

**ANTIBIOTIC SUSCEPTIBILITY PATTERN OF BACTERIAL  
UROPATHOGENS ISOLATED FROM PATIENTS IN NAKURU LEVEL 5  
HOSPITAL, KENYA**

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the Degree of Master of Science (Microbiology) in the School of Pure and  
Applied Sciences of Kenyatta University**

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**DECLARATION**

This thesis is my own original work and has not been presented for the award of a degree in any other university.

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**APPROVAL BY SUPERVISORS**

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## **DEDICATION**

This research project is dedicated to my beloved wife Salome Tibi and our beloved children Victor, Grace, Martha, Daisy, Caleb and Ann for their support, encouragement and prayer during my time of my study.

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**ABBREVIATIONS AND ACRONYMS**

AMC	Amoxicillin-Clavulanic Acid
AIDS	Acquired Immunodeficiency Disease Syndrome
AMP	Ampicillin
AMR	Antimicrobial Resistant
API	Analytical Profile Index
ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
CEF	Cefotaxime
CFU	Colony Forming Unit
CIP	Ciprofloxacin
CLED	Cystine Lactose Electrolyte Deficient
CLSI	Clinical and Laboratory Standards Institute
CXC2	Chemokine Receptor 2
DNA	Deoxyribonucleic acid
GET	Gentamicin
HIV	Human Immunodeficiency Syndrome
HPF	High Power Field
IRIS	International Remote Imaging Systems
KUEC	Kenyatta University Ethical Committee
LPS	Lipopolysaccharide
MARI	Multiple Antibiotic Resistance Indices
MDR	Multidrug Resistance
MIC	Minimum Inhibitory Concentration
MSU	Mid-Stream Urine

NACOSTI	National Commission for Science, Technology and Innovation
NAG	N-Acetyl-Beta-Glucosaminidase
NAL	Nalidixic Acid
NCCLS	National Committee for Clinical Laboratory Standards
NIT	Nitrofurantoin
NGU	Non-Gonococcal Urethritis
OIF	Oil immersion field
PCR	Polymerase Chain Reaction
PMN	Polymorphonuclear Neutrophils
Spp	Species
SPSS	Statistical Package for the Social Sciences
SXT	Cotrimoxazole
TLR4	Toll-Like Receptor
UPEC	Uropathogenic <i>E.coli</i>
USA	United States of America
UTI	Urinary Tract Infection
WBC	White Blood Cell

**ABSTRACT**

Urinary tract infections (UTI) are bacterial infections encountered in the hospital and community and is preventable. It is among bacterial infection encountered with increasing antibiotic resistance to uropathogens, although there is availability of antibiotics. Despite the wide spread of antibiotics, it remains the common bacterial infections. Antibiotic susceptibility testing therefore provides information that allows clinicians to select the most appropriate antimicrobial drugs. Over the years, the UTIs antimicrobial resistance patterns have been changing. The study was carried out to establish the prevalence of bacterial isolates and their drug susceptibility patterns among the study population. A descriptive cross-sectional study was conducted in outpatients and inpatients presenting with symptoms of UTI. Purposeful sampling was used to obtain 385 respondents. Mid-stream urine sample were obtained from respondents using sterile bottles and bacterial isolates identification was done using biochemical tests. Culture and sensitivity pattern of uropathogens were determined using disc diffusion method. A questionnaire was administered to consenting respondents and data associated with risk factors was collected and analyzed at  $\alpha=0.05$ . Out of 385 urine samples 112 (29 %) patients were confirmed positive for UTI. The prevalence of UTI was higher among females (62.1 %) compared to males (37.9 %). *Escherichia coli* 66 (55 %) was the most predominant followed by *Klebsiella pneumoniae* 12 (10 %), coagulase negative staphylococci 25 (20.9 %), *Staphylococcus aureus* 11 (9.2 %) and *Proteus mirabilis* 6 (5 %). Antimicrobial profiles of *E. coli* strains showed the following susceptibility pattern to nitrofurantoin (100 %), cefotaxime (86.3 %), ciprofloxacin (83.3 %), gentamicin (81.7 %), ampicillin (45.3 %) nalidixic acid (48.5 %) and cotrimoxazole (44.1 %). Further 85% of the isolates were observed to be multidrug resistant, limiting treatment of UTIs with routinely used antibiotics. Hence, there is need for constant monitoring of antibiotic resistance for better management of patients on antibiotic treatment. In addition, the collected data could be use in determination of trends in antimicrobial susceptibility patterns and therefore assisting in policy formulation on the currently used antibiotics for management of UTIs.

## CHAPTER ONE

### INTRODUCTION

#### 1.1 Background of the study

Urinary tract infection (UTI) is the colonization of the urinary tract by pathogenic microorganisms. Infection is caused by fungi, bacteria and viruses. The infection has prolonged admissions in hospital, morbidity in general population and high financial cost implications to the patients (Ramakrishnan and Scheid, 2005, Prakash and Saxena, 2013). Majority of UTIs are caused by bacteria that are found in the bowel and live as normal flora and often result from faecal and perineal areas. These organisms are capable invading the tissues of the urinary tract and adjacent tissues causing lower urinary tract infections and upper tract infections (Shilpi *et al.*, 2012; Kumar *et al.*, 2013). UTI is a common condition that is found in very young children as well as older people (Tamber *et al.*, 2006; Manikandan and Amsath 2013). In general population and hospital set up, UTI is a common infection although there are new and more powerful antibiotics in use but bacterial resistance persists (Patel *et al.*, 2012). The spectrum of causative agents and their antimicrobial resistance pattern has been dynamic worldwide (Annapurna and Lakshmi, 2013).

Urinary tract infection may lead to life threatening complications and death (Gupta *et al.*, 2001). Urine culture is the most effective diagnosis of UTI and treatment (Onuoha and Fatokun, 2014). Lower UTI (cystitis) and upper UTI (pyelonephritis) are the two clinical entities mostly found in patients with symptomatic UTI. Lesions caused by UTI are severe and contribute to morbidity in the population resulting in loss of renal function, which leads to long-term illness (Lane and Mobley, 2007).

Urine pass through the urethra allows the entry of uropathogens into the urinary tract initiating an inflammatory response, colonize urine in the urethra and if not washed out during urination culminating into a bacterial infection. Due to their anatomical orientation: that is the short distance between the anus and vagina women are at a higher risk of getting UTIs (Foxman, 2010). A second re-infection occurs in about 50 % of all women with a first UTI within six months (Ehinmidu, 2003). Bacteria establish infection in the urinary tract only after overcoming possible elimination by normal flora during micturation and innate host defense mechanism in the bladder (Gupta *et al.*, 2001).

Only about 2-5 % of documented UTIs are acquired hematogenously and usually result from bacteremia caused by relatively virulent organisms such *Salmonella* spp. and *Staphylococcus aureus* (Karlowsky *et al.*, 2002). Common symptoms of UTIs include burning sensation during urination, loss of bladder control, increased frequency of urination especially in small amounts, low back pain, cloudy and bloody or foul-smelling urine (Onifade *et al.*, 2011).

Multidrug resistance should be monitored worldwide and surveillance systems should be used to determine the aetiology for UTIs (Kimando *et al.*, 2010). There is a worldwide setback in management of many bacterial infectious diseases due to antibiotic resistance. It is estimated that globally 26 % of deaths are due to infectious diseases such as UTIs of which 98 % occur in low income countries. Kenya is among the low-income countries thus bears impact of urinary tract infections (Wamalwa, 2013).

## 1.2 Problem statement

Being the second most common infectious disease in the community and hospitalized patients, UTI has globally affected over 150 million people per year which costs global economy more than 6 billion US dollars (Alemu *et al.*, 2012; Onuoha and Fatokun, 2014). Worldwide, infectious diseases cause a significant amount of financial burden and morbidity (Kolawole *et al.*, 2009; Tiruneh *et al.*, 2014). In the USA, about 7 million patients who visit the clinicians are diagnosed with UTI while more than 100,000 are hospitalized annually. In community and hospital acquired bacterial infections there has been a growing concern worldwide due to UTIs caused by multidrug resistant uropathogens (Radyowijati and Haak, 2003; Alemu *et al.*, 2012). There is pressure resulting from intensive and indiscriminate use of antibiotics in treatment leading to a rapid spread of antimicrobial agent resistance genes to uropathogens. A global concern is on the rise over rapid dissemination of drug-resistant bacteria creating serious complications on the treatment of infectious diseases. A major concern to clinicians is the increase in the number of resistant and multi-resistant strains of bacteria and the decline in the number of new antibiotics available for treatment of UTIs (Annapurna and Lakshmi, 2013).

The prevalence of UTI in Uganda was reported as 10 % (Mwaka *et al.*, 2011) while in Kenya it was 24 % (Kebira *et al.*, 2009) and in Kenyatta National Hospital, Nairobi was 26.7 % (Nabbugodi *et al.*, 2015). In Kenya, like any other developing country, antibiotics are sold as half doses by quacks mainly due to limited resources and most clinicians prefer empirical treatment of infections hence minimal microbiological samples are taken for culture and sensitivity. Kenya is at risk of developing bacterial resistance due to self-prescription and defaulting on medication. Patients who suspect

that they have UTI often get over the counter drugs without getting culture and sensitivity tests which lead to antibiotic resistant and may end up being a threat. In addition, there is over the counter treatment and widespread self-medication by the patients leading to development of multidrug resistance (Wamalwa, 2013).

In Nakuru Level 5 Hospital, there is lack of resources such as skilled manpower and laboratory facilities to efficiently carry out culture and sensitivity (Kariuki *et al.*, 2012). This often leads to overuse of antibiotics thus causing antibiotic resistance. Therefore there was need to establish the prevalence and susceptibility pattern of uropathogens in this study area. Susceptibility testing of antibiotics is of great important to reduce reoccurrence of infections of UTIs and reduce treatment failure. Therefore it important to isolate and identify uropathogens, common microorganisms causing UTIs and evaluate their susceptibility patterns of the isolates causing UTIs among patients visiting this study area. To date, there are few or no studies carried out describing the uropathogens and their susceptibility pattern among patients in Nakuru Level 5 Hospital.

