

**DETERMINATION OF URINARY TRACT INFECTIONS AND
ANTIBIOTIC SENSITIVITY AMONG NON-INSULIN DEPENDENT
DIABETES MELLITUS PATIENTS VISITING KISII TEACHING AND
REFERRAL HOSPITAL, KENYA**

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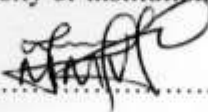
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**A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF
SCIENCE IN INFECTIOUS DISEASES (BACTERIOLOGY) IN THE
SCHOOL OF MEDICINE OF KENYATTA UNIVERSITY.**

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University or institution.


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DEDICATION

I dedicate this work to my loving wife Emily and our sons Joel and Jeremy. For your patience and love during this worth course, God bless you!

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ACRONYMS AND ABBREVIATIONS

| | |
|----------------|--|
| ASB | Asymptomatic Bacteriuria |
| BMI | Body Mass Index |
| CFU | Colony Forming Units |
| CLED | Cystine Lactose Electrolyte Deficient |
| CUP | Chaperone-usher Pathway |
| CVD | Cardiovascular Diseases |
| DM | Diabetes Mellitus |
| Ebp | Enterococcal biofilm-associated pili |
| GIT | Gastrointestinal Tract |
| HlyA | α -haemolysin |
| IBC | Intracellular Bacterial Community |
| IDF | International Diabetes Federation |
| KNH | Kenyatta National Hospital |
| KTRH | Kisii Teaching and Referral Hospital |
| KUERC | Kenyatta University Ethical Review Committee |
| MDR | Multi-drug Resistant |
| ME | Margin of Error |
| MHA | Mueller Hinton agar |
| NACOSTI | National Commission for Science, Technology and Information |
| NCD | Non-Communicable Diseases |
| NIDDM | Non-insulin Dependent Diabetes Mellitus |
| PMF | Proteus mirabilis-like Fimbriae |
| PMN | Polymorphonuclear |
| Pta | Proteus toxin agglutinin |
| SPSS | Statistical Package for Social Science |

| | |
|------------|---------------------------|
| SSA | Sub-Saharan Africa |
| UAE | United Arab Emirates |
| UK | United Kingdom |
| USA | United States of America |
| UTI | Urinary Tract Infection |
| UV | Ultra Violet |
| WHO | World Health Organization |

ABSTRACT

People with non-insulin dependent diabetes mellitus have been found to be prone to urinary tract infections. There is a wide gap of information in developing countries regarding the prevalence and antibiotic sensitivity of the pathogens causing urinary tract infections in diabetic patients. Developed nations also face a relatively huge burden of managing urinary tract infections among non-insulin dependent diabetics. The objective of this study was to determine the prevalence and antibiotic sensitivity of bacteria causing urinary tract infections among non-insulin dependent diabetic patients as well as determining the bacterial causative agents of urinary tract infections through biochemical confirmatory tests. The study was carried out in Kisii Teaching and Referral Hospital in Kisii County, Kenya. One hundred and eighty diabetic patients were enrolled in a cross-sectional study design. Clean catch mid-stream urine was collected from all participants and cultured in cysteine lactose electrolyte deficient agar for bacteria isolation. The isolates were later cultured in Mueller Hinton for antibiotic sensitivity testing. Classification of a positive culture for urinary tract infection was based on more than 100,000 ($\geq 10^5$) colony-forming units of a single bacterial species. All the data was extracted and analyzed with the statistical package for the social science (SPSS) version 20. The data was analyzed using frequencies, chi square ($P < 0.05$) and logic regression to find the odds ratio. One hundred and seven participants were male (59.4 %) and 73 (40.6 %) were female. Sixty-three participants (35 %) showed symptoms of urinary tract infections. The overall prevalence of urinary tract infections was 20.6 % with 37 participants testing positive for urinary tract infection. The duration of diabetes mellitus was revealed to be significant ($p = 0.01$) while age was also found to have a significant association with urinary tract infection ($p = 0.002$). Out of the 37 (100 %) isolates, 35 (94.6 %) were gram negative and the remaining 2 (5.4 %) were gram positive. *Escherichia coli* was the most predominant isolate with 21 (56.8 %) isolates followed by 10 (27 %) isolates of *Klebsiella pneumoniae* and then 4 (10.8 %) isolates of *Proteus mirabilis*. There were two (5.4 %) isolates of *Enterococcus faecalis*. Out of the 21 *E. coli* isolates, five isolates showed resistance to ampicillin, three isolates were resistant to nitrofurantoin and three isolates were resistant to co-trimoxazole. Out of 10 *K. pneumoniae* isolates, two were resistant to ampicillin, one was resistant to cephalixin and two were resistant to co-trimoxazole. Out of the four *P. mirabilis* isolates, there were three cases where one strain was each resistant to ampicillin, nitrofurantoin and co-trimoxazole. All 21 isolates of *E. coli* (100 %) were sensitive to gentamicin and cephalixin. All ten *K. pneumoniae* isolates (100 %) were sensitive to gentamicin and nitrofurantoin. These findings suggest an increasing antibiotic resistance among pathogenic causative agents of UTI among non-insulin dependent diabetic patients. A longitudinal study is recommended with a higher number of participants, in order to understand the risk factors of urinary tract infection among diabetic patients.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the study

Non-insulin dependent diabetes mellitus (NIDDM) is a disorder that is characterized by varying degrees of insulin resistance, high blood glucose levels and defective insulin secretion. Due to impairment of the immune system because of decreased cellular responses, urinary tract infections (UTI) are commonly found in patients with non-insulin dependent diabetes mellitus. People suffering from NIDDM are prone to contracting UTI since their weakened immune systems perform poorly in elimination of pathogens that cause UTI (Odegaard and Chawla, 2012). Poor metabolic control has also been found to be another reason why patients with NIDDM are prone to UTI (Hamdan *et al.*, 2015). High glucose production in urine encourages the growth of pathogenic bacteria that cause UTI in this category of patients. Considering the already weak immune system of the patients, the pathogens that cause UTI are able to thrive and colonize the urinary tracts. Autonomic neuropathy of the urinary tract hampers the complete emptying of the bladder. Dysfunctional voiding and retention of urine in the bladder reduces the amount of bacteria that is physically eliminated from the urinary tract through micturition (Rubin, 2006).

Lifestyle trends are fast changing and this has resulted in many cases of diabetes being recorded in young adults (Deshpande, Harris-Hayes and Schootman, 2008). The target population for UTI is expanding due to the increasing diabetic cases across the age category. Non-insulin dependent diabetes mellitus occurs later in life

and most of these cases are diagnosed a little too late for intervention to manage the condition. Besides diabetes, women also are predisposed to UTI due to hormonal changes in their body. In premenopausal women, lactobacilli make up to 90 % of the normal flora in the vagina (Charles and Emily, 2010). The bacteria safeguards against organisms that can cause UTI. At menopause, there is a significant loss of estrogen and consequently thinning of the vaginal epithelium with reduced amounts of glycogen. These conditions facilitate increase in the pH of the vagina, which makes the environment hostile to lactobacilli. Decreased numbers of vaginal lactobacilli predispose women to colonization with pathogens responsible for UTI (Jamison *et al.*, 2006).

Escherichia coli is the predominant organism that causes UTI both in diabetic and non-diabetic patients (Foxman, 2010). However, due to the frequency of UTI in diabetic patients, the susceptibility of pathogens causing UTI in diabetic patients has been found to differ from those causing UTI in non-diabetic patients. According to a study carried out in Sudan, it was found out that pathogens causing UTI in diabetic patients tend to be more resistant to administered antibiotics than pathogens causing UTI in non-diabetic patients (Hamdan *et al.*, 2015). This increased resistance to antibiotics could be attributed to the frequent use of antibiotics, which is prompted by frequent infection in diabetic patients (Arason and Sigurdsson, 2010).

Concerning socio-demographic characteristics relating to UTI and diabetes, there are contradicting findings that have been reported in various studies. In Sudan, study findings revealed that there was no association between age and duration of NIDDM and UTI among diabetics (Hamdan *et al.*, 2015). Another study carried out in

Sweden revealed an association between UTI among diabetics and age, duration of diabetes, metabolic control, diabetic cystopathy, frequent hospitalization, instrumentation of the urinary tract and recurrent vaginitis (Hammar *et al.*, 2010).

1.2 Statement of the problem

An estimated 150 million individuals are affected by UTI yearly on a global basis with a significant number of those affected being diabetics (Stamm and Norrby, 2008). Due to the weakened immunity and metabolic disorders among diabetics, the effects of UTI are adverse in this category of individuals. Complications associated with UTI cases among diabetics increases the financial burden of health authorities (Tiffany, 2013). There is a wide range of organisms that can cause UTI in humans and with a focus on people suffering from type 2 diabetes mellitus, these organisms are more resistant to the available antibiotic agents (Kayima *et al.*, 1996).

In order for health authorities to be able to curb UTI among diabetics, it is important that they have the information regarding the pathogens that are responsible for the UTI and their sensitivity to the available antibiotic agents. This information is largely missing in Kenya and this has created a big gap between the problem of UTI among NIDDM diabetics and the appropriate solutions from the healthcare stakeholders. There were no research findings available on the way pathogenic organisms causing UTI among NIDDM diabetic patients respond to the available antibiotics in at the study site. This is a big gap considering that this missing data is key to proper management of UTI cases among NIDDM diabetic patients.

1.3 Justification of the study

The International Diabetes Federation (IDF) reported that in the year 2014, there were 775,200 cases of patients suffering from diabetes in Kenya (International Diabetes Federation, 2015). Considering the fact that UTI is the leading cause of morbidity among diabetics, a high prevalence of diabetes is likely to translate to a high prevalence of UTI (Banerjee, 2012). According to a study carried out on asymptomatic bacteriuria among diabetic patients attending Kenyatta National Hospital, the results obtained showed that diabetes patients are commonly prone to UTI due to metabolic disorders and lowered immunity (Kayima *et al.*, 1996). The study also proved that the pathogens that cause UTI among diabetic patients are poorly sensitive to the regularly available antibiotic agents. Several studies on urinary tract infections and others on diabetes have been done. However, not much has been done to study the relationship between the two and the sensitivity patterns of antibiotics. Lack of this information and the pathogens that cause the infection hinders interventions towards management of UTI in diabetic individuals (Vora and Buse, 2012). This study provided information on antibiotic sensitivity patterns and risk factors predisposing diabetic patients to UTI. With the help of this information, health authorities can plan and execute appropriate measures in reducing the burden of UTI.

1.4 Research questions

- i. What is the prevalence of UTI and socio-demographic characteristics in non-insulin dependent diabetes mellitus patients?

- ii. What are the bacterial causative agents of urinary tract infections in non-insulin dependent diabetes mellitus patients?
- iii. What is the antibiotic sensitivity of bacterial agents causing urinary tract infections among non-insulin dependent diabetes mellitus patients?

1.5 Null hypotheses

Ho₁ There are no UTI cases in non-insulin dependent diabetes mellitus patients

Ho₂ Causative agents of UTI in non-insulin dependent diabetes mellitus patients are not sensitive to antibiotic agents.

1.6 Objectives

1.6.1 General objective

To determine the prevalence, socio-demographics and antibiotic susceptibility of uropathogens causing UTI in non-insulin dependent diabetes mellitus patients.

1.6.2 Specific objectives

1. To determine the prevalence of UTI and socio-demographic characteristics of non-insulin dependent diabetes mellitus patients testing positive for UTI.
2. To isolate the bacterial causative agents of urinary tract infections among non-insulin dependent diabetes mellitus patients.
3. To determine antibiotic sensitivity profile of bacterial agents causing urinary tract infections among non-insulin dependent diabetes mellitus patients.

1.7 Significance of the study

There is a need for people to understand the burden of UTI among NIDDM diabetics in the society. Managing UTI will reduce morbidity in diabetic patients since they are the leading cause of morbidity. The medical costs resulting from complications associated with UTI in NIDDM diabetic patients consume a lot of money. This can be stemmed if UTI are well managed and in a timely manner. This study will provide information about pathogens that cause UTI in NIDDM diabetic patients and how these microorganisms can be treated. It will be a bold step towards the fight against UTI in NIDDM diabetic patients.

1.8 Scope and limitations

The study was conducted in Kisii Teaching and Referral Hospital (KTRH) in Kisii County. Diabetic patients from the diabetes clinic were recruited to take part in the study. The study did not involve patients aged less than 18 years because non-insulin dependent diabetes is adult onset diabetes. This limited the study results to adult diabetic patients aged 18 years of age and older.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Non-insulin dependent diabetes mellitus

Non-insulin dependent diabetes mellitus accounts for over 90-95 % of all cases of diabetes in the world (Shaw *et al.*, 2010). Weight gain and physical inactivity are known to be responsible for the incidences of type 2 diabetes mellitus. Failure to utilize insulin produced by the pancreas leads to elevated blood sugar levels resulting in non-insulin dependent diabetes mellitus (Deshpande *et al.*, 2008). According to the Centre for Disease Control (CDC), one fifth of individuals presently over 60 years of age have diabetes, while two thirds of non-insulin dependent diabetes mellitus patients are aged over 60 years by 2025 (Codario, 2011). Complications and disabilities associated with non-insulin dependent diabetes mellitus are more severe among older adults. This is due reduced cognitive functioning, declined healthcare access and reduced financial resources (Codario, 2011).

Due to metabolic changes that occur in diabetic patients, various organs in the body become prone to a number of infections (Puska, 2012). Diabetes damages small blood vessels and nerves in the body. This disruption of blood flow and nerve function in the body leads to dysfunction of some organs in the body. Retinopathy and the tingly feeling that diabetic patients experience at the feet and hands are some of the results of neuropathy and disruption of blood flow (Banerjee, 2012). With the changing lifestyle that people are adopting, diabetes cases have been on the rise and the world health organization (WHO) has projected that by the year 2030, it will be the seventh leading cause of death (World Health Organization, 2017). Successful

response to this challenging burden is through coordinated intervention of medical and public health initiatives, patient provider and caretaker communication, patient education and financial assistance (Codario, 2011). It is evident that to curb this increasing prevalence of non-insulin dependent diabetes mellitus cases, prevention is critical. Additionally, it should be initiated at an early stage for successful management of diabetes (Odegaard and Chawla, 2012).

