), but in other study conducted in Iran (Mohamed *et al.,* 2006) age had not significant relation with asymptomatic bacteriuria. The higher prevalence of UTIs in female than male is also in agreement with previous findings done in Ethiopia (Wolday and Worku, 1997; Messele, 1983; Moges *et al*., 2002; Tessema *et al*., 2007), in Africa (Kayima *et al.,* 1996; Alebiosu *et al,* 2003; Adeyba *et al*., 2007) and in India (Goswami *et al.,* 2001; Janifer *et al*., 2009). The higher incidence of UTI in women could be due to

their shorter and wider urethra as well as its proximity to anatomical condition predisposing for ascending infection by organisms colonizing the perianal area (Willett and Radojcic, 1976, Wheat, 1980).

Further more our study showed that significant bacteriuria was more likely to occur in patient with glucoseuria than patients without glucoseuria. Higher glucose concentration in urine may serve as a culture medium for pathogenic microorganisms. This result was comparable with pervious result reported from Iran (Mohammed *et al.,* 2006). Some studies found microalbumiuria as a risk factor for developing bacteriuria (Greelings *et al.,* 2000; Greelings *et al.,* 2001). However our study did not confirm microalbuminuria as possible association and it agrees with a study conducted in Nigeria (Odetoyin *et al*., 2008), in Iran (Mohamed *et al.,* 2006) and in Netherlands (Suzane E., *et al.,* 2000).

We could not observe any relationship between type of diabetes and presence of bacteriuria in the present group of diabetic individuals. In the present study, duration of DM is not associated with bacteriuria. Even though some studies have shown that a longer duration of diabetes increases the risk of developing bacteriuria (Alebiosu *et al*, 2003)in Nigeria, (Jannifer *et al*., 2009) in India, while others could not confirm this notion (Odetoyin *et al*., 2008) in Nigeria, (Mohammad *et al*., 2006, Zamanzad and Moezzi, 2006) in Iran.

In this study, significant pyuria was observed in 41(48.23%) of DM patients with significant bacteriuria ($p<0.001$). Using urine culture as "Gold Standard" the sensitivity, specificity, positive predictive value and negative predictive values of pyuria in detection of urinary tract infection were 91.1%, 88%,48.2%, and 73.2%, respectively (Table 5). Similar findings have been reported in Ethiopia (Awole, 2001; Assefa *et al.*, 2008). Pyuria and hematuria are good indicators of an inflammatory response. The absence of pyuria should cause the diagnosis of UTI to be questioned until urine culture data are available. Conversely many disease of the urinary tract produce significant pyuria in the absence of bacteriurua. Tuberculosis is the well recognized example of abacterial pyuria, Staghorn calculi and stones of smaller size can produce intense pyuria with clumps of

White Blood Cells(WBCs) in the absence of UTI (Anthony and Edward, 2007; Greenwood *et al.,* 2007).

The simultaneous test to detect of bactriuria and pyuria, when used together, perform better than either test performs when used alone. In addition, combined tests have low sensitivity, high specificity, low positive predictive values and high negative predictive values. Taken together, the performance characteristics of these tests make them useful as a way to rule out bacteriuria on the basis of a negative test result (Wilson and Gadio, 2004).

The result also indicated that *Escherichia coli*, 34/46 (73.9%) was the most common bacterial pathogens isolated in both symptomatic and asymptomatic DM patients. *Klebsiella pneumoniae* was the second most frequently isolated urinary pathogens. The result from the present study showed that the etiologic pathogens of UTIs mainly belong to gram negative enteric microorganisms. This agrees well with the previous study conducted in Ethiopia (Wolday and Erge, 1997, Gedebou, 1983; Tessema *et al*., 2007; Feleke *et al.*, 2007; Assefa *et al.*, 2008), in Nigeria (Adeyba *et al*., 2007) in Kenya (Kayima *et al*, 1996), in Iran (Mohammed *et al.*, 2006), in India (Goswami *et al.*, 2001, Janifer *et al*., 2009), and in Netherlands (Suzane E., et al., 2000). In few studies, the microorganism was different. For example *Klebsiella* was the most common isolate in a study by Alebiosu *et al.* (2003) and staphylococcus was the most common isolates in a study by Odetoyin *et al.* (2008).