This study aimed at describing the major pathogens causing UTI among patients, the prevalence of UTI and establish susceptibility pattern of antimicrobial resistance in Nakuru County. The data will be used in guiding on the most effective drugs of choice on treatment of UTIs and identifying the most prevalent uropathogen. On the light of this, the following study was carried out.

### **1.3 Justification of the study**

Routine culture and sensitivity is not carried out for UTI patients in hospitals of developing countries therefore antimicrobial agents are administered before laboratory results of urine culture are available (Kariuki *et al.*, 2012). This phenomenon creates multidrug resistance (Kariuki *et al.*, 2012; Annapurna and Lakshmi, 2013). The estimation of local etiology and susceptibility profile could support the most effective empirical treatment. Therefore, investigating epidemiology of UTIs (prevalence, risk factors, bacterial isolates and antibiotic sensitivity) is fundamental for care givers and health planners to guide the expected interventions. Thus, the aim of this study was to determine bacterial etiologic agent of uropathogens and evaluate their in vitro susceptibility pattern to commonly used antimicrobial agents (Momoh *et al.*, 2011). This is due to lack of awareness, low hygiene and poverty. Uses of expired drugs in circulation are on increase. There is need to update on the increasing rate of antibiotic resistance among uropathogens in Nakuru Level 5 Hospital and other hospitals and determine their antibiotic susceptibility patterns regularly.

### **1.4 Research Questions**

- i) What is the prevalence of UTIs among patients visiting Nakuru Level 5 Hospital?
- ii) What is the most common uropathogen that causes UTI among patients in Nakuru Level 5 Hospital?
- iii) What are the susceptibility patterns of UTI bacterial isolates to commonly used antibiotics among patients in Nakuru Level 5 Hospital?

## **1.5 Hypotheses**

- i) Prevalence of UTI among patients visiting Nakuru Level 5 Hospital is not known
- ii) Bacterial isolates from patients visiting Nakuru Level 5 Hospital are not susceptible to antibiotics.
- iii) Levels and spectrum of antimicrobial resistance is of isolates obtained from Nakuru Level 5 Hospital is not known.

## **1.6 Objectives of the Study**

### **1.6.1 General Objective**

To determine the prevalence of urinary tract infection, isolate uropathogens, and determine their antibiotic susceptibility patterns among patients in Nakuru Level 5 Hospital.

### **1.6.2 Specific Objectives**

- i) To determine the prevalence of UTI among patients in Nakuru Level 5 Hospital
- ii) To determine the most common bacterial isolate that causes UTIs among patients in Nakuru Level 5 Hospital.
- iii) To determine the antimicrobial susceptibility patterns of the identified bacterial isolates of UTIs among patients in Nakuru Level 5 Hospital

## **1.7 Significance of the study**

An infection in the urinary tract involves the lower or the upper part of urinary tract each giving different symptoms but abdominal pain is common for both of them. It is

a common problem among patients in Nakuru Level 5 Hospital. These patients with abdominal pain are commonly treated for urinary tract infection without urine culture leading to overuse of antibiotics and causing resistance. Therefore it is important to establish sensitive and specific ways of diagnosing UTI and determine the involved bacteria and their sensitivity pattern in this hospital. In this regard physicians should avoid empirical treatment in order to minimize development of multidrug resistance. The results of this study will assist the clinicians to administer antibiotics after culture and susceptibility patterns are carried out and therefore reduce multidrug resistance in Nakuru level 5 Hospital.

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 Infections of the Urinary Tract

Microbial colonization of the urinary epithelial cells as well as tissue invasion and multiplication of uropathogens is termed as urinary tract infection (UTI). This is one of the site of bacterial invasions and a number of women have recurrent UTIs at a particular point in their life. Implicated microorganisms, could be bacteria, fungi, protozoa and viruses. Usually bacteria are more prevalent and invasive. The symptoms of UTIs are dysuria, polyuria, burning feeling in the bladder, fever, nausea, flank pain; urine is milky and may have a foul smell (Momoh *et al.*, 2011).

In most parts of the Sub-Saharan Africa, as well as in other developing parts of the world, UTI is a health problem occurring among patients of all ages (Tula and Lyoha, 2014). For the last thirty years uropathogens have caused multidrug resistant in urinary tract and has become a growing concern worldwide (Mitta *et al.*, 2009). Studies have shown that drug resistance problem in Africa comes from factors like indiscriminate use of antibiotics, inappropriate advertisement of medicines, lack of awareness and prescription by quacks over the counters (Khoshbakht *et al.*, 2013).

Bacteriuria increases with age in both men and women but has a higher prevalence among the very young and very old. The prevalence of UTI is significantly higher for women than men until men attain the age of 60. Women aged 15-29 have the highest distributions of symptomatic infection (Foxman, 2010).

## **2.2 The prevalence of UTI**

The prevalence of UTI is usually higher in females than in males. The higher prevalence of UTI in females is attributed to the nature of their urinogenital tract; the urethra of the female is much shorter and closer to the anus than in males and it also lacks the bacteriostatic properties of prostatic secretions. The UTI occur highest in the sexually active age group. This may be a result of increased sexual activity with sexually active group which predisposes them to UTI. The gender related prevalence of uropathogens among the patients gram negative rods are main cause of UTIs in both sexes. They are usually found in the perineum of the large intestines as commensals (Ogbukagu *et al.*, 2016).

## **2.3 Transmission of uropathogens**

Microorganisms move from normal flora in the rectum, enter the urinary tract via the urethra into the bladder in healthy patients (Kalantar *et al.*, 2008). Uropathogens consequently colonize epithelium of the urethra in the ascending route. This route enhances acceleration of microorganisms in female patients who are soiling around the perineum, use urinary catheters and spermicidal agents (Foxman, 2010). About half of the infections ascend into the upper urinary tracts in patient with cystitis and infections of pyelonephritis which are caused by ascension of the bacteria from the bladder through the ureters and into the renal pelvic region (Patel *et al.*, 2012). Pregnancy and urethral obstruction aid in attachment of uropathogens inhibiting urethral peristalsis. Microorganisms enter the renal parenchymal cells through the collecting ducts and reach the pelvic region resulting in inflammation of the urinary tract (Manikandan *et al.*, 2011).

## 2.4 Etiologic Agents of UTI

The Gram-negative rods *E.coli*, *Proteus*, *Klebsiella*, *Pseudomonas aeruginosa* and other Enterobacteriaceae are mostly found in hospital. They are common cause of UTI in hospital because of their resistance to antibiotics (Manikandan *et al.*, 2011; Onuoha and Fatokun, 2014). *Klebsiella pneumoniae* strains cause lesions such as urinary infections, nosocomial infection, respiratory tract infection and wound infection. The family of Enterobacteriaceae especially in *Klebsiella* spp. and *E. coli* cause most nosocomial infections, including urinary tract infection and are known to be antimicrobial resistance (Alemu *et al.*, 2012). Acquisition of plasmid that encode for the production of extended spectrum  $\beta$ -lactamase (ES $\beta$ C) from cephalosporin and penicillin, by *K. pneumoniae* causes resistance to antibiotics mainly cephalosporins and penicillins, (Alemu *et al.*, 2012). *Proteus mirabilis* isolates cause severe UTIs leading to acute pyelonephritis, chronic inflammation and bacteremia. There is frequent infection by *P. mirabilis* in inpatients and outpatients due to contaminated hospital equipments which increase the risks of nosocomial infection in hospital staff (Muder *et al.*, 2005). Due to increased antibiotic resistance, it has become necessary to control the spread of *P. mirabilis* strain isolated from community infections and in hospital environment. *Proteus mirabilis* strains are usually resistance to  $\beta$ -lactams and with prolonged use of these drugs will increase their resistance which can be reduced by culturing and setting susceptibility testing and use of correct prescription of the right antibiotics (Maczynska and Kalemba, 2007).

Staphylococci coagulase-negative are thought to be contaminants and had insignificant importance but now are known to be the major cause of UTIs. Among the Gram-positive species, *Staphylococcus saprophyticus* has been associated with

young sexually active women (Sarathbaby *et al.*, 2013). *Staphylococcus epidermidis* and *Enterococcus* species are associated with UTI in hospitalized patients and are multidrug resistant are difficult to treat in addition to causing of native-valve and prosthetic-valve endocarditis (Sarathbaby *et al.*, 2013). Coagulase-negative staphylococci have shown resistance to many classes of antimicrobial agents including penicillin therefore leaving less options for use of vancomycin treatment as the last resort narrowing the choice of regimen to choose from in case of resistant strains of uropathogens (Nicolle *et al.*, 2013 ; Sarathbaby *et al.*, 2013).

*S. aureus* mainly causes UTI in hospitalized and catheterized patients increasing the risk of *S. aureus* carriage to the urinary tract leading to staphylococcal bacteremia (Muder *et al.*, 2006; Ikeagwu *et al.*, 2008). Most UTIs caused by *S. aureus* are asymptomatic but bacteriuria occurs concomitantly with long term urinary catheterization. *S. aureus* being a normal flora of the skin is isolated from the urine of asymptomatic patients and from a clinical urinary tract infection (Ikeagwa *et al.*, 2008). It occurs as a secondary invader in urinary tract and may lead to septicaemia which affects about 10% of the people (Sarathbabu *et al.*, 2013)

Urinary tract infection may be caused by viruses and fungi. Fungi, such as *Candida*, is the second most cause of nosocomial UTI in patients, it can be spread systemically and can be life threatening (Samuel *et al.*, 2012). Fungi infections are seen in patients who are on long term antibiotics, patients who are immune-compromised, or patients using invasive devices like IVs, and catheters. *Candida* and fungal infections are more prevalent in children with Urinary tract Anomaly (UTA); it is associated with infections after instrumentation of the urinary tract (Mehta *et al.*, 2013). The

prevalence of UTI due to *Candida* increases gradually with the duration of hospitalization. Treatment of Candiduria includes stopping antibiotics, removing or changing indwelling catheters, and starting antifungal therapy with anti-fungal agents like oral fluconazole, parental or intravesical amphotericin B. Viral UTI can be caused by adenoviruses types 11 and 21, polyomavirus BK, and herpes simplex viruses (Samuel *et al.*, 2012).