Two prevention strategies can be employed in management of non-insulin dependent diabetes mellitus. Primary prevention strategies delay or halt the development of the disease. In most cases, it involves physical activity and weight loss. However, since these practices alone cannot maintain proper glycaemic control for a long period, pharmacotherapy is usually introduced to improve efficiency (Dalal *et al.*, 2011). Secondary prevention strategies are used to avoid development of complications among individuals who have already been diagnosed with non-insulin dependent diabetes mellitus. The focus in this case is on blood pressure, lipid and glycaemic control.

2.2 Urinary tract infections

The presence of microbial pathogens in the urinary tract is known as urinary tract infection. Depending on the site of infection, UTI can be classified as cystitis when the bladder is infected, pyelonephritis when the kidney is infected and bacteriuria when the bacteria are in urine (Foxman, 2002). Urinary tract infection can present with symptoms or without any symptom. These symptoms include pelvic pain, painful voiding of urine, deep coloured urine and fever. UTI can also be classified as

complicated and uncomplicated. Complicated UTI are found in individuals who have defective genitourinary tract and consequently are provided with instruments to aid the functioning of the genitourinary tract (Foxman, 2010). Indwelling urethral catheters are common examples of instruments used to rectify defective genitourinary tract. In most cases, complicated UTI are asymptomatic (Foxman, 2010). Uncomplicated UTI are found in individuals with normal functioning genitourinary tracts and are mostly symptomatic.

Bacteriuria is defined as presence of bacteria in urine and in quantities significant with infection. Depending on whether or not there are symptoms accompanying the isolation of bacteria, bacteriuria can be classified as symptomatic or asymptomatic bacteriuria (Flores-Mireles *et al.*, 2015). Antimicrobial treatment is not recommended in asymptomatic bacteriuria since it is not a serious condition. However, in some special populations like pregnancy, there is a need for antimicrobial therapy (Flores-Mireles *et al.*, 2015).

Urinary tract infection affects 150 million people in the world globally (Stamm & Norrby, 2001). The United States incurred approximately US\$ 3.5 billion per year due to urinary tract infections (Flores-Mireles *et al.*, 2015). Middle and low-income countries are projected to suffer a heavier burden due to inadequate facilities for early diagnosis and management of urinary tract infections.

Urinary tract infection affects both men and women but there is a higher prevalence in women than men. The short urethra in women and the close proximity of the urethra to the anus are some of the contributing factors to the high prevalence of UTI in women (Charles and Emily, 2010). During pregnancy, women also tend to be

more prone to urinary tract infections because of hormonal level variations. Sexual activity has been found to be another reason why UTI are passed on from one individual to another (Foxman, 2010).

Urinary tract infections are common across different age groups and in both sexes. They are some of the most common bacterial infections that can be acquired in a hospital set up as well as in the community (Hall, Thomsen, Henriksen and Lohse, 2011). Considering that UTI can be symptomatic or asymptomatic, sometimes antibiotic prescriptions are given based on the presence of symptoms alone. Antibiotic therapy without thorough laboratory testing to confirm UTI has contributed to the increasing antibiotic resistance (Arason & Sigurdsson, 2010). Proper antibiotic prescription will ensure that there is classification of first line drugs for use in uncomplicated infections (Bjorkman *et al.*, 2013).

2.2.1 Urinary tract infections risk factors

The normal human body is always in operation to keep at bay any pathogens that cause infections. When the host defence mechanisms become overwhelmed by pathogens, an infection occurs (Tandogdu and Wagenlehner, 2016). There is a wide range of bacteria that can infect the urinary tract leading to UTI. According to Walsh and Collyns (2017), there are factors that may facilitate progression of an infection faster than others may. These factors may be associated with the host or the pathogen. Host factors that predispose an individual to UTI include gender, kidney stones and an enlarged prostate (Al-Rubeaan *et al.*, 2013). It is projected that up to half of women will have at least one episode of UTI in their lifetime. Women have a

shorter urethra making it easier for bacteria to travel up to the bladder and cause an infection there (Hamdan *et al.*, 2015). The female anatomy also has the urethra closer to the anus, consequently making it easier for bacteria from the rectum to find their way to the urethra (Dielubanza and Schaeffer, 2011). Women who use spermicidal agents as a form of birth control are also known to be at a higher risk of contracting UTI. The normal flora in the vagina are greatly reduced by spermicides and this leaves the pathogenic bacteria which survive the effect of the spermicidal agents to cause an infection (Schmiemann *et al.* , 2010). According to Raz (2011), estrogen enhances growth and survival of lactobacillus as well as reducing the pH in the vaginal epithelium and consequently inhibiting colonization of the vagina with enterobacteriaceae.

Instrumentation is another risk factor of UTI in individuals. When foreign instruments, like catheters, are introduced in an individual, they provide a route for foreign organisms to find their way into the body. Pathogens invading the urinary tract also find it easy accessing the organs in the region through the indwelling catheter. Individuals with indwelling catheters are more likely to get UTI compared to those with no catheters (Hirji *et al.*, 2012). A catheter use means that voiding of urine does not completely occur. Retention of urine in the bladder encourages colonization of bacteria and a potential UTI (Mnif *et al.*, 2013).

Sexual activity is also a risk factor that predisposes both men and women to UTI. Due to the nature of the role played by women in a sexual encounter, women are more prone to UTI than men (Charles and Emily, 2010). Raz (2011), states that the more sexual partners an individual has, the more the risk of contracting UTI.

Suppression of the immune system allows various parts of the body to be infected by pathogenic organisms. UTI also take advantage of suppressed immune system in individuals. Diabetes mellitus is one of the diseases that interfere with the immune response of the body and consequently allowing UTI to develop (Hamdan *et al.*, 2015). Some special conditions like pregnancy also suppress the immune response of women and this is why pregnant women are prone to UTI (Aryal *et al.*, 2015).

An individual may have one or more risk factors making them prone to UTI. Depending on the risk factors and pathogenesis of the pathogen infecting an individual, UTI differ in terms of severity (Davis and Flood, 2011). The role played by the host defences also determines whether there will be a successful UTI or not. An infection will only occur when there is an imbalance between the host defences and pathogenesis of the organism infecting the urinary tract (Flores-Mireles *et al.*, 2015).

2.2.2 Bacterial causes of urinary tract infections

The urinary tract is prone to infection by a number of microorganisms ranging from viruses, bacteria, fungi and parasites (Bennett *et al.* , 2014). Due to their virulence factors, bacteria are the most common causes of UTI. In each single infection, a single bacterium species is mostly responsible for the infection (Bennett *et al.*, 2014). Every organism has its own virulence factors that enable it to successfully invade the urinary tract and cause an infection. The infection cycle involves attachment of the pathogen at the site of infection, evasion of host immune defences and ability to multiply within the host.

Escherichia coli utilize type 1 pili to enhance colonization, invasion and persistence. In order to ensure that the pathogen is not flushed out of the body, attachment to the sites of infection is critical for infection to progress. Type 1 pili are tipped with adhesins that are able to recognize integrin with stereochemical specific properties to start invasion into the cells. With the use of flagella, *E. coli* escapes into the cytoplasm to evade the innate immune responses. Development of biofilm-like intracellular bacterial community (IBC) helps *E. coli* to survive in the urinary tract, which is well supplied with immune defence mechanisms like cell exfoliation, urination and inflammation (Flores-Mireles *et al.*, 2015). *Escherichia coli* also secretes toxins which lead to pore formation on umbrella cells and membrane ruffling. Some of the toxins secreted by *E. coli* are α -haemolysin (HlyA) and cytotoxic necrotizing factor 1 (CNF1). In a calcium ion dependent manner, the HlyA integrates in the cholesterol-rich microdomains in the host cell membrane. This leads to pore formation and eventually cell lysing. It is through this that the bacterium gets to acquire iron and nutrients from the cells (Dhakal and Mulvey, 2012). Activity of CNF1 also prevents apoptosis of the colonized uroepithelium and this facilitates survival and protection of the niche.

Proteus mirabilis produces *P. mirabilis*-like fimbriae (PMFs) which are chaperone-usher pathway (CUP) pili that facilitate biofilm formation and colonization of the bladder and kidneys (Armbruster and Mobley, 2012). Adhesion and invasion mediated by the *Proteus* auto transporter play a critical role in infection of the kidney and bladder. Due to the selective nature of receptors where adhesion occurs, this explains the different tissue tropisms. *Proteus mirabilis* produce two toxins,

haemolysin and *Proteus* toxin agglutinin (Pta). These toxins play a role in tissue damage and dissemination of the pathogen in the body (Jacobsen *et al.*, 2008). Haemolysin inserts itself into the cell membrane and due to its calcium ion dependent activity, causes sodium ion efflux. Activity of *P. mirabilis* urease provides a convenient alkaline environment where Pta functions. *Proteus* toxin agglutinin punctures the cell membrane, causing leakage of the cytosol, osmotic stress and depolymerisation of actin filaments (Jacobsen *et al.*, 2008). Bladder and kidney damage result from such destruction of the structural integrity of the cell.

Chaperone-usher pathway pili are also utilized by *Klebsiella pneumoniae* in their colonization of the urogenital tract. Type 1 pili are used by *K. pneumoniae* for biofilm formation and bladder colonization. The type 1 pili in *K. pneumoniae* have been found to have varying binding specificities but with weaker adherence to the bladder than *E. coli*. In addition, *K. pneumoniae* encodes for more CUP pili including type 3 pili, which play an important role in colonization and biofilm formation (Murphy *et al.*, 2013).

There are several adhesion factors encoded by enterococci for successful colonization of the urogenital tract. Enterococcal surface protein, enterococcal polysaccharide antigen and enterococcal biofilm-associated pili (Ebp) are all required for persistence during infection (Arias and Murray, 2012). During catheterization, fibrinogen is released into the bladder as part of the inflammatory response. Accumulation and deposit of fibrinogen on the catheter facilitates binding of Ebp enhancing colonization.

Organisms that cause uncomplicated UTI depict stronger virulence factors and more specific and hence successful infection compared to organisms that cause complicated UTI in individuals with urinary tract structural deformities (Flores-Mireles *et al.*, 2015). Gram-negative bacteria are the main cause of UTI in both sexes with *E. coli* and *K. Pneumoniae* being the predominant isolates. *Escherichia coli* was found to be the main uropathogen by Hamdan *et al.*, in South Sudan (Hamdan *et al.*, 2015). *Escherichia coli* is naturally found in the gastrointestinal tract (GIT) but there are strains that have virulence factors that enable them to cause infections in areas outside the GIT (Todar, 2012). *Klebsiella pneumoniae* is the second most dominant bacterial pathogen responsible for UTI. The pathogen has virulence factors similar to those of *E. coli*. Out of various species of *Proteus*, *Proteus mirabilis* has also been identified as a common pathogen responsible for UTI in a number of cases. The virulence factors of *P. mirabilis* have made it a common uropathogen in individuals with abnormal urinary tract structures (Walsh and Collyns, 2017).

2.3 Epidemiology of urinary tract infections among non-insulin dependent diabetes mellitus

Urinary tract infections are classified as upper or lower and as complicated or uncomplicated (Foxman, 2010). When the infection is confined to the bladder, it is classified as upper urinary tract infection. Co-infection with urinary tract infection among people with diabetes is an emerging cause of morbidity across the globe. There is a huge burden in the African continent due to limited resources to manage the condition in good time (Hall *et al.*, 2011). A study carried out in Ethiopia showed a high prevalence of bacteriuria in diabetic patients. Symptomatic cases of bacterial

infection among diabetics were found to be 13.6 % while asymptomatic cases were 10.4 % (Yeshitela *et al.*, 2012). The burden of urinary tract infections in diabetic patients is shared between symptomatic and asymptomatic cases. While the symptomatic cases may receive treatment leading to recovery, most of the asymptomatic cases are not given medical attention and this may eventually contribute to morbidity among diabetic patients. A similar study carried out in Sudan showed the magnitude of asymptomatic urinary tract infections among diabetic patients (Hamdan *et al.*, 2015). Among 200 diabetic patients involved in the study, it was found out that 20.9 % had asymptomatic bacteriuria.

Studies have shown that diabetic patients are at a higher risk of contracting UTI than non-diabetic patients. In a study carried out in Netherlands, 7.1 % of women with diabetes were found to have relapsing UTI and 15.9 % had reinfection of the urinary tract compared to women without diabetes who had 2.0 % and 4.1 % relapse and reinfection respectively (Gorter *et al.*, 2010). Another study conducted in the United States of America (USA) with over seventy thousand diabetes mellitus type 2 patients found out that 8.2 % were diagnosed with UTI in the first year. The same study showed women to be more prone to UTI than men (Yu *et al.*, 2014). Gender was clearly brought out as a risk factor in contraction of urinary tract infections.

Complications of UTI are also common in diabetes mellitus type 2 patients. The weakened immune system in diabetic patients allows UTI to cause renal abscesses and other complications (Mnif *et al.*, 2013). Due to the wide range of UTI that diabetic patients have, the pathogens causing the infection have been found to develop resistance to the available antibiotic agents. Administration of the broad-

spectrum antibiotics leads to the antibiotic resistance, worsening management of the UTI (Munar and Singh, 2007). Some investigators (Abdelhafiz *et al.*, 2015) have found out that avoiding hospitalization also contributes to the increasing prevalence of infections in diabetic patients. The study found out that individuals living with diabetes for a long duration of time are prone to a number of health complications coupled with the aging process. Consequently, the burden of infections in diabetic patients above 75 years is large and keeps on increasing (Abdelhafiz *et al.*, 2015). Urinary tract infections among other complications found in diabetic patients are responsible for morbidity of old people with diabetes mellitus. According to a study carried out in the united states of America (USA) based on diabetes and hospital admission, it was found out that 43.7 % of the cases were due to acute conditions. Of these, 28.2 % were due to urinary tract infections. Hospitalization cases due to chronic conditions were found to be at 56.3 % with congestive heart failure, respiratory and urinary tract infections and chronic obstructive pulmonary disease accounting for 89.1 % of admissions (Hongsoo *et al.*, 2011).

Management of UTI becomes a difficult task when the people affected are diabetic patients. The high cost of medical intervention means that victims in developing nations are the worst affected when it comes to UTI in diabetes mellitus type 2 patients (International Diabetes Federation, 2015). The fact that prevalence of diabetes mellitus type 2 is on the rise means more people are at risk of contracting UTI and this is likely to increase the burden of the infection in the society. Studies have also shown that avoiding hospitalization contributes to the emergence of complications associated with diabetes mellitus and consequently increasing the cost

of management of the health conditions (Bennett *et al.*, 2014). There is a need to have an integrated care system that combines both primary and secondary care (Simkhada, 2013). A multifunctional approach focusing on early diagnosis of diabetes, health education and proper preventive measures to ensure that complications associated with diabetes mellitus do not occur will go a long way in reducing the cost of healthcare. Additionally, this will prevent long-term morbidity that arises from preventable complications like urinary tract infections among diabetic patients (Sinclair, 2011).