Mixed infections are more likely to occur in patients with underlying disorders that interfere with free urine flow and is also frequent in patients with indwelling catheter (Onifade *et al.,* 1992; Welday and Erge, 1997). In this study two bacteria were isolated from one symptomatic DM patients with prostatitis.

In this study, the susceptibility patterns of 46 bacteriuria strains isolated from DM patient against fourteen chosen antimicrobial agents were studied (Table 7). 80% of the gram negative isolates were sensitive to Amoxicillin-Clavulanic acid, ceftazidime, nitrofuantoin, ceftiraxone, norfloxacin, and nalidixic acid.

The sensitivity pattern of ciprofloxacin and nitrofuratoin were the highest. This observation is comparable to that of other researchers reported in Ethiopia (Wolday and Erge, 1997; Moges et *al*., 2002), in Nigeria (Alebiosu *et al.*, 2003; Mohammed *et al.,* 2006) and in Kenya (Kayima *et al*, 1996). The sensitivity pattern of ciprofloxacin (84.8%) may be explained by the fact that resistance is by chromosomal mutation rather by acquisition of plasmids (Volk et *al.,* 1996).In this study nitrofurantoin was highly effective over 80% of all isolates. The implication of this finding is that nitrofuratoin, though an old drug, it is still effective in the treatment of urinary tract infection. Thus, this drug could be included as a first choice of alternative in the empirical treatment of UTI in DM patients.

Amoxicillin-Clavulanic acid and ceftazidime were effective antimicrobial agents against (82.6%) of the urinary pathogens. Effectiveness of Amoxicillin-Clavulanic acid may be due to the activity of clavulanic acid. Clavulanic acid is strong inhibitors of bacterial beta-lactamases and can protect beta-lactam antibiotics from hydrolysis by these enzymes. The isolates were, highly sensitive to ceftiraxone 89.1% but require parenteral administration and, therefore, will not be suitable for treating outpatients.

Remarkably over (60%) of all isolates were resistances to ampicillin, tetracycline and Trimethoprim-sulphamethoxazole have been found in this study, which is comparable to other findings reported in Ethiopia (Gedebou 1983, Wolday and Erge, 1997; Moges *et al.*, 2002), and (Alebiosu *et al.,* 2003) in Nigeria. This may be due to the fact that many antibiotics are easily available for self medication and being used indiscriminately.

More than (70%) of urinary pathogens were resistance to two or more drugs. Though the number of isolates was low, multiple resistances were high in 33/46(71.7%). Since all isolates showed resistance against more than three combined antimicrobial agents. This is higher as the previous study done in Ethiopia by Moges *et al*. (2002), which showed 68% of the isolated bacteria from urine showed resistance for two to nine antimicrobials, but comparable to other studies done in Ethiopia (Assefa *et al.,* 2008; Tessema *et al*., 2007).This high drug resistance pattern may be a wide spread misuse of antibiotics which can cause increased prevalence of resistant organisms in a community.

LIMITATIONS OF THE STUDY

- The design of the study did not include fungi and other pathogens that are important causes of urinary tract infections due to budget constraints.

CONCLUSION

The prevalence of urinary tract infection in this study is high in women than men and also pyuria and glucosuria can be considered as strong association with bacteriuria. *Escherichia coli* was the most common bacterial pathogen isolated in both symptomatic and asymptomatic bacteriuria. Remarkably over (60%) of all isolates were resistances to ampicillin, tetracycline and Trimethoprim-sulphamethoxazole have been found in this study. Therefore, the investigation of bacteriuria in diabetic patients by screening for urinary tract infection is very important as it enables to properly treat and prevent the development of renal complications and eventually severe renal damage and failure.

RECOMMENDATIONS

Based on these findings the following recommendations are made: -

- The prevalence and drug susceptibility pattern of urinary tract infections should be done by including fungus and other micro-organism in Tikur Anbessa Specialized University hospital.
- Empirical treatment to urinary tract infections provoke drug resistance, therefore treatment should be based on the result of culture and sensitivity. In order to achieve this, the capacity of microbiology laboratory should be strengthened with trained manpower, budget and necessary laboratory equipments.
- There is a need for a continuous surveillance for resistant bacteria to provide the basis of alternative treatment.
- This study suggests that if one could not wait the culture results in DM UTI, Ampicillin, Amoxicillin, Tetracycline and Trimethoprim- sulphamethoxazole, are quite ineffective to treat these infections.