## **2.5 Bacterial Virulence Factors**

There are four main attributes of pathogens to display virulence; attachment, invasion, ability to damage the tissues of the host by toxins and capability of resisting host defense mechanism by encapsulation. Virulence of bacteria determines the level of infection and this determines its ability to invade the urinary tract. Virulence factors assists in attachment and invasion of the lower urinary tract by *E. coli* pathogenic strains and are present within bowel normal flora. They promote invasion and infection of urinary tract (Prakash and Saxen, 2013). Adherence of bacteria is dependent on three environmental factors; its own adhesive characteristics, epithelium receptive features and its pili (Alemu *et al.*, 2012).

Bacteria move and adhere to the mucosal surface of the host cell precipitating an inflammatory response. Attachment onto urinary tract is by adhesins found on the bacterial cell membrane and is responsible for initial invasion causing urinary tract infections (Foxman, 2010). *E. coli* infects and causes diseases of urinary tract express itself using adherence factors namely type 1 fimbriae and P fimbriae (Oladeinde *et al.*, 2011). Type 1 fimbriae extend from the surface of *E. coli* and other genera of the Enterobacteriaceae (Alemu *et al.*, 2012). Invasion of the urinary tract by *E. coli*

requires the fimbriae to bind to Mannose-containing oligosaccharide via the Fim H adhesive tip protein. Besides their primary function as adhesion molecules, type 1 fimbriae and P fimbriae also function to promote attachment and send signals to the epithelial cells leading to inflammation of host's epithelial cells (Ejaz *et al.*, 2006).

*E. coli* strains usually produce haemolysin and aerobactin. They are resistant to the bactericidal action of urinary tract tissues causing acute pyelonephritis and acute cystitis. Patients with structural or functional deformities of the urinary tract are generally susceptible to infections caused by bacterial strains that possess haemolysin and aerobactin (Oladeinde *et al.*, 2011). Other factors that may increase the risk for urinary tract infections include infrequent voiding, incomplete voiding, personal hygiene, and sexual activity, use of spermicidal contraception, genetics, hormonal status, diabetes and immunosuppressant substances (Komala and Kumar, 2013).

## **2.6 Pathogenesis and Pathology**

Healthy individuals produce sterile urine in the kidney which passes through the renal pelvic region and ureters where an infection is acquired by the ascending route from the urethra to the urinary bladder proceeding to the kidney leading to pyelonephritis (Oladeinde *et al.*, 2011). Women have a shorter urethra which predisposes them to infection than the males. The incidence of UTI particularly increases in sexually active women than among the abstinent women because tissue injury during sex. In adolescent males, UTIs are more common in the uncircumcised due to colonization of the inside of the prepuce and urethra (Kathleen, 2008; Alemu *et al.*, 2013).

Invasion and multiplication of uropathogens in the underlying epithelial cells leads to the establishment of a quiescent bacterial reservoir within the bladder tissue (Lewis, 2013). Uropathogens persist in the bladder epithelial cells for weeks therefore causing acute infection when they flux out of cell and colonize surrounding cells providing them with a mechanism to subvert host defense mechanisms (Foxman, 2010). The symptoms of UTI include dysuria, pain on urination, incontinence and polyuria. A full sensation in the rectum is experienced by men. Children with UTIs present with symptoms, such as irritability, incontinence, diarrhea, poor appetite, and fever (Kathleen, 2008).

## **2.7 Epidemiology**

UTIs are of two categories: community-acquired and hospital acquired. The hospital-acquired is associated with catheterization. Multidrug resistance increases both in developing and developed countries and is a global health problem. The distribution of antimicrobial resistance among different uropathogens differs between and within countries leading to selection of superior regimen of treatment in developed countries and raising the cost of treatment unaffordable to developing countries. When these resistant strains reach poor countries they may cause infections that are untreatable (Annpurna and Lakshmi, 2013).

Factors that contribute to the rapid emergence and spread of antimicrobial resistance include inadequate access to antibiotics, poor health care services, poverty, malnutrition (due low immunity) and incomplete doses of medicines that are routinely used. It is estimated that about 50 % of antibiotic use is inappropriate and therefore does not benefit the patients. The abuse of antibiotics increases selection pressure for

the emerging use of superior drugs which are expensive lower income earners who cannot afford them and therefore spread of antibiotic resistant (Oladeinde *et al.*, 2011).

## **2.8 Clinical Manifestations**

Urinary tract infections have traditionally been viewed as acute and often self-limiting infections. However, this concept has been challenged by recent findings demonstrating that an acute bladder infection results from a complex series of host pathogen interactions that can lead to bacterial invasion and persistence and that ultimately can determine the course of the infectious disease (Vila and Pal, 2010). In general, UTIs can be classified as asymptomatic bacteriuria, cystitis, or acute pyelonephrities. Cystitis predominantly involves colonization of the bladder (Oladeinde *et al.*, 2011).

Patients with cystitis usually report dysuria, frequency, urgency, and supra-pubic pain. The urine often becomes grossly cloudy and malodorous, and it is bloody in about 30 % of cases. White blood cells and bacteria can be detected by examination of un-spun urine in most cases. However, some women with cystitis have only  $10^2$  to  $10^4$  bacteria per milliliter of urine, and in these instances bacteria cannot be seen in a Gram stained preparation. Physical examination generally reveals only tenderness of the suprapubic area (Manikandan *et al.*, 2011).

The more severe upper urinary tract disease acute pyelonephrities involves colonization of the kidneys and represents an infection capable of progressing to bacteremia (Parham, 2005; Chenari *et al.*, 2012). Symptoms of acute pyelonephrities

generally develop rapidly over a few hours or a day and include fever, chills, nausea, vomiting, and diarrhea. Symptoms of cystitis may or may not be present. Besides fever, tachycardia, and generalized muscle tenderness, physical examination reveals marked tenderness on deep pressure in one or both costovertebral angles or no deep abdominal palpation. In some patient, signs and symptoms of gram-negative sepsis predominate. Most patients have significant leukocytosis and bacteria detectable in Gram-stained un-spun urine. Leukocyte casts are present in the urine of some patients, and the detection of these casts is pathogenic. Hematuria may be demonstrated during the acute phase of the disease; if it persists after acute manifestations of infection have subsided, a stone, a tumor, tuberculosis should be considered (Emiru *et al.*, 2013).

## **2.9 Laboratory Diagnosis of Urinary Tract Infections**

Diagnosis of UTI includes examination of urine for the presence of uropathogens. Technique for specimen collection is important in order to avoid contaminants (WHO, 2000; Annpurna and Lakshmi, 2013; Mehta *et al.*, 2013). It is also defined by the presence of more than  $10^5$  colony forming units (CFUs/ml) of single bacteria in cultured urine. Accurate diagnosis and treatment of UTI is essential to limit its associated morbidity, mortality and to avoid prolonged or unnecessary use of antibiotics (Piranfar *et al.*, 2014).

Evaluation of UTI relies on both laboratory investigations and clinical signs and symptoms and investigations include both urinalysis and urine culture. Diagnosis of UTI is not always straight forward because one cannot look at its appearance and conclude an infection (Kolawole *et al.*, 2009). Midstream urine sample is needed to confirm the diagnosis. Urine normally has no organism present, or only very few

(Mehta *et al.*, 2013). Ideally, the sample of urine should not come into contact with skin or other materials that may contaminate it with other bacteria ( Annpurna and Lakshmi, 2013; Mehta *et al.*, 2013). Adults and older children can do this by a midstream collection of urine. In young children, the usual way is to catch some urine in the specimen bottle whilst they are passing urine. In babies one method is to place a specially designed absorbent pad in a nappy (supplied by a doctor). Urine is sucked into a syringe from the wet pad. Another method is to use a plastic bag that sticks on to the skin and collects urine (Piranfar *et al.*, 2014).

Contamination poses frequent challenge depending on the method of collection used, thus a cut off of  $10^5$  CFU/ml is used for “clean catch” mid-stream sample. The use of “urine bags” to collect samples is discouraged due to the high risk rate of contamination (WHO, 2000). There is considerable evidence of practice variation in use of diagnostic tests, interpretation of signs or symptoms and initiation of antibiotic treatment such as drug selection, dose, duration and route of administration. Urine culture should be obtained for diagnosis of UTI in patients if there is high clinical suspicion, cloudy urine, or positive urine dipstick (Piranfar *et al.*, 2014). Prophylactic antibiotics may be used to reduce the risk of recurrent UTI (Mehta *et al.*, 2013).

### **2.9.1 Automated and Semiautomated Systems**

Automated screening systems are used for a large output with minimal labour and a rapid turn-around time compared with conventional cultures. These methods are expensive and often these costs can be justified only in laboratories that receive many samples (Emiru *et al.*, 2013). Several automated or semiautomated urine screening systems that are either bacterial growth independent or dependent are commercially

available. By examining images of un-centrifuged urine samples using a video camera one is able to recognize many cellular structures, including leucocytes, erythrocytes, epithelial cells and microorganisms. A walk-away robotic instrument has been introduced for urine screening using fluorescent stain probes to detect bacterial membrane from urine sample. After staining, the membrane is examined using fluorescent microscopy imaging technology to detect the presence of uropathogens in urine. Although this method is faster there is a need to culture negative urine specimens to eliminate few organisms which may not have been detected by this method (Parham *et al.*, 2005). The Coral UTI Screen system uses a somatic-cell which releases the adenosine triphosphate (ATP). On the contrary, in bacterial cells the bacterial ATP remains protected within the bacterial cell. This then is liberated and detected by the instrument which is directly proportional to number of present bacteria. This test has a sensitivity of 86 % and specificity of 76 % (Parham, 2005; Chenari *et al.*, 2012).