Antimicrobial therapy for organisms causing urinary tract infections in patients with diabetes mellitus type 2 is a key step towards reduced cases of morbidity (Geerlings, 2008). While some studies have shown little or no difference in the way pathogens responsible for urinary tract infections in diabetics respond to the antibiotics administered, there is evidence by some studies that the sensitivity to antibiotics administered differs from individuals with diabetes and those without diabetes (Fredricka *et al.*, 2011). Changes in the metabolic processes in diabetic patients affects the physiological functioning of the antibiotics administered and consequently influencing the antibiotic sensitivity of the organisms causing urinary tract infections (Simkhada, 2013).

2.4 Risk of UTI in non-insulin dependent diabetes mellitus

According to studies from all over the world, type 2 diabetes mellitus has been strongly associated with UTI (Davis and Flood, 2011). One study carried out in Saudi Arabia found out a number of factors predisposing individuals to UTI. Among

them were gender, hypertension, insulin therapy, body mass index (BMI) and nephropathy (Al-Rubeaan *et al.*, 2013). Patients with type 2 diabetes mellitus show most of these defects in their bodies due to the impaired immunity and physiological changes caused by diabetes (Todar, 2012). The proximity of the urethra and the perianal region in women makes it easy for microorganisms in the area to get access into the urethra to cause UTI. An observational study in the United Kingdom (UK) showed that the incidence rate of UTI in diabetic patients was 46.9 in 1000 patients compared to 29.9 in patients without diabetes (Hirji *et al.*, 2012). The more diabetes patients are exposed to UTI, the higher the burden of the infection. The epidemiology of diabetes associated UTI has been marked all over the world with developing nations suffering the greatest burden (Flores-Mireles *et al.*, 2015).

Gender has also been noted as a risk factor when it comes to diabetes patients contracting UTI. An American study involving 70,000 patients with diabetes type 2 showed that 8.2 % were diagnosed with UTI. Out of this, 12.9 % were women and 3.9 % were men (Hirji *et al.*, 2012). Microorganisms thriving at the perianal region can easily access the urethra in women due to the proximity between the two. This introduces the pathogens to initiate UTI in women (Todar, 2012). Women, therefore, are always at a higher risk of acquiring UTI than men.

There are inadequate studies linking age to prevalence of UTI but diabetes mellitus type 2 has been closely linked to elderly individuals. Besides other predisposing factors, research has shown that age also contributes to the risk of a diabetic patient contracting UTI. Diabetic women in the premenopausal, menopause and post-menopausal stage were found to be 4.1 times more likely to be diagnosed with

pyelonephritis than those without diabetes (Scholes *et al.*, 2005). Old age facilitates muscle dystrophy and consequently affecting completing voiding. Incomplete voiding encourages colonization of the urogenital tract leading to development of UTI (Walsh and Collyns, 2017).

An epidemiological study carried out in the year 2010 in sub-Saharan Africa found 12.1 million people to be living with diabetes (Hall *et al.*, 2011). Data collected from urban Kenya showed the highest prevalence of 12 % of people living with diabetes. With the close association of diabetes and UTI, it is evident that Africa is facing a great burden of UTI among diabetes patients (Jamison *et al.*, 2006). Poverty has been mentioned as a facilitating factor in the increasing burden of managing UTI among diabetes patients (Hall *et al.*, 2011). With poverty, there is weak financial ability to access quality health care and this makes individuals to be prone to UTI. Lack of proper nutrition also leads to malnutrition and this decreases the immune response to pathogens causing UTI.

When UTI are not managed at an earlier stage, they end up having complications that cost authorities more money in implementing interventions. Prior diagnosis and proper drug administration helps in keeping the infections under control and hence allowing the body to fight the pathogens. However, when antibiotics are prescribed without proper consideration of the causative agent, there is a risk of antibiotic resistance arising. Patient factors like poor compliance and refusal of treatment may also influence the way pathogens respond to administered antibiotics (Owens and Lautenbach, 2008). Coupling with the increasing diabetic cases in Africa, this makes UTI among diabetes an ever-growing health problem.

2.5 Pathogenesis of UTI among non-insulin dependent diabetes mellitus patients

Several physiological changes occur in diabetic patients and are potential mechanisms for proliferation of UTI (Nitzan *et al.*, 2015). Due to poor regulation of glucose in the body of diabetic patients, the high levels of glucose in urine may promote growth of pathogenic bacteria (Fünfstück *et al.*, 2012). There are no studies linking high glucose in the body and development of UTI but increased sugar levels in the urogenital region promotes growth and multiplication of microorganisms; these organisms are suspects for development of UTI (Fünfstück *et al.*, 2012).

Diabetic patients have a weakened immune system and this may contribute in the pathogenesis of UTI (Delamaire *et al.*, 1997). In a study aimed at monitoring the functioning of polymorphonuclear neutrophils (PMN) in diabetic patients without any infection, it was found out that the functioning of PMNs was altered in patients with diabetes (Delamaire *et al.*, 1997). This could be a potential explanation as to why diabetes patients have a wide range of infections with UTI being top of the list. A comparison study between diabetic patients with asymptomatic bacteriuria (ASB) and patients with ASB but without diabetes showed a lower level of interleukin -6 and -8 in patients with diabetes (Park *et al.*, 2006). Low interleukin 6 and interleukin 8 levels mean that there will be a defective clearance of pathogens and consequently increased infection.

There is improper urine retention and voiding when any nerve associated to the urinary tract is affected (Kaplan *et al.*, 1995). Dysfunctional urine retention and voiding promotes growth of pathogenic bacteria because there is a decreased clearance of these microorganisms through micturition (Kaplan *et al.*, 1995).

Frequent urination is experienced in diabetic patients but there is an undesired retention of urine that promotes growth and multiplication of bacteria and hence causing UTI in the affected persons (Rubin, 2006).

2.6 Bacterial causes of UTI in non-insulin dependent diabetes mellitus patients

Bacterial causative agents in most UTI cases are well known. *Escherichia coli* remains to be the most pathogenic organism isolated in diabetic patients with UTI (Todar, 2012). *Staphylococcus*, *Saprophyticus*, *Enterococcus*, *Klebsiella* and *Enterobacter* have also been found to cause UTI but in less common cases (Acton, 2013). Due to the weakened immunity in diabetes patients, there has been positive diagnosis of fungal UTI among diabetes type 2 patients (Winter and Signorino, 2002). Patients with diabetes are likely to have resistant pathogens causing UTI because of the antibiotic therapy that they undergo especially when they have ASB (Codario, 2011). A comparison of the prevalence of bacterial pathogens in diabetic and non-diabetic patients showed a higher prevalence in the former group (Todar, 2012).

Diabetic patients with catheters are more prone to *Pseudomonas* infections (Madigan *et al.*, 2014). According to a study carried out in the US, up to 70-80 % of complicated UTI are because of indwelling catheters (Flores-Mireles *et al.*, 2015). The morbidity and mortality of catheter associated UTI (CAUTI) increases when the affected individual is diabetic.

2.7 Diagnosis of urinary tract infections

Symptoms of UTI range from dysuria, turbid urine, foul smell in urine to vagina and urethral irritation (Geerlings, 2008). Diabetic patients may have complicated symptoms because of the defective physiological processes in the urinary tract. Clinical diagnosis of urinary tract infections is fundamentally based on the medical history of the patient (Winter and Signorino, 2002). A study carried out in Germany showed that women are likely to contract UTI but show no symptoms unlike male patients (Schmiemann *et al.*, 2010). Besides the medical history of the patient, urine is also used to conduct a number of tests to confirm any suspected infection (Crook, 2012). There is a close relationship between urine collection and the results acquired from the tests done. Since urine is collected outside the body, there is always a high chance of contamination with microorganisms found on the area around the urinary tract (Mnif *et al.*, 2013). Proper guidance is required in order for patients to collect the correct sample so that there is minimal chance of contamination.

There are two major diagnostic methods employed when diagnosing for urinary tract infections. The first method is dipsticks urinalysis while the second method is culture of the urine sample. The first method takes a shorter period compared to the second method. Once a patient has brought a urine sample to the laboratory, the sample is analyzed macroscopically before it is examined microscopically. Macroscopic examination looks at the physical appearance of the urine. Colour, turbidity and odour are some of the characteristics noted at this stage. The principle of this method is that if there is an infection in the urinary tract, the urine collected will have some anomaly in terms of colour, turbidity and odour. However, it is not obvious that

every UTI will be symptomatic. There are some cases where one will have a UTI but they do not show symptoms.

After taking note of the colour, turbidity and odour of the urine sample collected, urine dipsticks are also used in diagnosis of UTI because they are good detectors of biochemical defects in urine (Nitzan *et al.*, 2015). Nitrites and leukocyte esterase are the most preferred markers that signal a possibility of a UTI (Winter and Signorino, 2002). The dipsticks are laced with different biochemical detectors that change colour depending on the parameter they are set to detect. The resultant colour changes are then read against a chart provided with the strips. The urine sample is then centrifuged in order to get sediment that is then examined under a microscope. Centrifuging the urine sample is important as it separates the supernatant from any residue in the urine. This makes it simple for examination under the microscope to identify any residue of medical importance in diagnosis of UTI. This method gives an indication of whether one has a UTI or not but without specifying which bacteria is causing the infection.

Bacteriological urine culture remains to be the gold standard in testing whether one is having a UTI or not (Schmiemann *et al.*, 2010). Urine cultures give a precise identification of the bacteria causing the UTI. A count of more than 10^5 colony-forming units of a single bacterial species is a positive culture (World Health Organization, 2017). Urine cultures take more time when compared to the microscopic examination but they also give precise results regarding UTI.

2.8 Complications associated with urinary tract infections

Under normal circumstances, UTI should not have any dire consequences if managed in a good time. However, limited access of healthcare services in developing nations has resulted in a huge medical burden of UTI complications (Kayima *et al.*, 1996). A study carried out in South Korea found out that over 90 % cases of emphysematous pyelonephritis and 67 % of episodes of emphysematous cystitis were found in diabetes type 2 patients (Park *et al.*, 2006). Results of another study carried out in Greece reflected similar outcomes where a group of elderly patients with acute pyelonephritis were tested for bacteraemia. It was found out that 30.7 % of the individuals with bacteraemia had diabetes compared to 11 % without diabetes (Kofteridis *et al.*, 2009).

2.9 Treatment and management of urinary tract infections

Drugs for treatment of urinary tract infections can be classified into four. The classes are fluoroquinolones, cephalosporin, penicillin and nitrofurantoin (Simkhada, 2013). Ciprofloxacin, which is a fluoroquinolone, is a broad-spectrum antibiotic that inhibits activity of DNA gyrase and consequently stopping bacterial replication. Nitrofurantoin has the same mode of action where they attack ribosomal proteins and DNA to stop bacterial replication. Cephalosporins like ceftriaxone and penicillin like ampicillin disrupt the synthesis of the peptidoglycan layer of the cell wall and, therefore, inhibiting cell wall development in bacterial replication (Charles and Emily, 2010).

There are a number of factors to consider when managing UTI in diabetic patients. Generally, treatment in diabetic patients is similar to that of non-diabetic patients (Nitzan *et al.*, 2015). The choice of antibiotics should be guided by the susceptibility patterns of the pathogens. The only difference is that metabolic complications resulting in diabetic patients also need to be corrected as part of the treatment plan (Nitzan *et al.*, 2015). First line treatment options for various types of UTI are listed in the table below:

Table 2.1: First line antibiotics for urinary tract infection in patients with type 2 diabetes mellitus

| Type of UTI | Gender | Antibiotic treatment | Duration of treatment (days) |
|---|---------------|--|--|
| Asymptomatic bacteriuria | Men and Women | None | |
| Acute cystitis | Men and Women | Nitrofurantoin Fosfomycin | 5 days Single dose |
| Complicated lower UTI | Men and Women | Ciprofloxacin Ofloxacin Cefuroxime | 7 – 14* 7 – 14* 7 – 14* |
| Complicated pyelonephritis urosepsis | Men and Women | Ciprofloxacin Ofloxacin Gentamicin Amikacin | 10 – 14* 10 – 14* 10 – 14* 10 – 14* |

Source: Nitzan *et al.*, 2015.

* Length of treatment depends on severity of symptoms and patient response.

Earlier studies raised concerns that individuals with ASB stood a higher chance of developing symptomatic UTI and, therefore, need to have medication. However, subsequent studies have found that ASB does not increase the risk of symptomatic UTI (Nicolle *et al.*, 2005). In fact, these studies cautioned that use of antibiotics in people with ASB would contribute to antibiotic resistance and this increases the challenge of managing symptomatic UTI (Nicolle *et al.*, 2005). The choice of drugs

in diabetic patients should also consider interactions between antibiotics and antidiabetics so that there is no glucose homeostasis impairment (Winter and Signorino, 2002). In some cases, dosage adjustment is necessary so that renal function of the affected individuals is not worsened (Nicolle *et al.*, 2005).

It is evident that cases of diabetic mellitus type 2 patients who are suffering from UTI are on the rise all over the world. Trends of antibiotic resistance in diabetic associated UTI show a diverse pattern where pathogens are increasingly developing resistance to available antibiotics. Inadequate research studies in developing nations are making it difficult for intervention measures to manage these infections. The burden of UTI in diabetic patients continues to slow down development in the health sector. With the changing etiologies of this disorder, there is an urgent need to have extensive studies among vulnerable groups in the society (Owens and Lautenbach, 2008). Information regarding the prevalence, causative agents and their response to the available antibiotic agents will be of great help in managing and controlling UTI in diabetic mellitus type 2 patients.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

The study was done in Kisii Teaching and Referral Hospital in Kisii County. The county is located between latitudes $0^{\circ} 40'$ and $00.0'$ South and longitudes $34^{\circ} 45'$ and $00.0'$ East. The study site is shown on a map in appendix I. According to the 2009 census, the county had a population of 1,152,282 with a mean density of 874 people per square kilometre. The hospital has a patient handling capacity of approximately 1500 patients per day in the outpatient department and an inpatient bed capacity of 450. The county is highly populated bordering six other counties. The location of this referral hospital is a great boost to the high demand of healthcare in the region. Being a referral facility in the region, the hospital attends to a large number of patients who are referred here from other smaller health facilities. The facility also has a diabetic clinic where diabetic specialists attend to patients. This study area is an appropriate location as it attends to many patients from the surrounding counties and this minimizes sample bias due to the wide area covered and convergence at a central point.