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3.8. ANNEX

ANNEX I

QUESTIONNAIRE:

A DATA COLLECTION FORM

ADDIS ABABA UNIVERSITY FACULTY OF MEDICINE

DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY

AND PARASITOLOGY

QUESTIONNAIRES: Administered for investigation to determine prevalence of Symptomatic and asymptomatic bacteriuria and drug susceptibility pattern of urinary tract infections among diabetic patients.

I. Patient identification

Date of data collection.....

Name..... Age..... Sex.....Address…Urban ☐ Rural ☐

Card N^{o}. ……...Code No ……OPD……Ward……..Bed No……

Date of Hospitalization……

Educational Level: Illiterate ☐ Elementary ☐ High school ☐ College ☐ University ☐

Type of diabetic mellitus: Type I ☐ Type II ☐

Other specific types…….

Duration of diabetes ……(wks/months/years)

II. Clinical data

Do you have the following symptoms of UTI?

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

Urgency ☐ ☐

Burning during micturition ☐ ☐

Flank pain ☐ ☐

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

	YES	NO
Recent antibiotic use less than one month?	☐	☐
If yes specify……..……..		
Are you taking antibiotics currently?	☐	☐
If yes specify……		

Reason of taking antibiotics (Diagnosis)…….

III. Laboratory data

Current FBS value……..……..mg/dl

Recent Urea……..……...mg/dl

Recent Createnine…….mg/dl

LABORATORY FORMAT

URINALYSIS

Color_____

Appearance_____

CHEMSTRIP SCREEN

PH_____

Glucose +1 +2 +3 +4

Albumin +1 +2 +3 +4

Ketone +1 +2 +3 +4

MICROSCOPIC ANALYSIS

WBC_____/HPF

RBC_____/HPF

Casts_____/LPF

Others

CULTURE RESULT

Organism obtained……

Susceptibility Test

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

*S= Sensitive *I=Intermediate *R=Resistant

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

Name of principal investigator _____

Signature _____ Date _____

Annex. II

GENERAL INFORMATION FOR THE STUDY PARTICIPANTS

Date..... Patient code----------

Greetings:

Hello, how are you?

My name is ----------. I am currently a student in the Microbiology, Immunology and Parasitology, who is now going to conduct a cross sectional study on diabetic patient. The objective of this study is to determine the prevalence of Symptomatic and asymptomatic bacteriuria to identify bacterial pathogen and drug susceptibility pattern of urinary tract infections among diabetic patients, Complications in urinary tract infections cause devastating consequences in the socio-economics of our community in general and the patients in particular. Therefore, here we request your kindly participation in this study, which requires your willingness to give urine samples for laboratory examination, to respond to an interview. Your cooperation and willingness is very helpful in identifying the problems related to the issue. I assure you that this study doesn't have any risk and all information that you give will be kept strictly confidential. If we identify the pathogens, we will inform to your physician to adjust antibiotic treatment for you. Your participation is voluntary and you are not obliged to answer any question you do not wish to answer and non-participation will not affect service provided to you. If you are not comfortable please feel free to stop it at any level of the study. I appreciate your cooperation greatly.

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

Department of Microbiology, Immunology and Parasitology, AAU.

TEL. 0912018707 E-mail: biruk_23@yahoo.com

Addis Ababa University Medical Faculty, Institutional Review Board

TEL. 0115538734 E-mail: aaumfIRB@yahoo.com

አጠቃላይ መረጃ

ቀን---------- የደንበኛው መለያ ቁጥር----------

ስሜ ---------- ባላለሁ ። በአሁኑ ወቅት በአዲስ አበባ ዩኒቨርስቲ ሕክምና ፋክልቲ የማይክሮባዮሎጂ ትምህርት ክፍል የመጨረሻ ዓመት የሁለተኛ ድግሪ ተማሪ ነኝ። የመመረቂያ የምርምር ፅሁፌን ለመፃፍ ጥናት በማድረግ ላይ ነኝ። በሽንት ቧንቧ ኢንፌክሽን አምጪ ተህዋያን የስኳር ሕመማን ለተለያዩ ችግሮች ሲዳረጉ ይ ያል ። የምርምሬም ርዕሰ ጉዳይ በጥቁር አንበሣ ሆስፒ ል የስኳር ሕመማን በሽንት ቧንቧ ኢንፌክሽን ምልክት የሚያሳዩትንና የማያሳዩትን በየትኛው ተህዋስያን አንደተጠቁ ለይቶ ማውጣትና ተህዋስያኑ በየትኛው መድ ኒት ሊጠፉ ንደሚችል ለማመላከት ሲሆን ይህም ለሃኪሙ ህመማንን ለማከም የሚያግዝ ሲሆን በተጨማሪም ተያያዥነት ያላቸውን ችግሮች ማወቅ ና የመፍትሔ ርምጃ ንዲወሰድ ለማመላከት ነው።