### **2.9.2 Molecular identification of uropathogens**

Urine samples are collected from UTI patients with clean catch midstream technique. The samples are centrifuged and cotton swabs used for inoculation of brain heart infusion broth. The media then incubated for 3h at 37°C and the culture saved in refrigerator for deoxyribonucleic acid (DNA) extraction purpose (Ibraheam *et al.*, 2016). DNA is extracted from brain hearth infusion broth by participation of bacteria by 7000rpm/min. and extraction using genomic DNA kit (Geneaid, China). Polymerase chain reaction (PCR) assay is performed to detect *Lac Z* gene which is specific for the identification of *E. coli*. PCR reaction is conducted in 50µl of reaction mixture containing 25µl of green master mix, 2µl of each primer, 10µl DNA template

and 11µl of deionized water. Amplification is conducted using thermocycler Eppendorf programmed cycler for initial denaturation at 94°C for 3 min., 35 cycle of denaturation at 94°C for 30sec., annealing 59°C 30sec., extension 72°C 30sec, and 7min. of final extension at 72°C. PCR products were resolved by electro-phoresis on 2% w/v analytical grade agarose gels (Promega, USA) stained by ethidium bromide, with the use of 100 bp DNA ladder from (Intron, Korea) visualized using UV transeliminators and documented using digital camera (Sony, Japan) and run in TBE (1X) buffer, Gels were stained with ethidium bromide (0.5 µgml<sup>-1</sup>) and analyzed using UV eliminator. The molecular weight identification of resolved band was based on their correspondence to the ladder bands (Ibraheem *et al.*, 2016). *E. coli* represent the highest UTI's causal organisms among other causal organisms in both classical culture method (66%) and in PCR method (60%). Most of the bacteria which often seen in UTI are fecal bacteria, these bacteria were mostly found in feces, while anaerobic bacteria rarely cause UTI. Most of UTI (90%) in patient with normal anatomic structure are caused by *E. coli*, 10-20% of UTI infection is caused by *Staphylococcus saprophyticus* (young sexually active females) and 5% is caused by *Enterobacter* (Emiru *et al.*, 2013).

### **2.9.3 Urine collection**

Laboratory urine specimens are classified by the type of collection conducted or by the collection procedure used to obtain the specimen. Random is the specimen most commonly sent to the laboratory for analysis, primarily because it is the easiest to obtain and is readily available. This specimen is usually submitted for urinalysis and microscopic analysis, although it is not the specimen of choice for either of these tests (Samuel, 2012). Specimen can sometimes give a random inaccurate view of a

patient's health if the specimen is too diluted and analyte values are artificially lowered. Pediatric specimens, who routinely undergo chemistry and microscopic analysis, are generally of this type. First morning specimen is the specimen of choice for urinalysis and microscopic analysis, since the urine is generally more concentrated (due to the length of time the urine is allowed to remain in the bladder) and, therefore, contains relatively higher levels of cellular elements and analytes such as protein, if present. Also called an 8-hour specimen, the first morning specimen is collected when the patient first wakes up in the morning, having emptied the bladder before going to sleep. Catheterization is done for patient who is uncooperative or unable to void, but introduction of bacteria in the bladder occurs at 1-2% (Otajevwo, 2013).

Midstream clean catch specimen is the preferred type of specimen for culture and sensitivity testing because of the reduced incidence of cellular and microbial contamination. Patients are required to first cleanse the urethral area with a soap. The patient should then void the first portion of the urine stream into the toilet. These first steps significantly reduce the opportunities for contaminants to enter into the urine stream. The urine midstream is then collected into a clean container (any excess urine should be voided into the toilet). This method of collection can be conducted at any time of day or night (Parham *et al.*, 2005). Catheter collection specimen is conducted when a patient is bedridden or cannot urinate independently (Patel *et al.*, 2012).

For infants and small children, a special urine collection bag is adhered to the skin surrounding the urethral area. Once the collection is completed, the urine is poured into a collection cup or transferred directly into an evacuated tube with a transfer straw. Urine collected from a diaper is not recommended for laboratory testing since

contamination from the diaper material may affect test results (Otajevwo, 2013). Urine samples must be tested immediately after collection, but if urine cannot be tested and cultured within 4 hours of collection, the sample should be refrigerated or preserved with boric acid (NICE, 2012). Urine can be stored in the refrigerator for up to 24 hours (Tonagho and Mcaninch, 2004).

Microscopic examination is performed by preparing a gram stain that indicates the morphology of the organism. The presence of one organism per oil-immersion field in an uncentrifuged sample correlates with 100,000 bacteria/ml. White blood cells  $> 10$  WBC/mm<sup>3</sup> it only signifies the presence of inflammation. Sterile pyuria is associated with urinary tuberculosis, chlamydial, and fungal infections. Hematuria, non-specific, may indicate other disorders such as calculi or tumor. Proteinuria is found in the presence of infection. Dipstick test for nitrite; bacteria in the urine reduce nitrate to nitrite and false negatives may be caused by *Pseudomonas* that do not reduce nitrate. leukocyte esterase dipstick test indicates presence of enterobacteria in urine. One organism per oil immersion field correlates with 100,000 CFU/ml by culture (Parham *et al.*, 2005).

## **2.10 Treatment of UTIs**

Antibiotic treatment should be used when culture results become available to avoid drug resistance and therefore antimicrobial sensitivity test should be used to direct therapy (Beyene and Tsegaye, 2011). Management of uncomplicated UTIs should be done on two important principle organisms especially *E.coli* which accounts for more than half of all urinary isolates and *Staphylococcus saprophyticus* which accounts for

less than a quarter of the urinary isolates. Nosocomial and uncomplicated community acquired UTIs rate the highest in antibiotic resistance (Soon and Gupta, 2012).

In the treatment of UTIs the following antibiotics are recommended ciprofloxacin, ofloxacin and trimethoprim-sulfamethoxazole (TMP-SMX). Their efficacy is seen when given for 3 days to treat acute symptomatic and uncomplicated lower urinary tract infections (Hilbert, 2011). To reduce UTIs, guidelines recommend that TMP-SMX be used for empirical treatment of uncomplicated UTI unless it's resistance in a community exceed 10 % to 20 % cutoff which is related to clinical and economic considerations of the patients. Beta-lactams antimicrobial agents are used to treat fluoroquinolone-resistant bacteria and trimethoprim-sulfamethoxazole-resistant bacteria for patients with uncomplicated UTIs caused by uropathogens. The combination of these drugs showed that the clinical outcomes and clinical cure rates were effective to more than 60 % (Perfetto *et al.*, 2004).

Fluoroquinolone antimicrobial agents are the drugs of choice for the management of UTIs. These drugs have a high microbiological and clinical cure rates and low rates of resistance among most common urinary pathogens (Schaeffer, 2002). They are prescribed for the management of acute uncomplicated UTIs and complicated UTIs, in patients of all ages (Schaeffer, 2002). Ciprofloxacin (fluoroquinolone) is widely used and has high efficacy against uropathogens (Blondeau, 2004). Fluoroquinolones are administered twice daily and therefore help in patients' adherence. Their properties include a broad spectrum of coverage, low rate of bacterial resistance and few side effects (Schaeffer, 2002).

Ciprofloxacin can be available for administration as immediate-release tablet, extended-release tablet, an oral suspension and solution forms. It rapidly distributes into the tissues after a loading dose administration and is maintained within a 24 hour dosage interval. Immediate release formulation of ciprofloxacin used is effective for conventional therapy of uncomplicated UTI and pyelonephritis improving the patient's adherence to treatment and thereby reducing the risk of re-infection, recurrence and emergence of multidrug resistance UTIs (Blandeau, 2004).

Other first line agents include nitrofurantoin and fosfomycin which are effective and safe. Their range of activity has proven to be highly effective (Maczynska and Kalemba 2007; Manikandan *et al.*, 2011). Nitrofurantoin is a commonly prescribed antimicrobial agent because it does not have cross-resistance with other uropathogen drugs and its widespread use is justified for routine use. The following factors should be considered in the selection of antibiotics: pharmacokinetics, spectrum of activity, resistance to community, potency of the drug, side effects, adverse effects and duration of therapy (Manikandan *et al.*, 2011).

### **2.11 Antimicrobial Resistance**

Factors favouring antimicrobial resistance are mutations, acquiring new genetic material, exposure to cells with new genetic material and use of antimicrobial agents as growth promoters in animal feeds destined for human consumption give rise to multidrug resistance. However, misuse of antimicrobial agents has led to a post antibiotic era which is a current situation worldwide (Vila and Pau, 2010).

Global data shows an increasing multidrug resistance among uropathogens to conventional drugs. Drug resistance has emerged even to newer and more potent antimicrobial agents. The resistant pattern has a regional variability and has been changing continuously due to excessive use of antibiotics. However, many studies have indicated the presence of multidrug resistance in organisms causing UTIs is increasing. High multidrug resistance is due to the mal-administration of antibiotics, incorrect use of antibiotics for the prophylaxis of recurrent UTIs and use of drugs over the counter without prescription of the clinicians. However, many studies have indicated the presence of multidrug resistance in organisms causing UTIs (Tula and Iyoha, 2014).

The worldwide drug resistance is increasing for both developing and developed countries but in developing countries is multiplied and increased. There is little or no infection control due to financial constrains to implement accepted guidelines related to susceptibility testing in these countries (Pecoul *et al.*, 1999). This compounded by low general education level of the population and their lower standards of living has led to misuse of antibiotics and therefore leading to drug resistance (Vila and Pau, 2010).