3.2 Research design

A cross-sectional study was adopted in this study.

3.3 Sample selection technique

Patients already enrolled for diabetic clinic were recruited in this study. One hundred and eighty diabetic patients were selected using a simple random selection method to

participate in the study. Sample selection was done using the simple random sampling technique to reduce bias and maintain uniformity in the study (Black, 2011).

3.4 Inclusion criteria

Diabetic patients who had not been diagnosed with UTI and were not on antibiotic therapy for the last two weeks were recruited. Only individuals who gave consent were allowed to take part in the study.

3.5 Exclusion criteria

Individuals diagnosed with UTI and those on antibiotic therapy were excluded from the study.

3.6 Sample size determination

The appropriate sample size was determined by using the Fischer (1998) method:

$$n = \frac{z^2 pq}{d^2}$$

Where:

n = Desired sample size

z = the standard normal deviation, set at 1.96

p = Postulated prevalence (12 %) (Hall *et al.*, 2011)

q = (1.0 - p)

d = the degree of accuracy desired, set at 0.05 corresponding to 1.96

$$\text{In substitution, } n = \frac{1.96^2 \times 0.12 \times (1 - 0.12)}{0.05^2} = 162$$

n = 162 samples

A sample size of 180 samples was targeted. This would allow the minimum sample size to be met after excluding mixed growth cultures and any refusals if any.

3.7 Laboratory validation

In order to ensure performance testing was properly done, *Staphylococcus aureus* and *Proteus mirabilis* were used as control organisms in the CLED medium to be used. Additionally, to ensure that the antibiotic sensitivity results obtained were accurate, two control organisms, *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were used as per the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines.

3.8 Sample collection

In accordance with a clean-catch procedure, the participants were asked to provide a morning, midstream urine sample of about 30 ml. The collection containers were UV-sterilized plastic bottles with airtight screw cap tops. Clear instructions were given to the participants on how they would collect the samples. In order to avoid contamination of the urine sample collected, female participants were advised to ensure that they practise front-rear wiping after a long call. Wiping in the rear-front direction introduces organisms from the anus and this is likely to contaminate the

urethra and consequently lead to urinary tract infection (Charles and Emily, 2010). To collect midstream urine samples, the participants were instructed to void the first portion of the urine into the toilet before collecting the urine sample into the containers provided. About 30 ml of urine was collected per participant. The samples were then labelled with a reference code, gender and time of collection. The samples were transported to a laboratory in a cooler box at 4°C and processed within two hours of collection. All the samples were analysed by microbial culture, macroscopically and microscopically and thereafter with a urine dipstick.

3.9 Urine dipstick

After urine samples were collected, they were analysed by use of urine dipstick strips. This analysis was conducted within the first two hours of sample collection. Urinalysis reagent strips (REF D-U10100) with a 10-parameter scale manufactured by Rapid Labs limited were used. The parameters were leukocytes, nitrites, urobilinogen, proteins, pH, blood, specific gravity, ketones, bilirubin and glucose. The strip was dipped in the urine samples and then put on a flat surface (on top of the urine container caps) for up to two minutes before the results were read against the available chart on the container of the urine strips. According to the manufacturer's instructions, each parameter had a period after which the result would be read ranging from thirty seconds to two minutes. The purpose of doing the urine dipstick was to give an idea of what to expect in the urine samples in regard to UTI.



Figure 3.1: Rapid labs limited sample urine dipstick

3.10 Microbial culture method

All samples were cultured in cystine lactose electrolyte-deficient (CLED) agar. The procedure followed in preparation of the culture medium is attached in appendix VII. From each batch of prepared culture media plates, three plates were taken and kept in the incubator overnight. This was done to test for the sterility of the culture plates. No growth in the plate after 24 hours meant that the culture media was sterile and ready for use. Before all culture media plates were inoculated, they were also examined visually to ensure that there was no change that would suggest contamination or deterioration.

The media was used to determine pathogens causing UTI among diabetics. A positive culture for urinary tract infection was based on more than 100,000 ($\geq 10^5$) colony-forming units of a single bacterial species. Each urine sample was mixed by rotating the container. A sterile calibrated wire loop (0.02 inches) was then used to

inoculate the urine samples. The plates were labelled matching the urine samples and then incubated at 35-37⁰ C for 24-48 hours (Cheesbrough, 2010). To ensure that single colony cultures were obtained, the urine sample was streaked on the culture plates as per the recommended streaking method (Figure 3.1). Bacterial isolates were then identified through various biochemical tests.



Figure 3.2: Streaking technique

Inoculation was started in quadrant one then spread in subsequent quadrants as shown above. The intention was to have a single colony growth.

3.11 Gram staining

Gram staining was used to identify pathogens in various specimens and cultures following their gram reaction (Cheesbrough, 2010). Urine samples were centrifuged at 2000 rpm for about two minutes and the deposit transferred onto a glass slide to make a smear. The urine smears were air dried and then covered with crystal violet stain for 30-60 seconds. The stain was then gently rinsed with clean water. The smears were then covered with lugol's iodine for 30-60 seconds after which they were rinsed with clean water. Acetone was used to decolourize the smears for a few

seconds followed by washing off the acetone with clean water. The smears were then covered with neutral red for 2 minutes as a counter stain. The stain was rinsed with clean water after 2 minutes and the slides wiped clean at the back (Cheesbrough, 2010). The slides were then examined microscopically, first with 40X objective and then with the oil immersion objective.

3.12 Biochemical Tests

Biochemical tests used to identify the individual bacterial species were chosen based on the characteristics of the colonies observed in the culture media plates. Lactose fermenters were identified with urease, citrate, indole and triple sugar ion (TSI) tests. *Enterococcus faecalis* being a non-lactose fermenter was identified using the strep-tex test.

Sterile Christensen's modified urea broth was used to carry out the urease test. The test organism was introduced into a 3 ml medium with a phenol red indicator in a bijou bottle. The medium was then incubated at 35-37° C for 12-18 hours and the results recorded afterwards.

To carry out a citrate test, a thick bacterial suspension of the organism was prepared in 0.25 ml sterile physiological saline. A citrate tablet was then added into the solution and the tube incubated at 35-37° C for 12-18 hours. The results were recorded as soon as they were observed after the incubation period.

In order to carry out the indole test, the test organism was inoculated in a bijou bottle with 3 ml sterile tryptone water and then incubated at 35-37° C for 24 hours. After

the incubation period, 0.5 ml of Kovac's reagent was added. The mixture was shaken gently and examined within 10 minutes for results.

The TSI test was also carried out by inoculating the test organism in a TSI agar. The agar was first stabbed through the centre to the bottom of the tube and then a streak made on the slope with the help of a sterile straight wire. The tube was closed with a loose-fitting cap and incubated at 35-37° C for 24 hours. The slant and butt were all observed for any colour change and cracks that would signify production of gas during fermentation.

3.13 Antibiotic sensitivity testing

Mueller Hinton (MH) media was used to carry out the antibiotic sensitivity testing. One of the main reasons why this media is preferred for antibiotic sensitivity is its batch-to-batch reproducibility (Erika, 2014). The procedure on how the Mueller Hinton agar was prepared is outlined in the appendix VII.

The Kirby Bauer test technique was used to perform the sensitivity testing on the isolated pathogens. With the help of a sterile wire loop, 4 to 5 well-isolated colonies of same morphological type were touched and emulsified in 3-4 ml of sterile physiological saline. The inoculum was used to make a turbid suspension standardized to 0.5 McFarland standard solution. Within 15 minutes after adjusting the turbidity of the inoculum, a sterile swab was dipped into the suspension. The swab was rotated and pressed firmly on the inside wall of the tube to remove excess inoculum from the swab (Erika, 2014). The inoculum was streaked over the entire surface of the MH agar plate. The procedure was repeated two more times and the

plate rotated at 60° each time to ensure even distribution of the inoculum. The plate tops were replaced to allow any excess surface moisture to be absorbed for 5 minutes.

Appropriate disks were placed evenly with the help of a sterile forceps. Not more than five disks were used in any single culture plate. The plates were inverted and placed in an incubator at 35°C within 15 minutes after disks were applied. The plates were incubated aerobically. Zones of complete inhibition were measured after 16-18 hours of incubation. The measurements acquired were recorded accordingly in reference to the EUCAST provided table. The chart shown in table 3.1 was used to interpret the zone sizes of complete inhibition by the antibiotic disks:

Table 3.1: Antimicrobial specific disc content and inhibitory zone diameter tested against *Enterobacteriaceae* and *Enterococcus faecalis* bacteria

| Organism / Antimicrobial | Disk content | Zone diameter | |
|----------------------------------|--------------|---------------|--------------|
| <u>Enterobacteriaceae</u> | | S ≥ | <R |
| Ampicillin | 10 µg | 14mm | 14mm |
| Gentamicin | 10 µg | 17mm | 14mm |
| Nitrofurantoin | 100 µg | 11mm | 11mm |
| Cephalexin | 30 µg | 14mm | 14mm |
| Co-trimoxazole | 1.25-23.75µg | 16mm | 13mm |
| <u>E. faecalis</u> | | | |
| Ampicillin | 10 µg | 14mm | 14mm |
| Amoxicillin-clavulanic acid | 20 µg/10 µg | 18mm | 13mm |
| Nitrofurantoin | 100 µg | 11mm | 11mm |
| Ciprofloxacin | 5 µg | 22mm | 19mm |

(Erika, 2014)

3.14 Socio-demographic data collection

A pretested questionnaire was used to collect socio-demographic data. The questionnaire was tested in the same facility where the study was carried out to confirm that it would be efficient. The participants involved in testing the questionnaire were not included in the actual study. Every participant was taken through the questionnaire and shown how to fill it in. The participant was then given time to read the questionnaire and answer all the questions. Age, gender and education level were demographic variables considered. Any question that was not clear to the participant was elaborated upon inquiry. All the information extracted from the questionnaire was provided upon by the participant (Appendix III).

3.15 Data Analysis

With the help of the Statistical Package for Social Science (SPSS) version 20, chi square and odds ratio were used to analyse the data. Considering the fact that analysis was looking at the way two variables associate, chi square was the appropriate formula used to analyze data. Urinary tract infection was compared to age, level of education, symptoms of UTI and duration of diagnosis of NIDDM prior to the study to see if there was an association between them. Odds ratio was used to give the likelihood of a participant-testing positive for UTI when compared with other variables.

3.16 Ethical consideration

The proposal was approved by the Kenyatta University graduate school paving way for submission to the ethics review committee. Ethical approval was granted by the Kenyatta University Ethics Review Committee (Appendix V). The research permit was given by the National Commission for Science Technology and Innovation (Appendix IV). Authorization was granted by Ministry of Medical services through Director of Medical Services, Kisii County (Appendix VI).

CHAPTER FOUR

4.0 RESULTS

4.1 Socio-demographic information

The background characteristics of non-insulin dependent diabetes mellitus patients included gender, age, level of education, duration of diabetes mellitus and symptoms of urinary tract infections. This information is recorded in Table 4.1 below.

Table 4.1: Background characteristics of diabetic patients

| Variables | Frequency | Percentage (%) |
|---|-----------|----------------|
| Gender | | |
| Male | 107 | 59.4 |
| Female | 73 | 40.6 |
| Age in years | | |
| 50-54 | 11 | 6.1 |
| 55-59 | 139 | 77.2 |
| 60-64 | 26 | 14.4 |
| 65-69 | 4 | 2.2 |
| Level of education | | |
| Primary school | 16 | 8.9 |
| Secondary school | 39 | 21.7 |
| Tertiary institution | 125 | 69.4 |
| Duration of diabetes mellitus in years | | |
| 1-4 | 47 | 26.1 |
| 5-8 | 131 | 72.8 |
| 9-12 | 2 | 1.1 |
| Symptoms of UTI | | |
| Present | 62 | 34.4 |
| Absent | 118 | 65.6 |

4.1.1 Gender

Among the diabetic patients who took part in the study, 59.4 % (107 diabetic patients) were male, while 40.6 % (73 diabetic patients) were female.

4.1.2 Age of diabetic patients

The ages of diabetic patient were categorized in four groups as shown in table 4.1. The age group between 55 to 59 years old had the highest proportion of diabetic patients at 77.2 % (139 patients). This was followed by the group between 60 to 64 years old at 14.4 % (26 patients), then the group between 50 to 54 years old at 6.1 % (11 patients) and finally the age group between 65 to 69 years old had the least proportion of diabetic patients at 2.2 % (4 patients).

4.1.3 Level of education

The diabetic patients were categorized in three levels of education as in table 4.1 above. The majority of the patients were graduate from a tertiary institution at 69.4 % (125 patients), followed by secondary school graduates at 21.7 % (39 patients) and finally the primary school graduates at 8.9 % (16 patients).

4.1.4 Duration of diabetes mellitus

The patients were categorized into three groups based on duration since diagnosis of non-insulin dependent diabetes mellitus. The group between 5 to 8 years had the highest proportion of diabetic patients at 72.8 % (131 patients), followed by the group between 1 to 4 years at 26.1 % (47 patients) and the least was the group between 9 to 12 years at 1.1 % (2 patients).

4.1.5 Urinary tract infection symptoms

Among the diabetic patients who participated in this study, 65.6 % (118 patients) did not have symptoms of urinary tract infection, while 34.4 % (62) exhibited symptoms of urinary tract infection.

4.2 Prevalence of urinary tract infections among diabetes mellitus patients

Of the 180 diabetic patients who participated in this study, 37 participants had urinary tract infection making the prevalence to be at 20.6 %.

4.2.1 Urinary tract infections in non-insulin dependent diabetes mellitus patients by gender

The gender of diabetic patients exhibited no significant difference in terms of urinary tract infection (chi square (χ^2) = 0.568; degree of freedom (df) = 1; p value = 0.45). As shown in figure 4.1, among the diabetic patients who were positive for urinary tract infections, 64.9 % (24 patients) were male, while 35.1 % (13 patients) were female. Fifty-eight percent (83 patients) of diabetic patients who were negative for urinary tract infection were male, while 42 % (60 patients) were female. Among the male diabetic patients, 22.4 % (24 patients) were positive while 77.6 % (83 patients) were negative. Of the female diabetic patients, 17.8 % (13 patients) were positive while 82.2 % (60 patients) were negative. Gender revealed no significant difference in terms of UTI (p=0.45). The male participants were 1.335 (odds ratio) times more likely to test positive for UTI than female participants were. This can be attributed to collaborating variables like how well the diabetes is controlled and one's response to the diabetic medications.