በመሆኑም ለዚህ ምርምር ይጠቅመኝ ዘንድ የሽንት ምርመራ በማድረግ ና የቀረቡትን መጠይቆች በመመለስ ንዲተባበሩኝ በአክብሮት ጠይቆ ለሁ ። ይህ ጥናት ምንም ጉዳት የማያስከትል መሆኑንና የሚሠጡት ነፃ አስተያየትዎ በሚስጥር የሚያዝና ለማንም የማይገለፅ መሆኑን አረጋግጥልዎ ለሁ ። መጠይቆቹን ለመመለስ ፈቃደኛ ካልሆኑ ወይም ካልተመቸዎት ንዲመልሱ አይገደዱም ። ጥናቱ በማንኛውም ሁኔ ካልተመቸዎት በማንኛውም የጥናቱ ደረጃ ማቋረጥ ይችላሉ ።በጥናቱ ባለመሳተፍዎ በሚያገኙት የህክምና አገልግሎት ላይ የሚደርስቦት ችግር የለም። ጊዜዎትን መስዋዕት አድርገው ስለተባበሩኝ ከልብ አመሰግናለሁ ።

ይህንን ጥናት አስመልክቶ ጥያቄ ካለዎት ወይንም የጥናቱም የመጨረሻ ውጤት ለማወቅ ከፈለጉ በሚከተለው አድራሻ ልያገኙኝ ይችላሉ ።

አዲስ አበባ ዩኒቨርስቲ ህክምና ፋኩልቲ ማይክሮባዮሎጂ ፡ ኢሚዩኖሎጂና ፓራሲቶሎጂ ትምህርት ክፍል የስልክ ቁጥር 0912 01 87 07 ኢሜይል፡ biruk_23@yahoo.com

አዲስ አበባ ዩኒቨርስቲ ህክምና ፋኩልቲ ኢንስቲትዩሽናል ሪቪው ቦርድ

የስልክ ቁጥር 0115538734 ኢሜይል፡ aaumfIRB@yahoo.com

CONSENT FORM

For adult patients who are able to respond:

I _____, after being fully informed about the detail of this study, hereby give my consent to participate in this study as the doctors find best for me.

Signature_____ Date_____

For families or attendants of patients unable to respond:

I_____ parent/guardian/attendant, after being fully informed about the purpose of this study, here by give my consent on the patient's participation in this study as the doctors find best for the patient.

Signature: _____ Date_____

<u>የስምምነት መጠየቂያ ቅጽ</u>

በዚህ ጥናት ለሚዳሰሱ ጥናቶች *ሐሳባቸዉን መግለጽ ለሚችሉ*

ኔ _____, የዚህን ጥናት ዓላማ በግልፅ ስለተረዳሁ በጥናቱ ለመሳተፍና ሐኪሙ ያመነበትን አካሄድ ለመከተል መስማማቴን በፊርማዬ አረጋግጣለሁ፡፡

ፊርማ _____ ቀን _____

ሐሳባቸዉን መግለጽ ለማይችሉ

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Annex III

Laboratory procedure for Gram staining technique:

Gram staining technique

1. Labeling the slides clearly with the date and patient's name and 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 (methanol).

5. Cover the fixed smear with crystal violet stain for 30-60 seconds.

6. Rapidly wash off the stain with clean water. If the tap water is not clean, use filtered water or clean boiled rainwater.

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 objectives 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

- Gram-positive bacteria ----------dark purple
- Gram-negative bacteria ----------pale to dark red (Cheesbrough, 2006).

Annex IV

Laboratory procedure for collection and culturing of urine sample:

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

Clean catch urine specimen

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

- The patient should urinate a small amount and this is discarded.
- The urine that comes next, the mid-stream specimen, should be collected into a sterile container of 30 to 50ml.
- After obtaining the specimen the patient continues to urinate and this is discarded

Blood agar, CHROMagar and Cystine Lysine Electrolyte Deficient (CLED) agar: Mix the urine well by inverting the container several times. Using a sterile calibrated wire loop, inoculate a loopful of urine on blood agar, CHROMagar-orientation and CLED agar.