Omulo *et al.*, 2015 describes the emergence of AMR as interplay of human being, ecological and pathogen-related factor. In sub-Saharan Africa, high prevalence of UTIs infections has increased the claim for antimicrobial therapies both for prophylaxis and management. However, shortfalls in the healthcare environment ranging from inadequate diagnostic capacity and resources, over the counter access to antibiotics, constrained access to health facilities and limited education with respect to

antibiotic use (Alliance for the Prudent Use of Antibiotics (APUA), 2011; Shears, 2001) have increasingly contributed to the demand for antibiotics. Over use of antibiotics in the animal feeds and management is also contemplated to contribute to antibiotic resistance in humans although little is known of their contributions especially in Sub-Saharan Africa. Our ability in Kenya to assess these contributions is limited largely by little or no surveillance on antibiotic use both for curative or prophylaxis. Unfortunately, only limited resources have been devoted to researching of this problem (Global Antibiotic Resistance Partnership (GARP)-Kenya Working Group, 2011).

## **2.12 Factors influencing resistance of antibiotics**

The factors that contribute to multidrug resistance of uropathogens to antibiotics in low income countries:

- i) **Low potency of drugs:** The antibiotics issued in these countries have low potency because of degradation or alteration of the drug dose or because of the presence of a lower concentration of active ingredient (Lewis *et al.*, 2013). For instance, standard concentration of ampicillin and tetracycline has been found in low concentration in Algeria (Reyes *et al.*, 1997). In addition, expired drugs with altered or removed expiry dates have been detected in developing countries (Pecoul *et al.*, 1999). Some drugs produced in industrialized countries have been found to have expired on distribution in developing countries (Reyes *et al.*, 1997). Finally, the antibiotics provided to these countries may be poorly transported and stored, leading to inactivation and therefore having low potency (Pecoul *et al.*, 1999).

- ii) Limited laboratories that can do culture and sensitivity: Few hospitals in these countries perform urine culture for diagnosis of uropathogens. World health organization guidelines and quality control for susceptibility testing service is not available or affordable. The information about the etiology of infectious diseases or antimicrobial susceptibility is not available. Bacterial infections are treated empirically with broad-spectrum antibiotics. In order to control of multidrug resistance in the laboratories they should have capacities to isolate and identify isolates. These methods needs be standardized and data on multidrug resistance compiled so that a policy can be developed. Structures should be put in place in local, regional and national laboratories with equipments and competencies that can carry these tasks (Vila and Pal, 2010).
- iii) Buying of antibiotics over the counter: Antibiotics are bought without prescription in drug chemists, local shops, supermarkets and from street vendors in most developing countries. Most drugs are costly and most patients purchase incomplete doses and they discontinue treatment when the symptoms disappear (Sosa *et al.*, 2012).
- iv) Use of antibiotics as growth promoters in animal feeds: Growth promoters in animals feeds, poses a risk to human health due to bacterial drug resistance transferred to humans through the food chain (Sosa *et al.*, 2010).

To improve this situation, medical practices should well be understood and one thorough research should be conducted in this area in developing countries (Byarugaba, 2004). Health education can be used to improve on correct use of antibiotics and consequences of low dosage should be explained, correct on mistrust and unfounded believes concerning antibiotics (Radyowijati and Haak, 2003). The

government should put heavy penalties on unauthorized dealers who sell antibiotics to uninformed patients. The health care providers should only prescribe antibiotics after getting culture reports to help in correct diagnosis in the developing countries. This is a moral obligation for both industrialized and developing countries to help reduce the emergence and spread of antibiotic resistance (Vila and Pal, 2010).

Drug resistance is still a global setback in management of many bacterial infectious diseases. The burden is more pronounced in developing countries where infectious diseases are rampant. It is estimated that globally 26 % of deaths are due to infectious diseases of which 98 % occur in developing countries. East Africa and Africa in general consist of low-income countries thus bear the highest encumbrance of impact of infectious diseases. In East African countries, like many developing countries, there is uncontrolled use of antibiotics mainly because of limited resources. Most clinicians opt for rational treatment of infections hence minimal bacteriological samples are taken for culture and sensitivity analysis before initiating patient treatment. In addition there is high extensive over the counter treatment with widespread self-medication by community. With convenience of travel across the world, this does not only remain an East African problem but rather a worldwide drawback particularly because these countries are frequently visited by citizens from high-income countries for reasons such as tourism, business and diplomatic reasons. Therefore, they can easily acquire resistant strains of microorganisms and carry these strains to their countries of origin (Wamalwa, 2013).

### **2.13 Mechanisms of action of antimicrobial agents**

In order to appreciate the mechanisms of resistance, it is important to understand how antimicrobial agents act. Antimicrobial agents act selectively on vital microbial functions with minimal effects or without affecting host functions. Different antimicrobial agents act in different ways. The understanding of these mechanisms as well as the chemical nature of the antimicrobial agents is crucial in the understanding of the ways how resistance against them develops. Broadly, antimicrobial agents may be described as either bacteriostatic or bactericidal. Bacteriostatic antimicrobial agents only inhibit the growth or multiplication of the bacteria giving the immune system of the host time to clear them from the system. Complete elimination of the bacteria in this case therefore is dependent on the competence of the immune system (Samuel, 2012). Bactericidal agents kill the bacteria and therefore with or without a competent immune system of the host, the bacteria will be dead. However, the mechanism of action of antimicrobial agents can be categorized further based on the structure of the bacteria or the function that is affected by the agents. These include generally the following:

- Inhibition of the cell wall synthesis
- Inhibition of ribosome function
- Inhibition of nucleic acid synthesis
- Inhibition of folate metabolism
- Inhibition of cell membrane function (Byavugaba, 2010).

### **2.13 Prevention of UTI and control**

Creating awareness to women on the effects of frequently using low dose antibiotics to treat symptomatic UTIs and prevent recurrent infections will be of great importance. Women have high risk of contracting recurrent UTI than men and they are advised use a single dose of trimethprime-sulfamethoxazole (160/800mg) before and soon after intercourse. Other antibiotics used for prophylaxis for recurrent UTIs are Norfloxacin and Fluoroquinolone. They can only be used after bacteriuria has been eradicated with a full dose treatment regimen (Saint and Chenoweth, 2003).

UTI can be prevented by regular intake of fluids which can help flush microorganisms from the urinary system. The individual should urinate when the urge arises to avoid multiplication of microorganisms when urine stays for long period in the bladder. Females should wipe from front to back after visiting toilet to prevent fecal flora microorganisms entering urethra. Tight-fitting jeans and nylon under wears trap a lot of moisture and hence encourage multiplication of microorganisms leading to UTI instead cotton underwear (Soon and Gupta, 2012).

### **2.16 Susceptibility to antimicrobial agents**

In spite of the availability and use of the antimicrobial drugs, UTIs caused by bacteria have been showing increasing trends in recent years. Much of the increase has been related to emerging antibiotic resistance among urinary tract pathogens. Increasing multidrug resistance in bacterial uropathogens is an important and evolving public health challenge. Accurate bacteriologic records of culture results provide guidance on empirical therapy before sensitivity patterns are available. Since most UTIs are

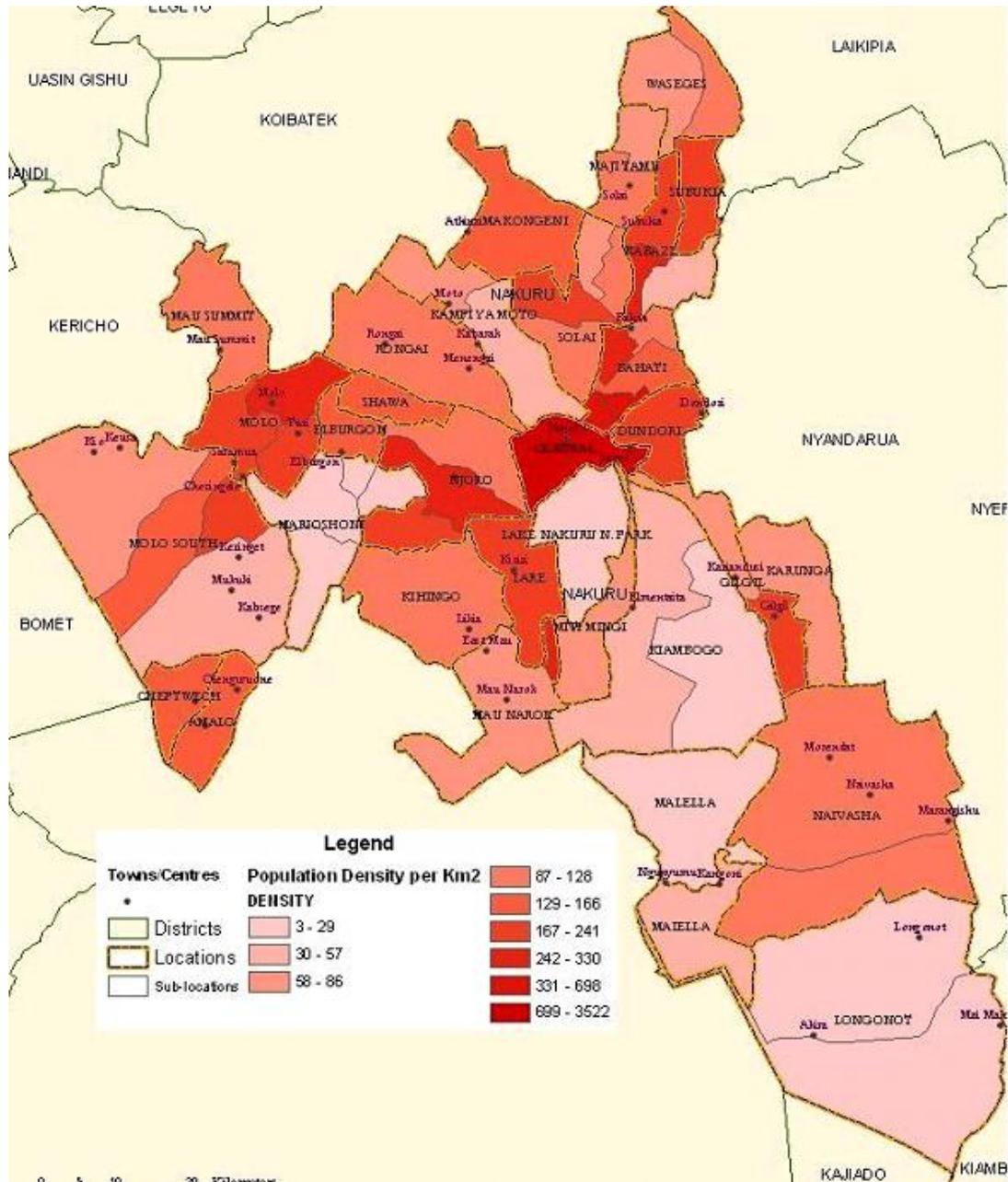
treated empirically, the criteria for the selection of antimicrobial agents should be determined on the basis of the most likely pathogen and its expected resistance pattern determined (Malachy *et al.*, 2016).