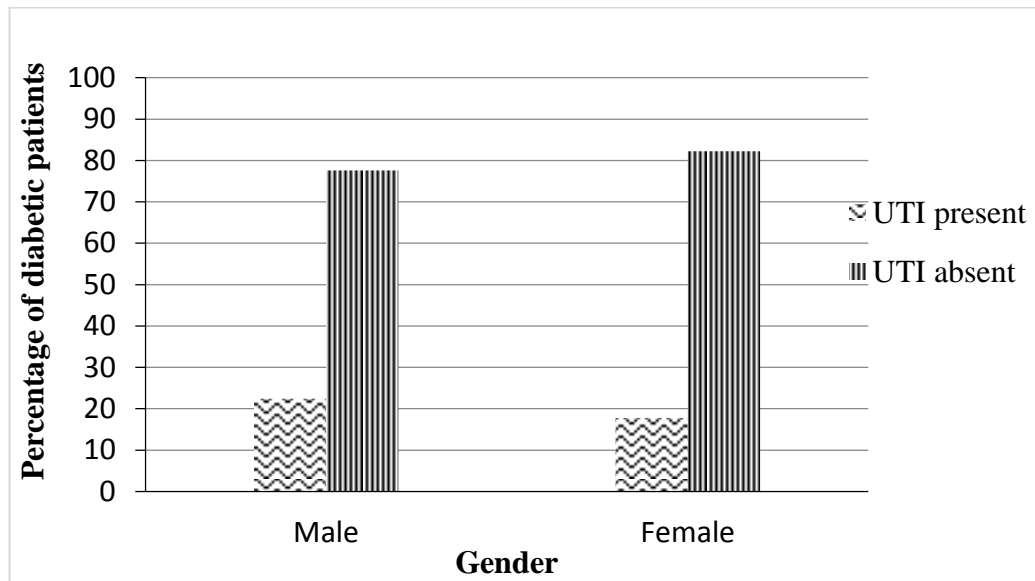


Figure 4.1: Distribution of urinary tract infections among diabetic patients by gender

4.2.2 Urinary tract infections in non-insulin dependent diabetes mellitus patients by age

The age of the diabetic patients revealed significant difference in urinary tract infections ($\chi^2 = 14.876$; $df = 3$; $p = 0.002$). As shown in figure 4.2, no diabetic patients (11 patients) between the ages of 50 to 55 years old was positive for UTI. Eighteen percent (25 patients) of diabetic patients between the ages of 55-59 years old were positive for UTI, while 82.0 % (114 patients) were negative for urinary tract infections. Among the diabetic patients between 60 to 64 years old, 46.2 % (12 patients) were positive while 53.8 % (14 patients) were negative. All the diabetic patients (2 patients) between the ages of 65 to 69 years were negative for urinary tract infections. Among the diabetic patients who were positive for urinary tract infections, 67.0 % (25 patients) were between 55 to 59 years old, while 46.2 % (12 patients) were between 60 to 64 years old. Of the diabetic patients who were negative for urinary tract infections, 7.7 % (11 patients) were between 50 to 54 years old, 79.7

% (144 patients) were between 55 to 59 years old, 9.8 % (14 patients) were between 60-64 years old and 2.8 % (4 patients) were between 65 to 69 years old. There was a significant difference between age and UTI ($p= 0.004$). As the age of participant increased by five years, the probability of a participant to test positive for UTI increased by 1.324 (odd ratio) times. This is probably due to the deteriorating state of the immunity that comes because of the diabetic condition of the participant.

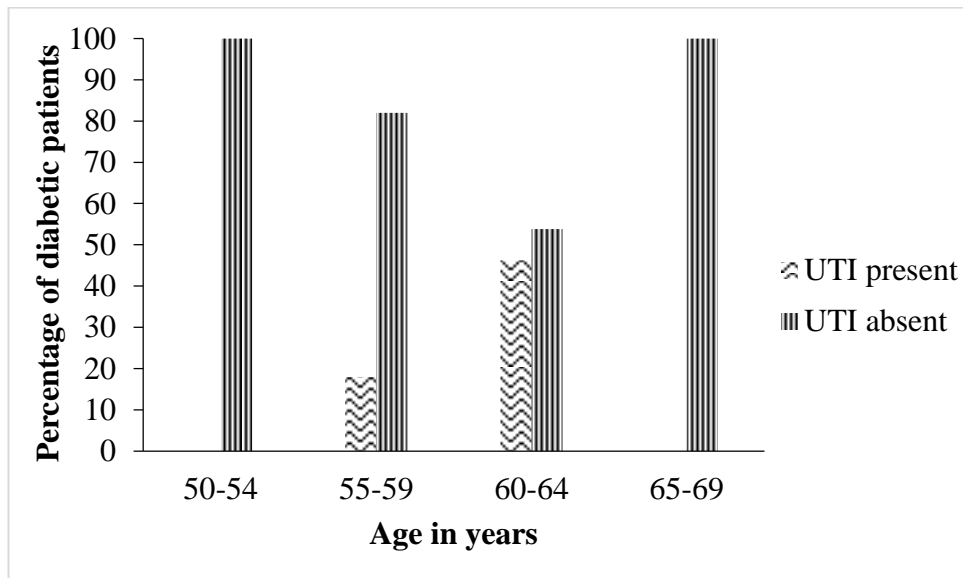


Figure 4.2: Distribution of urinary tract infections among diabetic patients by age

4.2.3 Urinary tract infections in non-insulin dependent diabetes mellitus patients by level of education

The level of education showed no significant difference in terms urinary tract infections among diabetic patients tested ($\chi^2 = 4.51$; $df = 2$; $p = 0.11$). As shown in figure 4.3 below, among the diabetic patients who were primary school leavers, 37.5 % (6 patients) were positive while 62.5 % (10 patients) were negative for urinary tract infections. Ten secondary school leavers (25.6 %) were positive while 29 from

the same category (74.4 %) were negative for urinary tract infections. Among the tertiary institution graduates with diabetes, 16.8 % (21 patients) were positive while 83.2 % (104 patients) were negative for urinary tract infections. Majority of the participants that were positive for UTI were from tertiary institutions (56.8 %) followed by secondary school (27.0 %) and lastly primary school leavers (16.2 %).

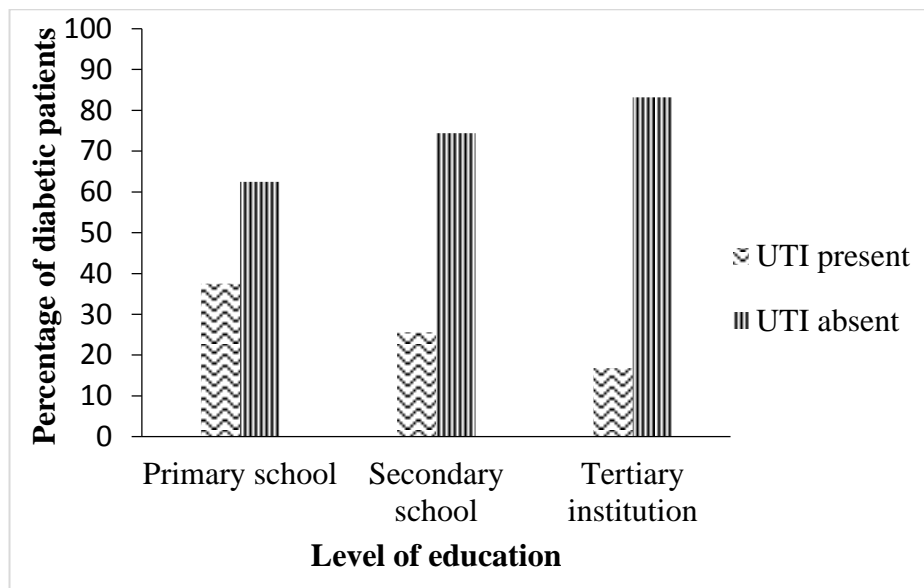


Figure 4.3: Distribution of urinary tract infections among diabetic patients by level of education

4.2.4 Urinary tract infections in non-insulin dependent diabetes mellitus patients by duration of diabetes mellitus

The duration of diabetes mellitus among diabetic patients exhibited significant difference in terms of urinary tract infections ($\chi^2 = 8.566$; $df = 2$; $p = 0.01$). As shown in figure 4.4, of the diabetic patients who had 1 to 4 years duration of diabetes diagnosis prior to the study, 6.4% (3 patients) were positive while 93.6% (44 patients) were negative for urinary tract infections. Twenty five percent (33 patients) of diabetic patients with 5 to 8 years duration of diabetes diagnosis prior to the study

tested positive for urinary tract infections, while 74.8 % (98 patients) tested negative for urinary tract infection. One diabetic patient (50 %), among the two with 9 to 12 years duration of diabetes diagnosis prior to the study were positive for urinary tract infection. Among the diabetic patients who tested positive for urinary tract infections, 8.1 % (3 patients) had been diagnosed with DM for a period between 1 to 4 years prior to the study. Thirty-three patients (89.2 %) of those who tested positive for UTI had been diagnosed with DM for duration between 5 to 8 years prior to the study, while one patient (50 %) out of the two that had been diagnosed with diabetes for a period of 9 to 12 years was positive for UTI. Of the diabetic patients who were negative for urinary tract infection, 30.8 % (44 patients) had been diagnosed for DM for 1 to 4 years prior to the study, 68.5 % (98 patients) between 5 to 8 years and 0.7 % (1 patient) between 9 to 12 years. There was a significant difference between the duration of diabetes mellitus and UTI ($p = 0.01$). As the duration of diabetes mellitus increased by one year, the probability of a participant to test positive for UTI category increased by 2.08 (odds ratio) times. This can be attributed to the decreased cellular activity of immune cells in diabetic patients.

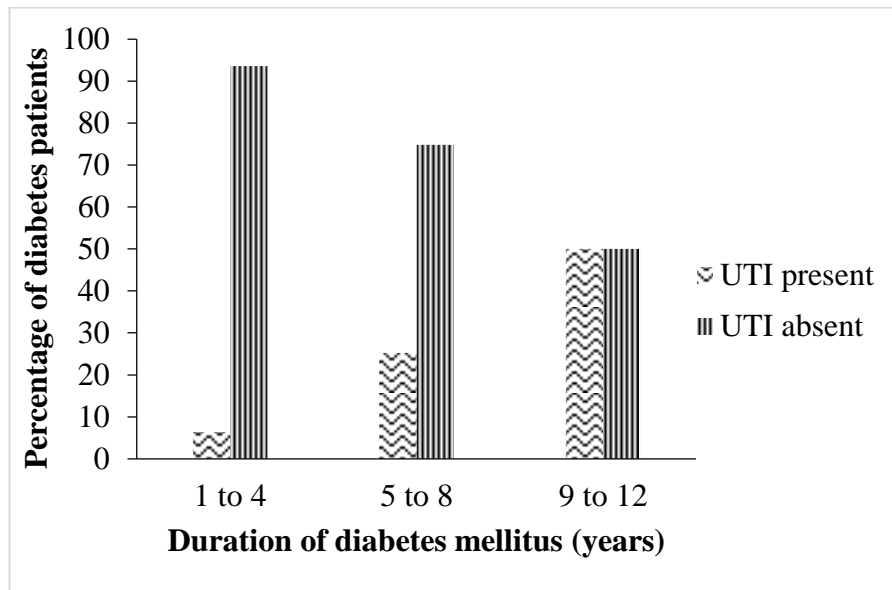


Figure 4.4: Distribution of urinary tract infections among diabetic patients by duration of diabetes mellitus

4.2.5 Urinary tract infection in non-insulin dependent diabetes mellitus patients by symptoms of urinary tract infections

The symptoms of urinary tract infections showed no significant difference in terms of urinary tract infections among diabetes patients tested ($\chi^2 = 33.783$; $df = 2$; $p = 0.50$). As shown in figure 4.5, among the diabetes patients who showed symptoms of urinary tract infections, 17.7 % (11 patients) tested positive for urinary tract infections, while 82.3 % (51 patients) did not test positive for urinary tract infections. Among diabetic patients that never exhibited symptoms of urinary tract infections, 22.0 % (26 patients) tested positive for urinary tract infections, while 78.0 % (92 patients) tested negative for urinary tract infections. Participants with UTI symptoms were 1.31 (odds ratio) times likely to test negative for UTI than those without the symptoms. This is because a number of the symptoms for UTI can be observed as a result of diet and physical activity and, therefore, not necessarily because one has a UTI.

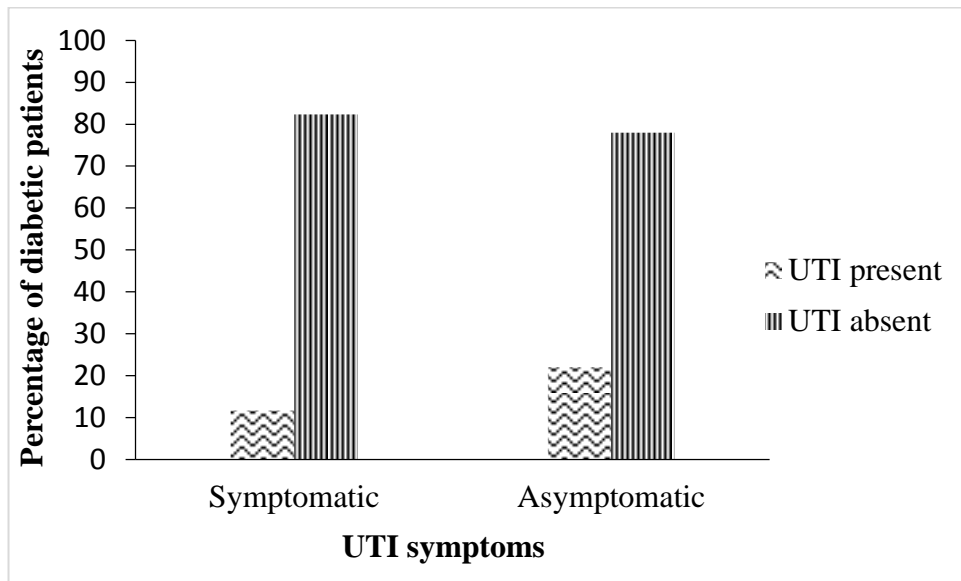


Figure 4.5: Distribution of urinary tract infection symptoms among study participants

4.3 Causative agents of urinary tract infections among non-insulin dependent diabetes mellitus patients

The bacterial isolates from diabetic patients that caused urinary tract infection included Gram-negative *E. coli*, *K. pneumonia*, *P. mirabilis* and Gram-positive *E. faecalis*. Identification of the bacteria started by observation of colonies obtained after culture. The bacteria colonies were either lactose fermenters or non-lactose fermenters. Lactose fermenters changed the colour of the media to yellow. A gram stain was then done to differentiate gram positive and gram-negative organisms. Biochemical tests were then carried out to isolate specific bacteria organisms according to the test results obtained.