The loop must be held vertical and only the loop must be dipped in the urine. If the stem is also immersed, more than the required volume of urine will be inoculated.

Incubate the inoculated plates aerobically at 35-37 ^{o}C over night.

Estimating bacterial numbers:-It is necessary to estimate the approximate number of bacteria in urine because normal specimens may contain small numbers of contaminating organisms, usually less than 10,000(10^4) per ml of urine. Urine from a person with an untreated urinary infection usually contains 100,000(10^5) or more bacteria per ml (Cheesbrough, 2006).

Annex V

Urinalysis

- Collect specimen in clean, well-rinsed container, free of detergents.
- Use only well mixed, non centrifuged urine which should not be older than four hours
- Do not touch test areas of the reagent strip
- Immerse the test strip in the urine (approximately two second), remove excess urine from the strip
- Compare the reagent areas on the strip with the corresponding color chart on the container 60 seconds after immersion (Cheesbrough, 2006).

Examination of a wet preparation

- Aseptically transfer about 10ml of well mixed urine to a labeled conical tube
- Centrifuge at 500-1000 g for five minutes. Pour supernatant fluid
- Remix the sediment, transfer one drop of the well mixed sediment to a slide and cover with cover slide
- Examine the preparation microscopically using 10x and 40x objective with the condenser iris closed sufficiently to give good contrast

Pyuria-Significant pyuria was determined according to the presence of >=10 leucocytes per HPF (Cheesbrough, 2006).

Annex VI

Laboratory procedure for disc diffusion sensitivity testing:

1. Emulsify several colonies of similar appearance of test organism a small volume of sterile peptone water, nutrient broth, or quarter strength ringer solution.
2. Match the turbidity of the suspension against the turbidity standard which has a similar appearance to an over night broth culture. When comparing turbidity it is easier to view against a printed card or sheet of paper.

Note: If preferred, the test organism can be subculture in sterile peptone water or nutrient broth to give a growth, which matches that of the turbidity standard.

3. Using a sterile loop of about 4mm diameter, apply a loopful of the test organism suspension (subculture) to the center of the sensitivity testing plate use a sterile dry cotton wool swab to spread the inoculums' evenly across the third of the plate.
4. Using a similar inoculation technique, inoculate an over night broth culture of the control organism evenly across the upper and lower third of the plate, leaving a distance of no more than 5 mm on each side of the test organism.
5. Allow the inoculums' to dry for a few minutes with the Petri dish lid in place.
6. Using sterile forceps or a needle mounted in a holder, place the antimicrobial discs (previously warmed to room temperature) between the test and control inoculums.
7. With in 30 minutes of applying the disks incubate the plate aerobically at 35-37^{o}C over night.
8. Read the tests after checking that the bacterial growth of the test and control organism is neither too heavy nor too light and the control inhibition zone, from the edge of the disc to the edge of the zone the end point of inhibition is growth starts.

Interpretation of results

Report the reaction of the test organism to each antibiotic as 'sensitivity', 'intermediate', or 'resistant', as follows:

Sensitivity (S): Zone of radius is wider than, equal to, or not more than 3mm smaller than the control.

A pathogen reported as sensitivity suggests that the infection it has caused is likely to respond to treatment of the drug to which it is susceptible is used in normal recommended dose.

Intermediate (I): Zone radius is more than 3mm smaller than the control but not less than 3mm.

A pathogen reported as being intermediately sensitive suggests that the infection it has caused is likely to respond to treatment if the drug to which it is susceptible is used in larger doses than normal.

Resistant (R): No zone of inhibition or zone radius measure 2mm or less.

A pathogen reported as resistant implies that the infection it has caused will not respond to treatment with the drug to which it is resistant irrespective of dose or site (CLSI, 2007).

DECLARATION

I, the undersigned, declare that this MSc. thesis is my original work, has not been presented for a degree in Addis Ababa University or any other universities. I also declare that all sources of materials used for the thesis have been duly acknowledged.

Name of the candidate _____

Signature _____

Date of submission _____

Name of advisor _____

Signature _____

Name of advisor _____

Signature _____