## CHAPTER THREE

### MATERIALS AND METHODS

#### 3.1 Study Area

The study was carried out among UTI patients in Nakuru Level 5 Hospital. It is the biggest hospital in Nakuru County that gives health care to over 1.6 million people and is used as teaching and referral hospital and covers an area of 7235.3km<sup>2</sup> and is located between longitudes 35° 28' and 35° 36' and latitudes of 0° 12' and 1° 10' to the South (Appendix viii). Agriculture is the mainstay of Nakuru's economy. The county's weather is conducive for large-scale farming, horticulture and dairy farming. Food crops grown in Nakuru include maize, wheat, beans, peas, cabbages, tomatoes, kales and carrots. The produce is consumed locally and sold to consumers in neighboring towns and cities. The livelihoods in the County also depend on employment and business ([www.nakuru.org](http://www.nakuru.org)). This hospital serves other counties like Nyandarua, Laikipia, Koibatek, Kericho, Bomet, Kajiado, and Kiambu.



Source: [www.wikipedia.com](http://www.wikipedia.com). Accessed on 20<sup>th</sup> October 2012 at 1200Hrs GMT+3

**Figure 3.1:** Map of Nakuru County showing area of study

### 3.2 Study Population

The study targeted both male and female outpatients and inpatients presenting with symptoms and signs of UTI which include dysuria, polyuria, fever, nausea, and flank pain were sampled for this study. Baseline demographic data including age, sex, level of education and risk factors such as catheterization, history of UTI, also out and in

patients were also collected. Samples were collected during the period between June and December 2013.

### 3.3 Study design

The study employed a cross-sectional survey design including in and out patients presenting symptoms of UTI were analyzed.

### 3.4 Sampling design

Purposive sampling was used to select patients with UTI symptoms and then simple random sampling was used to select 10 patients per week until sample of 385 was reached. This design was appropriate to the study since it provided baseline information concerning antibiotic susceptibility pattern of bacterial uropathogens inpatients and outpatients in Nakuru Level 5 Hospital.

### 3.5 Sample Size Determination

According to Fisher *et al.*, (1993) prevalence of 50 % was considered since the prevalence of UTI of the patient was not available in this study area. The sample size was determined as follows:

$$n = \frac{Z^2(pq)D}{d^2}$$

Where; n=require sample,

p= anticipated prevalence which was 50 % (0.5) in this study,

q = failure which was calculated as 100-50 giving 50 % (0.5),

Z= is the appropriate value from the standard normal deviate at 95 % level of confidence (1.96) in this study,

d=degree of precision set at 5 %.

$$\text{Sample size was } n = \frac{1.96^2(0.5 \times 0.5)}{0.05^2}$$
$$= 385$$

Random sampling was used to obtain 385 respondents that is, as patients' samples came that day they were analyzed.

### **3.6.1 Inclusion Criteria**

- i) Adult patients and children who parents/guardians who consented.
- ii) Those patients presenting with clinical symptoms associated with UTI.

### **3.6.2 Exclusion Criteria**

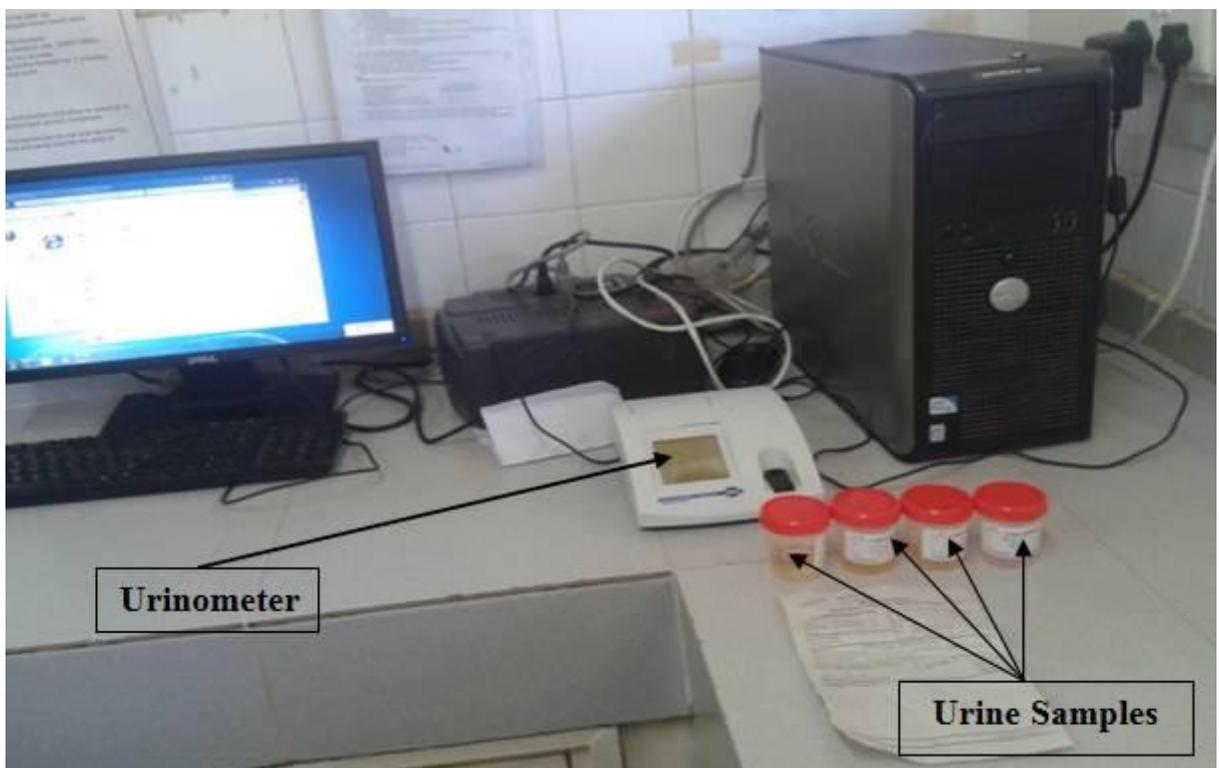
- i) Patients who or whose relatives declined to sign consent forms.
- ii) The patients who were on antibiotic therapy within one week were excluded.

## **3.7 Sample Collection and urinalysis**

### **3.7.1 Sample Collection**

Urine samples were collected from 385 patients using midstream technique for adults and urine bags for infants. In women, samples were taken after vulva swabbing with clear water. All specimens were analyzed as soon as possible after collection to avoid deterioration of leucocytes. Processing of the specimen was done to meet the required

number set for that day (Onifade *et al.*, 2011). Urinalysis was carried out using Uryyxon ® Relax (Bonn,Germany) (Urinometer). Drops of urine were put on to the strip and the strip inserted into it and sample was read before culturing to avoid sample contamination. It was used to identify the enzyme leukocyte esterase which indicates leukocytes; nitrite which is an indicator of enterobacteria in urine, Protein indicates presence of infection and red blood cells indicates urinary tract infection (Pate 3.1).



**Plate 3.1:** Processing of urine specimens in sterile containers

### 3.7.2 Microscopy examination

In the first step of microscopic evaluation of UTI, 10 ml of urine samples were centrifuged at 2000-3000 x g for 5-10 minutes. After centrifugation, supernatant was removed and one drop of deposit was placed onto the microscope slides, covered with cover slips and examined using light microscope under 10x and 40X objectives. Any

bacteria (1 - 4) was defined as bacteriuria and leukocytes more than 5 in one high power field (hpf) was defined as pyuria ( Plate 3.2) (Oladeinde *et al.*, 2011).



**Plate 3.2: Analysis of urine specimens**

### **3.8.1 Isolation and Identification of Microorganisms**

The urine specimens from all the patients were cultured on CLED agar (Oxoid LTD, UK) and identified to determine the microorganisms involved. Inoculation of urine specimen was done using sterile calibrated wire loop inoculating 0.001mL of urine specimen onto CLED agar (Oxoid LTD, UK). The cultured media were then incubated at 37°C for 24 hours. For the media which had no growth after 24hrs incubation were further incubated up to 48 hours before declaring absence of bacterial growth/negative. The numbers of isolated bacterial colonies were enumerated and were multiplied by dilution factor for the estimation of bacterial load per milliliter (ml) of urine sample. Urine samples with colony  $\geq 10^5$  Cfu/ml were taken as

significant growth (Positive urine culture =  $10^5$  CFC/ml). The significant growth was identified further using biochemical reactions (Kolawale *et al.*, 2009).

### **3.9.2 Biochemical Identification of Isolates**

#### **3.9.2.1 Catalase Test**

This is an enzymatic test that breaks down hydrogen peroxide into water and oxygen which is indicated by production of bubbles of air and it was used to identify *Staphylococcus* from other Gram-positive cocci. Hydrogen peroxide forms an oxidative end product of aerobic carbohydrate metabolism. Staphylococci produce catalase enzyme which reacted with hydrogen peroxide thereby producing bubbles of oxygen (Ochei and Kolhatkar, 2000).