Escherichia coli was identified by changing the colour of the medium to yellow confirming that it was a lactose fermenter. Gram stain test was negative and indole test was positive. Both urease and citrate were negative. In TSI, there was fermentation of sugar at the slant and butt of the tube with production of gas that was

observed in the cracks in the medium. *Klebsiella pneumoniae* was also a lactose fermenter but tested negative for indole test. Both citrate and urease test were positive. There was fermentation of sugar in TSI with the slant and butt both turning yellow. Gas was also produced as this was evidenced by cracks in the medium. *Proteus mirabilis* was identified as a non-lactose fermenter as the colour of the culture medium was translucent blue meaning lactose was not fermented. Gram stain and indole test were negative while citrate and urease tested positive. The slant in TSI was red meaning that sugar was not fermented but there was production of gas shown by cracks in the medium. The butt was also black, evidence that hydrogen sulphide was also produced. *Enterococcus faecalis* was identified as a lactose fermenter and gram positive. Catalase test was negative prompting the streptex rapid agglutination test that confirmed *E. faecalis*.

Among the bacteria which caused urinary tract infection in the diabetic patients, *E. coli* caused the majority of urinary tract infections at 56.8 % (21 isolates), followed by *K. pneumoniae* at 27 % (10 isolates), then *P. mirabilis* at 10.8 % (4 isolates) and finally *E. faecalis* at 5.4 % (2 isolates) (figure 4.6).

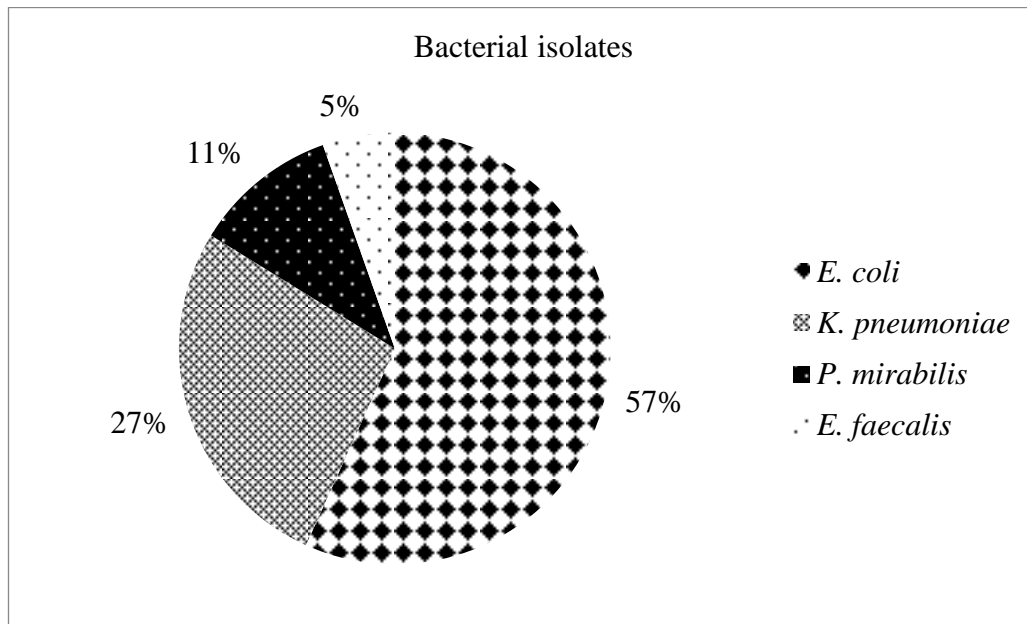


Figure 4.6: Percentage of urinary tract infection bacterial causative agents

4.4 Antibiotic sensitivity of Gram-negative bacteria causing urinary tract infection in non-insulin dependent diabetes mellitus patients

Among the antibiotics used, gentamicin was found to be the most effective drug as all the three gram-negative isolates were susceptible to it. Ampicillin was the least effective having five *E. coli*, two *K. pneumoniae* and one *P. mirabilis* isolates being resistant to it. The choice of drugs was based on the guidelines of managing UTI drafted by the hospital where the study was carried out.

4.4.1 *Escherichia coli* sensitivity to antibiotics

Majority of the *E. coli* isolates that caused urinary tract infections in diabetic patients were susceptible to ampicillin at 76.2 % (16 isolates) and resistant at 23.8 % (5 isolates). All the *E. coli* isolates (21 isolates) were susceptible to gentamicin and cephalexin drugs. The *E. coli* isolates were susceptible to nitrofurantoin and

cotrimoxazole drugs at 85.7 % (18 isolates) and resistant to nitrofurantoin and cotrimoxazole at 14.3 % (3 isolates) as indicated in figure 4.7 below.

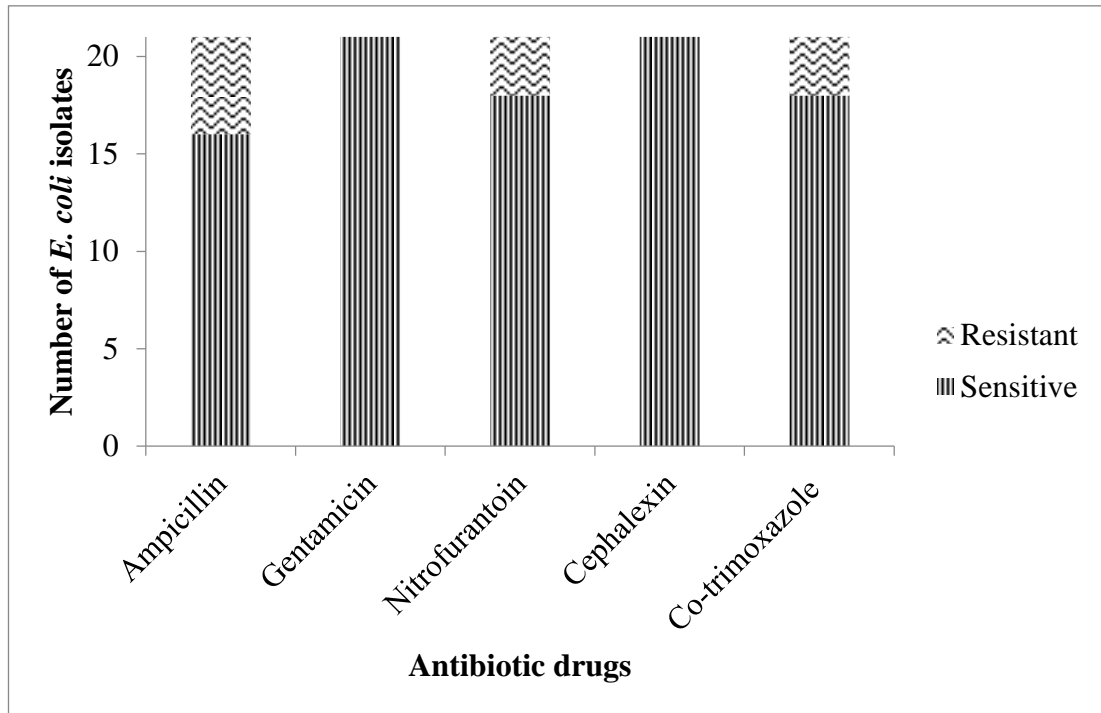


Figure 4.7: Sensitivity of *E. coli* to antibiotics

4.4.2 *Klebsiella pneumoniae* sensitivity to antibiotics

The *K. pneumoniae* isolates that caused urinary tract infections among the diabetic patients were susceptible to ampicillin and cotrimoxazole drugs at 80 % (8 isolates) and resistant to ampicillin and cotrimoxazole at 20 % (2 isolates) ($\chi^2 = 3.60$; $df = 1$; $p = 0.04$). All the *K. pneumoniae* isolates (10 isolates) were susceptible to gentamicin and nitrofurantoin drugs. The majority of *K. pneumoniae* were susceptible to cephalixin at 90 % and resistant to cephalixin at 10 % (1 isolate) as shown in figure 4.8.

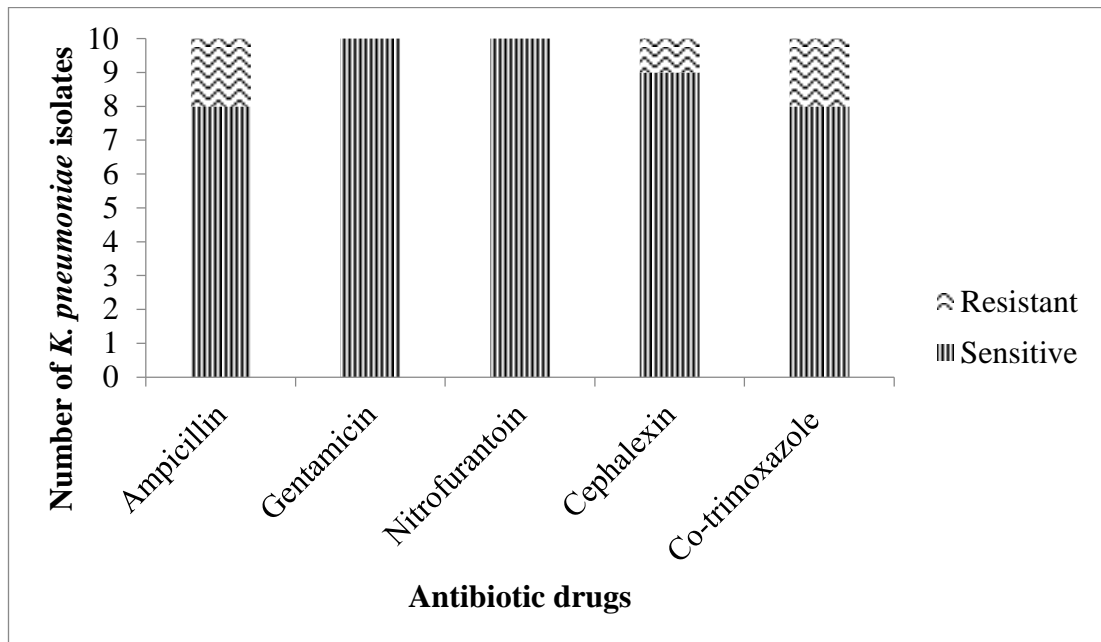


Figure 4.8: Sensitivity of *K. Pneumonia* to antibiotics

4.4.3 Sensitivity of *Proteus mirabilis* to antibiotics

The *P. mirabilis* isolates that caused urinary tract infections among diabetic patients were susceptible to ampicillin, Nitrofurantoin and cotrimoxazole drugs at 75.0 % (3 isolates) and resistant to ampicillin, nitrofurantoin and cotrimoxazole at 25.0 % (1 isolate). All the *P. mirabilis* isolates were sensitive to gentamicin and cephalixin (figure 4.9).

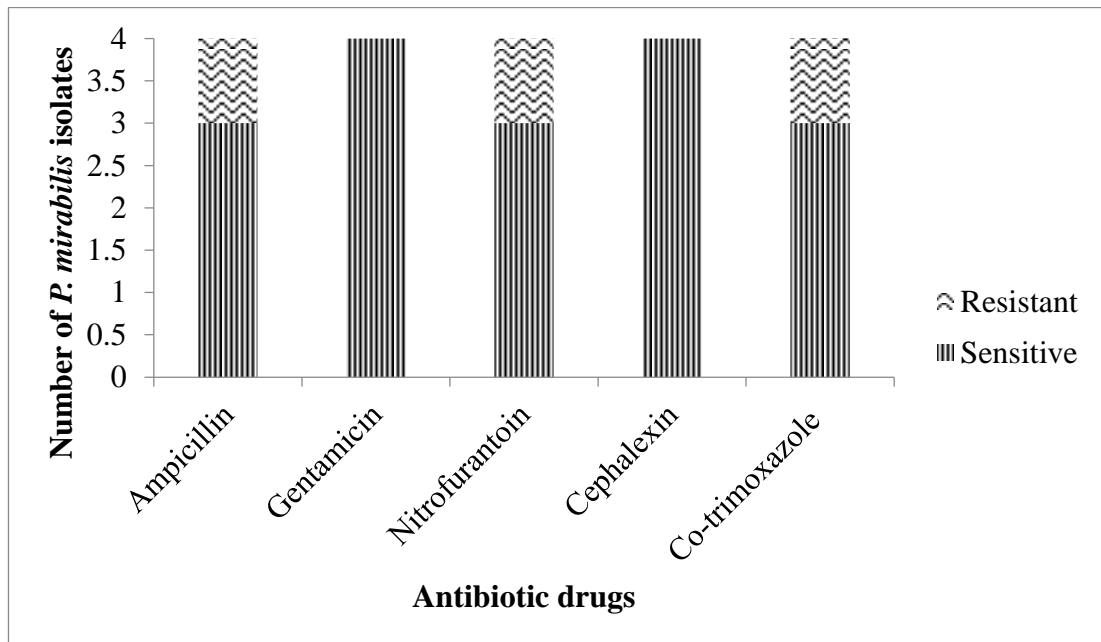


Figure 4.9: Sensitivity of *P. mirabilis* to antibiotics

4.5 Antibiotic sensitivity of Gram-positive bacteria causing urinary tract infections in diabetic patients

The two isolates of *E. faecalis* were tested for susceptibility to four antibiotics. There was 100 % susceptibility towards amoxicillin and nitrofurantoin whereas 50% susceptibility was observed to ampicillin and ciprofloxacin (Table 4.2).

Table 4.2: Sensitivity of Gram-positive bacteria to antibiotics

| ISOLATE | | ANTIBIOTIC AGENT | | | |
|--------------------------|---|------------------|---------------------------------|--------------------|-------------------|
| | | Ampicillin (%) | Amoxicillin-Clavulanic acid (%) | Nitrofurantoin (%) | Ciprofloxacin (%) |
| <i>E. faecalis</i> (n=2) | S | 1(50) | 2(100) | 2(100) | 1(50) |
| | R | 1(50) | - | - | 1(50) |

**KEY: S – Susceptible
R – Resistant**

4.5.1 Sensitivity of *Enterococcus faecalis* to antibiotics

All the *E. faecalis* isolates (2 isolates) were susceptible to Amoxicillin-Clavulanic acid and nitrofurantoin drugs. Of the *E. faecalis* isolates (2 isolates), one isolate was susceptible to ampicillin and the other resistant to ciprofloxacin (Figure 4.10).