#### **3.9.2.2 Coagulase Test**

Coagulase is an enzymatic test which catalyzes the formation fibrin clot in blood plasma inoculated with test organism. This differentiates *Staphylococcus aureus* from other coagulase negative staphylococci. The fibrin in blood plasma appears within 2-3 hours but if no fibrin it is further incubated for 24 hours. If no fibrin clots after this period the test is declared as negative (Cheesbrough, 2007).

#### **3.9.2.3 Oxidase Test**

This is an enzymatic test used in microbiology in the identification of enterobacteria which are non lactose fermenting Gram negative rods. In this analysis it was used to differentiate between *Pseudomonas* from *Proteus*. The enzyme will oxidize a redox dye such as tetramethyl paraphenylene diamine dihydrochloride to give deep purple

colour which is a positive test. This enzyme is produced by some aerobic microorganisms as part of their respiratory oxidation (Jaiswal *et al.*, 2013).

#### **3.9.2.4 Triple Sugar Iron (TSI) Agar Test**

This media was used for detecting enterobacteria especially non lactose fermenting organisms. This is achieved when sugars are fermented with production of gas and hydrogen sulfide. This media combines multiple biochemical tests in a single medium. It assists in separation of the enterobacteria from other non-lactose Gram negative bacteria. In this case it was used in identification of *E.coli*, *Proteus* and *Klebsiella* (Ochei and Kolhatkar, 2000).

#### **3.9.2.5 Indole Test**

This test is used for indole production in differentiation of Gram negative bacilli. The enterobacteria produce aromatic amino acid tryptophan which is present in the medium. A bright red ring with formation indicating that bacteria had broken the amino group and indole was produced. After an overnight growth Kovac's reagent was added to the broth culture which reacted with indole to form a bright red ring colour at the surface of the bijou bottle which was indicative of a positive test. The test was negative when Kovac's reagent was added and no colour change occurred. Indole test was used to differentiate between *E.coli* from *Klebsiella pneumoniae* (Cheesbrough, 2007).

#### **3.9.2.6 Motility Test**

The test was used to determine if an organism was motility. The motility was demonstrated by growth and spread of an organism throughout the medium from the

stab. Non-motile microorganisms were seen to grow only in stabbed area of the medium. In this case it was used in identification of *E.coli*, *Proteus* and *Klebsiella* (Jaiswal *et al.*, 2013).

#### **3.9.2.7 Citrate Test**

The citrate agar (green) slants and butt and was streaked with test organisms containing citric acid, which was a tricarboxylic acid and Brom Cresol agar. The citrate was metabolized to acetoin and carbon dioxide. The isolates with citrate permease allowed intake of citric acid, causing alkaline end products that change pH indicator from green to blue. The isolate was identified by the ability to utilize citrate as the source of carbon, and ammonium as its source of nitrogen. Uropathogen that changed slant to blue was considered as a positive test. Those organisms turned slant green were negative. The test was used in identification of enterobacteria such *E.coli* and *Klebsiella* (Cheesbrough, 2007).

#### **3.9.2.8 Urease Test**

The isolates were inoculated into urea broth medium, which contained phenol red indicator. Microorganisms contained urease enzyme decomposed urea to form carbon dioxide and ammonia. The ammonia produced reacted with water to form ammonium hydroxide causing a change in pH which was indicated by phenol red indicator. Broth became red-purple color when the test was positive due to production of ammonium hydroxide. If test was negative, the broth remained orange. The test was used for differentiating enterobacteria such *E.coli*, *Proteus* and *Klebsiella* (Jaiswal *et al.*, 2013).

### 3.9 Antibiotic Susceptibility Testing

This test was performed using disc diffusion method as described by Cavalieri, (2005). In this technique organisms isolated were inoculated in normal saline with the help of sterile wire loop. Briefly, colonies were taken from 24 hours culture plates into nutrient broth. The turbidity formed was adjusted to an equivalent of 0.5 McFarland. The test organism was streaked over the surface of Muller Hinton agar plates using sterile cotton swabs. Discs impregnated antibiotics which were commercially available were placed on plates firmly by means of sterile forceps aseptically and the inoculated plates were incubated for 24 hours at 37°C. Afterwards diameters of zone of inhibition were measured in mm. The antibiotics used and their zones of sensitivity were measured using veneer caliber and graded according to sensitive, intermediate or resistant (Bano *et al.*, 2011; Mirzarazi *et al.*, 2013). The inoculated plates were air dried, and antibiotic discs (ABTEK BIOLOGICAL LTD., UK) were placed on Muller-Hinton agar using sterile forceps and gently pressed down to ensure contact. The following 8 antibiotic discs were used; Nitrofurantoin (NIT, 300µg), Cefotaxime (CEF, 10µg), Amoxicillin-clavulanic acid (AMC, 10µg), Gentamicin (GET, 10µg), Nalidixic acid (NA, 30µg) Ampicillin (AMP, 10µg), Ciprofloxacin (CIP, 25µg) and Cotrimoxazole (SXT, 25µg). Standard strains of *Escherichia coli* ATCC 25922 and *S. aureus* 25923 were used as control during antimicrobial susceptibility testing (Bauer *et al.*, 1996; Barbara *et al.*, 2000).

**Table 3. 1:** Standard antimicrobial inhibition zones according to Clinical Laboratory Standards Institute

<b>Antibiotics</b>	<b>Resistant</b>	<b>Intermediate</b>	<b>Sensitive</b>
Ampicillin (10µg)	≤13mm	14-16mm	≥16mm
Ciprofloxacin(30µg)	≤12mm	14-16mm	≥17mm
Co-trimoxazole (30µg)	≤10mm	11-15mm	≥16mm
Gentamicin(10µg)	≤12mm	13-14mm	≥15mm
Amoxicillin-lavulinic acid(10µg)	≤13mm	14-16mm	≥17mm
Nitrofurantoin(10µg)	≤14mm	15-16mm	≥17mm
Cefotaxime(10µg)	≤14mm	11-15mm	≥18mm
Nalidixic acid(30µg)	≤13mm	14-18mm	≥19mm

### 3.10 Data Management and Analysis

Data collected and analyzed. Patients names were not used, numbers and letters were used to label the samples. The raw data was entered into excel spreadsheets and later imported to Statistical package for social sciences (SPSS version 15) for analysis. The antibacterial activity was reported in terms of diameters of the zones of inhibition (mm). Comparison of means of zones of inhibition was done using student t-test since there was more than one variable in consideration and values of ( $p < 0.05$ ) were regarded as significant. Chi square test was used in findings on comparison of positive UTI cases according to individual characteristics. Evaluations were carried out at 95 % confidence level and  $P < 0.05$  was considered statistically significant.

### 3.11 Scope and limitation of the study

The study encountered several limitations. The study was restricted to all patients with UTI symptoms who sought medical attention at Nakuru Level 5 Hospital. Finally

this was a cross sectional survey study. Consequently data on key variables was only collected once at a given period of time. Further the trends in the resistance of bacterial isolates to commonly used antimicrobials in the study community over time could not be established. Despite taking all due precautions, and instructing patients and children and their guardians how to take a clean catch, urine specimen, the possibility of contamination cannot be completely eliminated.

### **3.12 Ethical Consideration**

The study obtained approval Medical Superintendent of Nakuru level 5 Hospital. This study also applied and was granted ethical clearance from the Kenyatta University ethical review committee and National Commission for Science, Technology and Innovation (NACOSTI) (Appendix VII and VIII). Informed patient's consent was sought and obtained through writing from the participants parents/guardians. All patients' data and bacterial isolates gathered in this study were handled confidentially by the researcher. Further, acceptable protocols of handling patient data were strictly adhered to. In addition, laboratory coding was used to identify patients from whom the data was obtained. The patients were educated on how to participate on this study. For the infected patients they were taken to the responsible clinicians for management of urinary tract infection (appendix I and II).

## CHAPTER FOUR

### RESULTS

#### 4.1 Prevalence of urinary tract infection

Of the 385 urine specimens processed, 112(29.0 %) showed significant growth whereas the majority of the urine samples that is 273 (71 %) showed no growth. Fresh samples were collected from patients by mid-stream catch method in sterile universal bottles (Plate 1). The assessment of associated risk factors showed gender ( $\chi^2= 0.116$ ,  $P=0.0412$ ), age group ( $P=0.0120$ ), History of UTI ( $\chi^2=0.555$ ,  $P=0.004$ ) and symptoms of UTI ( $\chi^2=0.895$ ,  $P=0.017$ ) were significant. Level of education ( $\chi^2=2.742$ ,  $P=0.523$ ) and catheterization ( $\chi^2=0.17$ ,  $P=0.054$ ) were not significant (Table 4.1).

The age between 25-34 years had the highest number of positive samples 125 (32.4 %) followed the age between 15-24 years which had 124 (32.2 %). Age group of 55 and above had least number of positive samples 12 (3.1 %) (Table 4.1). Female, 239 (62.1%) were the highest while male were 146 (37.9%). Among the patients in difference sex showed significance difference ( $\chi^2=0.116$ ,  $P=0.0412$ ). Patients who had history of UTI were 341 (88.6%) while those having no History of UTI were 42 (10.9%).