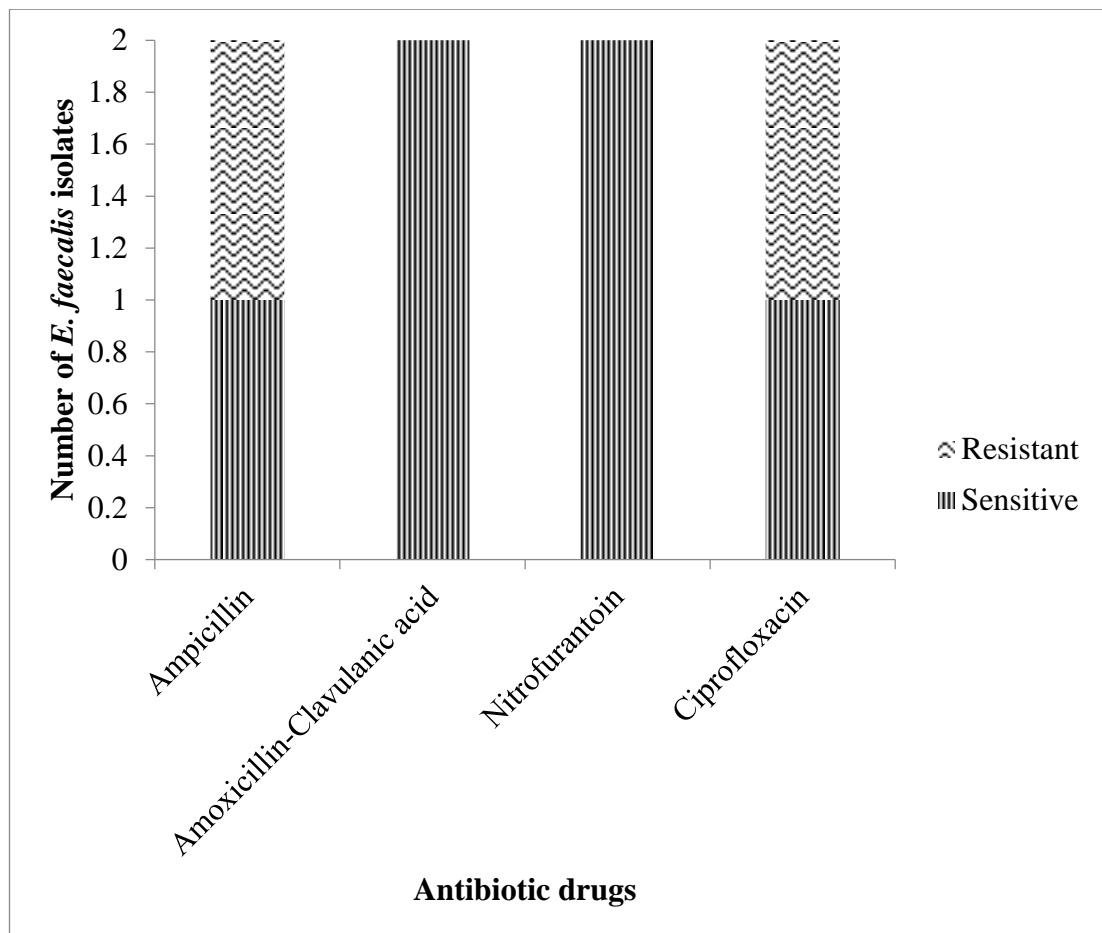


Figure 4.10: Sensitivity of *E. faecalis* to antibiotics

Findings from the study showed that majority of UTI cases are caused by gram-negative bacteria. It was also found out that gram positive can also cause UTI but in lower frequency when compared to gram-negative bacteria. A varying trend of

antibiotic resistance was also noted across different organisms and the available antibiotic drugs used.

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

This study determined the prevalence of urinary tract infections among diabetic patients. It further found out the bacterial causes of urinary tract infections and the antibiotic sensitivity patterns of these bacterial isolates to antibiotics.

The study found the overall prevalence of urinary tract infections among diabetic patients to be 20.6 % while the prevalence of ASB was found to be 22.0 %. These findings are higher when compared to a similar study carried out in KNH, where the prevalence was found to be 11 % (Kayima *et al.*, 1996). The study carried out in Kenyatta National Hospital was aimed at finding the prevalence of ASB among diabetics. The difference is likely because this study covered both asymptomatic and symptomatic cases. Findings from this study are comparable to those obtained from a study in Sudan where the overall prevalence of UTI among type 2 diabetic patients was 19.5 % (Hamdan *et al.*, 2015). This study's results gave a low prevalence of ASB when compared to a similar study conducted in Nigeria whereby ASB was estimated to be 36.1 % (Mubarak, *et al.*, 2012). The high prevalence in Nigeria could be attributed to emergence of antibiotic resistance bacteria that causes UTI in non-insulin dependent diabetes mellitus. Additionally, it is possible that the study population of diabetics could have also contributed to the high prevalence of urinary tract infection since diabetic patients are known to be highly susceptible to UTI.

These findings were lower compared to those obtained from a prospective study of UTI among diabetic participants carried out in India, whose prevalence was

estimated at 30.0 % (Nitzan *et al.*, 2015). Another study carried out in Netherlands also gave an approximate prevalence of ASB among diabetics at 26.0 % (Geerlings, 2008). This was also high when compared to the results obtained from this study. When the results obtained from this study are compared to those obtained from a similar study in Ethiopia, prevalence of UTI among diabetics is higher in this study than the one in Ethiopia, which had 10.4 % in ASB and 13.6 % in symptomatic bacteriuria among diabetics (Hamdan *et al.*, 2015). An older study population, which was also diabetic, could be a reason why the prevalence of UTI was higher in this study compared to other findings from previous studies. The immune system gets weaker with age and this predisposes an individual to UTI.

In comparison to the results obtained in this study, the prevalence of UTI among diabetics in America was low than what was obtained in this study. According to an American database study, it was found out that the prevalence of UTI type 2 diabetic patients is 12.2 % (Nitzan *et al.*, 2015). The variation in the prevalence of UTI among diabetics obtained from different studies could be because of the differences in the age among participants.

There was no statistical difference of gender distribution among participants in this study. More male than female participants tested positive for UTI, however, without statistical significance, 24 (22.4 %) vs. 13 (17.8 %), $p=0.45$. These findings are consistent with the findings of a study carried out in Sudan where the prevalence of UTI in males was higher than in females, 22.3 % vs 15.1 % (Hamdan *et al.*, 2015). However, the results of this present study contrast with results obtained in a study conducted in the United Arab Emirates (UAE) where gender was found to have a

statistical significance that diabetic females have a high prevalence of UTI than diabetic males 37.0 % vs. 34.0 % (Mubarak *et al.*, 2012). Another study contrasting findings of this present study was carried out in Saudi Arabia where it was found out that there was an association between gender and UTI among diabetics (Al-Rubeaan *et al.*, 2013). The age of participants may influence the vulnerability of an individual towards UTI. For instance, menopause is a contributing factor towards the risk of women contracting UTI. Hormonal changes at this stage facilitates colonization of the urogenital tract with pathogenic bacteria and hence development of UTI. This could contribute to the differences that have been observed in various studies especially concerning gender as a variable.

The age of participants in this study was statistically significant ($p=0.002$) in terms of the influence it had towards prevalence of UTI in diabetics. A study by Neil, 2011 also found out that the prevalence of ASB increases with age, a result that is consistent with the results of this study. In support of the findings of this present study, age was found to have an association with UTI in diabetic patients in a study carried out in Canada (Shah and Hux, 2003). An epidemiological study carried out in Sub-Saharan Africa found out that type 2 diabetes mellitus patients were at a higher risk of getting UTI than other groups of patients (Hall *et al.*, 2011). Another study that supports this finding was carried out in the UAE by Mubarak *et al.*, 2012 to determine the prevalence of UTI among diabetics and non-diabetics. The results demonstrated a significant association between UTI and age as well as an association between age and diabetes. Interestingly, this finding contrasts the finding by Hamdan *et al.*, (2015) in Sudan. It was found that there was no association between the investigated factors (patient age, duration and type of DM) and UTI (Hamdan *et al.*,

2015). Type 2 diabetes mellitus is an adult onset disorder and this means that age is a key risk factor for its development. Urinary tract infections are common in diabetic patients due to weakened immunity and defective physiological process (Flores-Mireles *et al.*, 2015). The relationship between age, type 2 diabetes mellitus and UTI is well supported by many studies that have confirmed the association of the three factors (Hall *et al.*, 2011; Mubarak *et al.*, 2012; Hamdan *et al.*, 2015).

According to the results of this study, the level of education showed no significant difference regarding the prevalence of UTI among the participants ($p=0.11$). The results were consistent with the findings of Hamdan *et al.*, 2015 in Sudan. Another study that also supported this finding was conducted by Nitzan *et al.*, 2015 while researching on UTI, its prevalence, diagnosis and management in Israel. The results did not find the level of education to be a risk factor for UTI in any way. Irrespective of the level of education an individual has attained, they are not at any higher risk of testing positive for UTI than those who have lower or higher level of education. Although the level of education did not show any significant difference in the prevalence of UTI, there was an interesting observation that was made in terms of prevalence of UTI and the level of education attained. There was a decreasing trend on the prevalence of UTI as the level of education attained moved higher. It is possible that attaining higher levels of education empowers an individual to seek medical attention earlier and maintain good habits that avoid contracting UTI.

Findings in this study revealed that there is an association between the duration of type 2 diabetes mellitus and UTI ($p=0.00$). The finding was consistent with the results obtained from a study in the UAE in a study to determine prevalence of UTI

among diabetics and non-diabetics (Mubarak *et al.*, 2012). The results showed that the longer the duration of diabetes (in years), the higher the risk of one having UTI. Shah and Hux, 2003 also had results consistent with the ones obtained in this study whereby they concluded that the duration of DM has an association with UTI in diabetics. Type 2 DM is known to weaken the immune system consequently making patients prone to UTI. According to these findings, it is possible that the longer one suffers from type 2 DM, the more their immunity is weakened and likely to test positive for UTI.

According to findings of this study, contrasting results were obtained when compared with those in Sudan and Saudi Arabia. While this study found out that there was an association between the duration of DM and UTI, studies by Hamdan *et al.*, 2015 and Al-Rubeaan *et al.*, 2013, found otherwise. However, Hamdan *et al.*, 2015 acknowledges that there are previous studies that have shown an association between the duration of DM and UTI. A longer duration of DM means that there is continued deterioration of the physiological functioning of the urinary system and consequently, increased chances of urinary tract infections. A longer duration of DM also translates to a weakened immunity considering the pathophysiology of diabetes.

The results obtained from this study did not show any significant association between symptoms of UTI and actual presence of UTI ($p=0.50$). Similar results were acquired in a study carried out in Sudan by Hamdan *et al.*, 2015. Results from the study in Sudan exclusively stated that symptoms of UTI were not risk factors of UTI. A study by Kayima *et al.*, (1996) conducted in KNH (Kenya) also concluded that there are no association between symptoms of UTI and actual infection with bacteria

that cause UTI. Parasitic infestation, fungal and viral infections may present with symptoms similar to those presented by UTI caused by bacteria. In this case, only bacteria isolates were tested and, therefore, it is possible that some participants presented with UTI symptoms with aetiologies other than bacteria. As a result, it is not accurate to say that individuals with symptoms of UTI are more likely to test positive for UTI caused by bacteria than those without the symptoms.

Both gram positive and gram-negative bacteria isolates were identified in this study. *Escherichia coli* was the predominant isolate with 21 isolates followed by *K. pneumoniae* and *P. mirabilis*. *Enterococcus faecalis* was only the only gram-positive isolate that was identified in the study. Kayima *et al.* (1996) also found the same result in their study in KNH when studying ASB in diabetic patients. In their findings, *E. coli* was the leading isolate (40.0 %) that was responsible for UTI in diabetic patients. Another study that obtained similar results was carried out in Sudan and found out *E. coli* to be the leading causative agent (56.4 %) of UTI in diabetic patients (Hamdan *et al.*, 2015). Another study that supports this finding was done in Greece where *E. coli* was isolated as the leading uropathogen (34.4 %) in both diabetic and non-diabetic patients (Papazafiropoulou *et al.*, 2009).

When comparing the prevalence *E. coli* isolated in this study with previous studies, the only finding that compares well is the one in Sudan (56.4 %). Other findings in KNH in Kenya (40.0 %) and Greece (34.4 %) recorded a lower prevalence. This finding could be so because *E. coli* is a normal flora in the GIT and the close proximity of the anal region to the urethra facilitates contamination. Introduction of *E. coli* onto sterile sites may lead to development of UTI. This finding could also be

attributed to the increasing prevalence of diabetes in the society. Non-insulin dependent diabetes mellitus predisposes individuals to UTI and an increase in the former means that UTI cases are also likely to be on the rise. Excretion of glucose into urine is the reason for the increasing cases of UTI in non-insulin dependent diabetes mellitus. Glycosuria provides nutrients for bacteria colonizing the urinary tract and this facilitates development of UTI. Additionally, glycosuria is also known to be an immune suppressor condition.

Isolation of *K. pneumoniae* in this study was comparable to previous studies. In their study in Sudan, Hamdan *et al.*, (2015) found out that the prevalence of *K. pneumoniae* was 23.0 %. This was slightly lower than the prevalence of the same pathogen isolated in this study (27.0 %). Another study conducted in Greece found *K. pneumoniae* to be prevalent at 8.3 % (Papazafiropoulou *et al.*, 2009). Increasing antibiotic resistance could be attributed to the observed high prevalence of *K. pneumoniae* in this study. *Klebsiella pneumoniae* is a known pathogen that is commonly isolated in respiratory infections. A comparison between the resistance patterns observed in a study carried in Sudan and this study showed an increased resistance trend. Use of antibiotics during respiratory infections could be attributed to the resistance *K. pneumoniae* develops against some of the antibiotics.

Proteus mirabilis was another uropathogen that was isolated in this study. Similar results were acquired in studies carried out in Sudan and Greece (Hamdan *et al.*, 2015; Papazafiropoulou *et al.*, 2009). The prevalence of *P. mirabilis* isolation was 7.65 % and 7.3 % in Sudan and Greece respectively. These findings are low compared to the prevalence of *P. mirabilis* isolation in this study (10.8 %). The high

prevalence acquired in this study can be attributed to the increasing vulnerability of diabetic patients over time. Other bacterial pathogens that were found in this study included *Enterococcus faecalis*, which is a gram-positive bacterium. This finding was consistent with the results Hamdan *et al.*, 2015 obtained in their study in Sudan. However, a comparison between the two results shows a high prevalence in Sudan (14.7 %) whereas that obtained in this study was 5.4 %. Gram-positive bacteria are not common uropathogens (Hamdan *et al.*, 2015). However, due to contamination, they have been found to always invade the urinary tract infection and cause UTI (Hirji *et al.*, 2012). Considering the fact that the target population for this present study was made up of diabetic patients only, it is possible that this explains the difference in the prevalence of bacteria isolates compared to other studies that coupled both diabetic and non-diabetic participants. Antibiotic resistance has also been observed in gram-positive bacteria. Over time, this is likely to facilitate colonization of the urogenital tract and development of UTI caused by gram-positive.

All isolated bacterial pathogens were tested for drug sensitivity with a given selection of antibiotics. Choice of antibiotics was guided by the guidelines of the health facility where the study was carried out. Gentamicin and cephalexin were the best performing antibiotics that were used in this study. All gram-negative isolates (100.0 %) were sensitive to gentamicin and cephalexin with an exception of one *K. pneumoniae* isolate that was resistant to cephalexin. Hamdan *et al.* (2015) in Sudan acquired a similar finding in their study. A few isolates showed resistance towards ampicillin and co-trimoxazole, a trend that is consistent with the results obtained in Greece (Papazafiropoulou *et al.*, 2009). It is also important to note that *E. coli* had the highest number of ampicillin resistance (23.8 %) in this study. This is consistent

with the findings of a study in Sudan where Hamdan *et al.*, 2015 found out *E. coli* resistance against ampicillin to be at 27.2 %. However, the result in Sudan is slightly higher when compared to the one obtained in this study (27.2 % vs. 23.8 %).

Four antibiotic agents were used to test for sensitivity of the isolated gram-positive bacteria. Both isolates (2) of *E. faecalis* were susceptible to Amoxicillin-Clavulanic acid and nitrofurantoin. This finding was identical to what was concluded in a similar study in Sudan where all isolates of *E. faecalis* (100.0 %) were found to be susceptible to Amoxicillin-Clavulanic acid and nitrofurantoin (Hamdan *et al.*, 2015). Out of the two *E. faecalis* isolates, one was resistant to ciprofloxacin and ampicillin. The resistance pattern of *E. faecalis* between the findings of this study and the results of the study in Sudan were inconsistent. While 50.0 % of the isolates were resistance to ampicillin and ciprofloxacin in this study, 20.0 % of the isolates were resistance to ampicillin and ciprofloxacin in Sudan (Hamdan *et al.*, 2015). There is an increase in the resistance of isolated bacterial organisms to available antibiotics.

Resistance of gram-positive isolates could be attributed to frequent antibiotic administration orally. Since most of the gram-positive bacteria are found in the GIT, continued oral ingestion of antibiotics is likely to contribute to the possible antibiotic resistance development (Sheerin, 2011).

5.2 Conclusions

1. The prevalence of UTI among non-insulin dependent diabetic patients in Kisii County is 20.6 %.
2. Among the bacterial isolates from the study, *Escherichia coli* was found to be the leading bacterial causative agent of UTI among non-insulin dependent diabetes mellitus patients.
3. Multi-drug resistance is a possible threat going by the rate of incidence of UTI among diabetics. This is likely to increase the burden of management of UTI among diabetic patients.

5.3 Recommendations

1. Urinary tract infections among non-insulin dependent diabetes mellitus patients should be diagnosed through urine culture as this will give help in isolating the bacterial causative agent, development of antibiograms and hence effective treatment.
2. Gentamicin should be used in treatment of gram-negative bacteria causing UTI among non-insulin dependent diabetes mellitus patients as all isolates were found to be susceptible to it.
3. Nitrofurantoin and amoxicillin clavulanic acid should be used in treatment of gram-positive bacteria causing UTI among non-insulin dependent diabetes mellitus patients as all isolates were found to be susceptible to it.

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APPENDICES

Appendix I: The map of Kisii County



Appendix II: Consent form

Title of the study

My name is Mageto Vincent Mogaka. I am a Masters student from Kenyatta University. I am conducting a study on “Determination of prevalence and socio-demographic characteristics of urinary tract infections and antibiotic sensitivity among non-insulin dependent diabetes mellitus patients visiting Kisii teaching and referral hospital in Kenya”. This information will be used by the ministry of health to improve access and quality for screening and treatment of urinary tract infections among diabetics in this hospital.

Procedures to be followed

Participation in this study will require that I ask you some questions and examine you in order to screen you for urinary tract infections. A urine sample will be taken from you for further tests.

You have the right to refuse participation in this study. You will get the same care and medical treatment whether you agree to join the study or not and your decision will not change the care you will receive from the clinic today or that you will get from any other clinic at any other time.

Please remember that participation in the study is voluntary. You may ask questions related to the study at the time.

You may refuse to respond to any questions and you may stop an interview at any time. You may also stop being in the study at any time without any consequences to

the services you receive from this clinic or any other organization now or in the future.

Benefits

If you participate in this study, you will help us to learn how to provide effective screening services that can improve the health of diabetic patients and reduce the risk of urinary tract infections. You will also benefit from being screened for urinary tract infections and if you are found to have a problem, you will be advised on the appropriate treatment.

Risks

Some of the questions you will be asked are personal and may make you uncomfortable. However, you may refuse to answer these questions if you choose to. The interview may also delay your medical services with approximately 20 minutes.

Confidentiality

The interviews and examinations will be conducted in a private setting within the clinic. Your name will not be recorded anywhere and the collected data will be kept safely. Everything will be kept private.

Withdrawal privilege

If you decide to withdraw from the study, then you are free to do so at any time without penalty or prejudice. This however will not have any consequences to the services you receive from this hospital now or in the future.

Contact Information

If you have any questions you may contact me through 0721 344 630 or Dr Scholastica Mathenge on 0722 936 884 or Dr Njoroge Wachuka on 0722 737 669 or the Kenyatta University Ethical Review Committee Secretariat on chairman.kuerc@ku.ac.ke, secretary.kuerc@ku.ac.ke, ercku2008@gmail.com.

Participants Statement

The above information regarding my participation in the study is clear to me. I have been given a chance to ask questions and my questions have been answered to my satisfaction. My participation in this study is voluntary. I understand that my records will be kept private and that I can leave the study at any given time. I understand that I will still get the same care and medical treatment whether I decide to leave the study or not and my decision will not change the care I will receive from the clinic today or that I will get from any other clinic at any other time.

Code of participant

Signature or Thumbprint Date

Investigator's Statement

I, the undersigned, have explained to the volunteer in a language s/he understands the procedures to be followed in the study and the risks and benefits involved.

Name of investigator

Investigator's signature..... Date

Appendix III: Questionnaire

**URINARY TRACT INFECTIONS AND ANTIBIOTIC SENSITIVITY
AMONG NON-INSULIN DEPENDENT DIABETES MELLITUS PATIENTS
VISITING KISII TEACHING AND REFERRAL HOSPITAL IN KENYA**

Code of participant

NB. Please mark the correct box with a tick (✓)

SECTION A: SOCIO-DEMOGRAPHIC CHARACTERISTICS

1. What is your gender?

Male

Female

2. How old are you? (In years)

20 – 25

26 – 30

31 – 35

36 – 40

41 – 45

46–50

51–55

56–60

61–65

66–70

3. What is your highest level of education attained?

University / College

Secondary School

Primary School

4. Do you have any history of UTI in the recent past? (Approximately 2 weeks)

Yes

No

5. Have you been under antibiotic therapy in the recent past? (Approximately 2 weeks)

Yes No SECTION B: CLINICAL CHARACTERISTICS

Have you experienced the following symptoms in the recent past? Please tick YES or NO accordingly.

1. A strong persistent urge to urinate

Yes No

2. A burning sensation when urinating

Yes No

3. Passing frequent, small amounts of urine

Yes No

4. Passing urine that appears cloudy

Yes No

5. Passing urine that appears red, bright pink or cola-coloured

Yes No

6. Passing strong smelling urine

Yes

No

7. Pelvic pain

Yes

No

Appendix IV: NACOSTI letter



NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

Telephone: +254-20-2213471,
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When replying please quote

9th Floor, Utalii House
Uhuru Highway
P.O. Box 30623-00100
NAIROBI-KENYA

Ref. No. **NACOSTI/P/17/88572/17706**

Date: **6th July, 2017**

Vincent Mogaka Mageto
Kenyatta University
P.O. Box 43844-00100
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on "*Determination of urinary tract infections and antibiotic resistance patterns among type 2 diabetes mellitus patients visiting Kisii Level 6 Hospital, Kenya,*" I am pleased to inform you that you have been authorized to undertake research in **Kisii County** for the period ending **6th July, 2018.**

You are advised to report to **the County Commissioner, the County Director of Education and the County Director of Health Services, Kisii County** before embarking on the research project.

On completion of the research, you are expected to submit **two hard copies and one soft copy in pdf** of the research report/thesis to our office.

**GODFREY P. KALERWA MSc., MBA, MKIM
FOR: DIRECTOR-GENERAL/CEO**

Copy to:

The County Commissioner
Kisii County.

The County Director of Education
Kisii County.

Appendix V: Ethical approval letter



KENYATTA UNIVERSITY
ETHICS REVIEW COMMITTEE

Fax: 8711242/8711575
Email: kuerc.chairman@ku.ac.ke
kuerc.secretary@ku.ac.ke
Website: www.ku.ac.ke

P. O. Box 43844,
Nairobi, 00100
Te: 8710901/12

Our Ref: KU/ERC/APPROVAL/VOL.1 (48)

Date: 11th May 2017

Mageto Vincent Mogaka
Kenyatta University,
P.O Box 43844,
Nairobi

Dear Mageto Vincent Mogaka

APPLICATION NUMBER PKU/610/1694 "DETERMINATION OF URINARY TRACT INFECTIONS AND ANTIBIOTIC SENSITIVITY AMONG NON-INSULIN DEPENDENT DIABETES MELLITUS PATIENTS VISITING KISI TEACHING AND REFERRAL HOSPITAL, KENYA.

1. IDENTIFICATION OF PROTOCOL

The application before the committee is with a research topic application Number PKU/610/1694 "Determination of Urinary Tract Infections and Antibiotic Sensitivity among Non-insulin dependent Diabetes Mellitus Patients visiting Kisi Teaching and Referral Hospital, Kenya" Received on 18th April 2017 and Approved on 8th May 2017

2. APPLICANT

Mageto Vincent Mogaka

3. SITE

KISI

4. DECISION

The committee has considered the research protocol in accordance with the Kenyatta University Research Policy (Section 7.2.1.3) and the Kenyatta University Review Committee Guidelines AND APPROVED that the research may proceed for a period of ONE year from 11th May, 2017.

ADVICE/CONDITIONS

- i. Progress reports are submitted to the KU-ERC every six months and a full report is submitted at the end of the study.
- ii. Serious and unexpected adverse events related to the conduct of the study are reported to this committee immediately they occur.
- iii. Notify the Kenyatta University Ethics Committee of any amendments to the protocol.
- iv. Submit an electronic copy of the protocol to KUERC.


When replying, kindly quote the application number above.

If you accept the decision reached and advice and conditions given please sign in the space Provided below and return to KU-ERC a copy of the letter.


DR. TITUS KAHIGA,
CHAIRMAN ETHICS REVIEW COMMITTEE



I, Mageth Vincent accept the advice given and will fulfill the conditions therein.

Signature  Dated this day of 18th May 2017.

Appendix VI: KTRH authorization letter

MINISTRY OF HEALTH



Telegramme "medical" Kisii
Telephone: (058) 31310 Kisii
Email: kisiihospital@gmail.com
Web: www.kisiihospital.org.ke

DEPARTMENT OF RESEARCH
THE KISII TEACHING & REFERRAL HOSPITAL
P.O. BOX 92
KISII

REF. NO.

DATE: 25th July, 2017

MAGETO VINCENT MOGAKA

RE: DATA COLLECTION

This is to inform you that the research department of Keumbu Sub- County Hospital, Kisii County has reviewed your proposal titled,

"Determination of prevalence and socio-demographic characteristics, of urinary tract infections and antibiotics sensitivity diabetes mellitus patients visiting Kisii Teaching and Referral Hospital in Kenya."

The following are our comments

- 1) You have been authorized to proceed with data collection upon payment of two thousand shillings (Kshs. 2,000/=).
- 2) Ensure confidentiality for your study subjects.
- 3) Ensure data collected is used for academic purposes only.
- 4) Ensure a copy of the final report is submitted to this office for retention and use.

For

DR. E.B. MASANTA -MBCHB (UoN), MPH (Epidem) (JOUST),
PGDPM (KIM) Applied Epidem & Bio (UoN).

FOR: CHIEF EXECUTIVE OFFICER
KISII TEACHING AND REFERRAL HOSPITAL

Appendix VII: Preparation of CLED media

1. Suspend 36 g of the medium in one litre of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 50°C, mix well and dispense into plates.
5. When the medium is solidified, invert the plates to avoid excess moisture.

The prepared medium should be stored at 8-15°C

Appendix VIII: Preparation of Mueller Hinton agar

1. Suspend 38gm of the medium in one litre of distilled water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes. Cool to room temperature.
4. Pour cooled Mueller Hinton Agar into sterile petri dishes on a level, horizontal surface to give uniform depth.
5. Allow to cool to room temperature.
6. Check for the final pH 7.3 ± 0.1 at 25°C.
7. Store the plates at 2-8 °C.

Appendix IX: Preparation of Christensens urea base broth

1. Suspend 24.01 grams in 950 ml distilled water.
2. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 115°C for 20 minutes.
3. Cool to 50°C and aseptically add 50 ml of sterile 40 % Urea Solution and mix well.
4. Dispense into sterile tubes and allow setting in the slanting position. Do not overheat or reheat the medium as urea decomposes very easily.

Appendix X: Preparation of Triple Sugar Iron agar

1. Suspend 64.42 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Mix well and distribute into test tubes.
4. Sterilize by maintaining at 115°C for 30 minutes.
5. Allow the medium to set in sloped form with a butt about 2.5cm long.

Appendix XI: Sample urine dipstick



Appendix XII: prepared CLED media plates