**Table 4. 1:** Risk factors associated with UTI at Nakuru Level 5 Hospital, June to December 2013 (N=385).

	<b>N = 385</b>	<b>Frequency (%)</b>	<b>Positiv e</b>	<b>Negativ e</b>	<b>Chi – square</b>	<b>P – value</b>
<b>Gender</b>						
Male	146	37.9	41	105	0.116	0.0412
Female	239	62.1	71	108		
<b>Age Groups years</b>						
1 – 14	32	8.3	8	24	2.918	0.012
15 – 24	124	32.2	39	85		
25 – 34	125	32.4	35	90		
35 – 44	68	17.7	22	46		
45 – 54	24	6.2	4	20		
55 and above	12	3.1	4	8		
<b>Education</b>						
Illiterate	12	3.3	9	3	2.742	0.523
Primary	202	55.6	41	161		
Secondary	138	38.1	19	119		
Tertiary	11	3	2	9		
<b>Catheterization</b>						
Yes	57	14.8	17	40	0.17	0.0504
No	328	85.2	95	233		
<b>History of UTI</b>						
Yes	341	88.6	99	242	0.555	0.004
No	42	10.9	12	30		
Total	385					
<b>Outpatient/ Inpatient</b>						
Outpatient	355	92.2	83	272	72.024	0.000
Inpatient	30	7.8	29	1		
<b>Symptoms</b>						
Yes	338	98	98	236	0.895	0.017
No	47	14	14	37		

#### 4.2 Identification of uropathogens isolated using biochemical tests

Bacterial isolates were 120; Gram-negative bacteria were more 82 (68.3 %) and prevalent than Gram positive isolates 38 (31.7 %). The highest bacterial isolates were *Escherichia coli* 66 (54.8 %) followed by coagulase negative Staphylococci 25 (20.8 %), *S. aureus* 11(9.2 %) and *K. pneumoniae* 12(10 %) (Table 4.2).

**Table 4. 2:** Isolates from the study population (n=120), at Nakuru Level 5 Hospital, June to December 2013

Microorganism	No. of isolates	Percentage (%)
<b>Gram negative rods</b>		
<i>Escherichia coli</i>	66	55
<i>Klebsiella pneumoniae</i>	12	10
<i>Proteus vulgaris</i>	6	5
<b>Gram positive cocci</b>		
<i>Staphylococcus aureus</i>	11	9.2
Coagulase negative staphylococci	25	20.8
	<b>120</b>	<b>100</b>

*E. coli* isolates were identified by lactose fermentation hence producing yellow colonies on CLED agar. Colonies were raised, smooth irregular edge and moist while others were dry. *E.coli* isolates were citrate negative; they did not utilize citrate and were identified by their green colour. They were urease test negative since they lacked the enzyme urease for breaking down urea. They produced tryptophase enzyme which cleaves tryptophan in the media producing indole which was detected by adding of Kovac's reagent into it reacting with indole forming a bright pink colour hence termed as indole positive. Motility of *E.coli* is detected when the broth was inoculated by stabbing vertically and growth spread within the entire medium forming a cloudy medium hence they were said to be motile.

*K. pneumoniae* isolates were subjected to the same procedures of biochemical tests, the bacteria reacted giving result (4.5). On triple sugar iron (TSI) agar, *K. pneumoniae* fermented all the three sugars producing acids which turned the butt yellow and slant yellow with production of a gas which was not hydrogen sulphide.

*P. mirabilis* isolates were subjected to the same procedures of biochemical tests, the bacteria reacted giving the results (Table 4.3). On TSI, they fermented all the sugars producing acids which turned the butt yellow and slant red and utilized thiosulfate in the medium as a terminal electron acceptor reducing it to hydrogen sulphide ( $H_2S$ ). This then reacted with ferrous sulphate in the medium producing ferrous sulphide that gave a black precipitate. It was a positive test for TSI with an alkaline slant and acid butt producing hydrogen sulphide ( $H_2S$ ). These reactions helped in identification of *P. mirabilis*. *S. aureus* isolates were lactose fermenting microorganism hence produced yellow colonies on CLED agar. Colonies are round, smooth, raised, and measuring about 1-2 mm in diameter. The biochemical reactions were catalase and coagulase positive while D-mannitol gave a positive fermentation test (Table 4.3).

**Table 4. 3:** Biochemical results for Gram negative isolates

<b>Biochemical tests</b>	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Proteus vulgaris</i>
(TSI) agar	+Ve	+Ve	+Ve
Citrate utilization	-Ve	+Ve	+Ve
Urease production	-Ve	+Ve	+Ve
Motility	+Ve	-Ve	+Ve
Indole production	+Ve	-Ve	+Ve
Voges-proskauer test	-Ve	+Ve	N/A
Oxidase test	N/A	N/A	-Ve

*-Ve = Negative, +Ve = Positive and N/A = Not applicable*

Coagulase negative staphylococci were lactose fermenting organisms hence producing yellow colonies on CLED agar. Colonies were round, smooth, raised, and measuring about 1-2 mm in diameter. The biochemical reactions showed that coagulase and D-mannitol gave a negative fermentation test while catalase gave a positive result (Table 4.4).

**Table 4. 4:** Biochemical results for Gram positive isolates

<b>Biochemical tests</b>	<b>CNS</b>	<i>Staphylococcus aureus</i>
Catalase test	+Ve	+Ve
Coagulase test	-Ve	+Ve
D-Mannitol fermentation	+Ve	-Ve

*-Ve = Negative, +Ve = Positive, N/A = Not applicable and CNS = Coagulase negative Staphylococci*

### 4.3 Antimicrobial susceptibility of bacterial uropathogens

**Table 4. 5:** Antimicrobial susceptibility pattern Gram- negative uropathogens isolated from urine culture for patients (n=82) at Nakuru Level 5 Hospital, June to December 2013

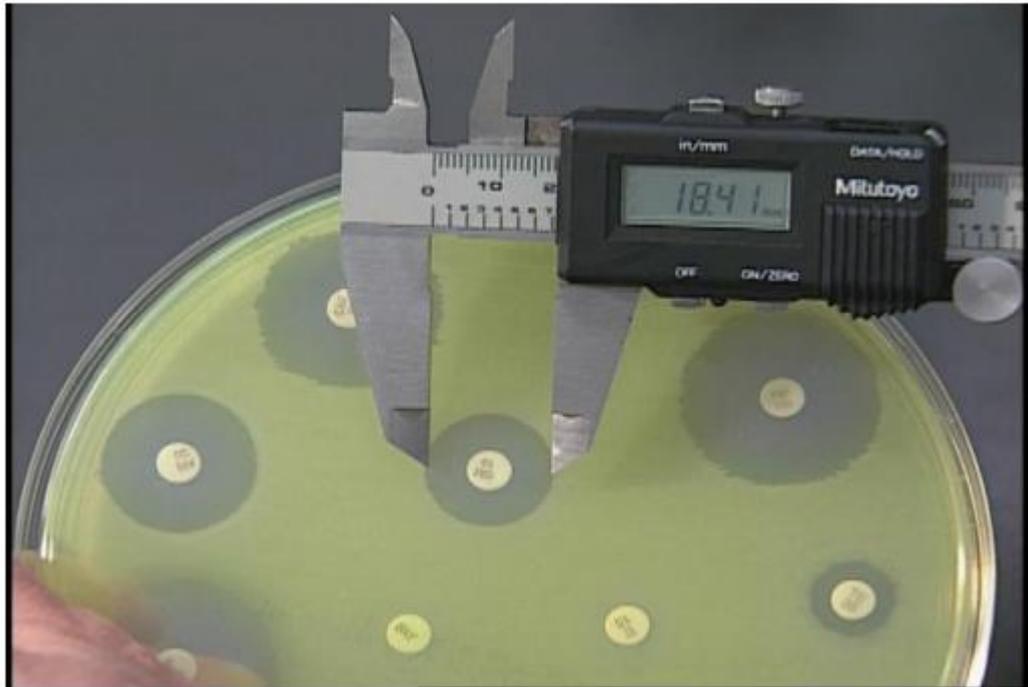
Bacterial isolates	No. of isolates (n)	Patterns	NIT	CET	AMC	GET	NAL	CIP	AMP	SXT
<i>E. coli</i>	66	S	53(80.3%)	45(68.2%)	20(30.3%)	44(66.7%)	19(28.8%)	46(69.7%)	22(33.3%)	12(18.3%)
		I	13(19.7%)	12(18.1%)	14(21.2%)	10(15.1%)	13(19.7%)	9(13.6%)	14(11.7%)	17(25.8%)
		R	0(0.0%)	9(13.7%)	32(48.5%)	12(18.2%)	34(51.5%)	11(16.7%)	30(45.5%)	41(62.1%)
			$X^2=39.00$	Df=36	P=0.006					
<i>Klebsiella vulgaris.</i>	12	S	7(58.3%)	9(75.0%)	8(66.7%)	3(25.0%)	6(50.0%)	7(58.3%)	0(0%)	0(0.0%)
		I	5(41.7%)	3(25.0%)	4(33.3%)	3(25.0%)	4(33.3%)	2(16.7%)	2(16.7%)	2(16.7%)
		R	0(0.0%)	0(0.0%)	0(0.0%)	6(50.0%)	2(16.7%)	3(25.0%)	10(83.3%)	10(83.3%)
			$X^2=25.80$	df=18	P=0.0104					
<i>Proteus ssp.</i>	6	S	4(66.7%)	3(50.0%)	3(50.0%)	3(50.0%)	3(50.0%)	3(50.0%)	2(33.3%)	3(50.0%)
		I	2(33.3%)	2(33.3%)	2(33.3%)	2(33.3%)	1(16.7%)	1(16.7%)	3(50.0%)	2(33.3%)
		R	0(0.0%)	1(16.7%)	1(16.7.3)	1(16.7%)	2(33.3%)	2(16.7%)	1(16.7%)	1(16.7%)
			$X^2=21.536$	Df=8	P=0.006					

AMP = ampicillin, CIP = ciprofloxacin, SXT = co-trimoxazole GET = gentamicin, AMC = amoxicilin + clavulanic acid, NIT= nitrofurantoin, CET= Cefotaxime, NAL= nalidixic acid. S=Sensitive, I=Intermediate, R=Resistant.

The results of antimicrobial susceptibility pattern of Gram-negative isolates ranged from 0-100 % (Table 4.5). All the isolates were sensitive to nitrofurantoin 84 (100 %). Most Gram-negative isolates were sensitive to cefotaxime 74(89.3 %), ciprofloxacin 68 (80 %), gentamicin 65(67.6 %) amoxicilin-clavulinic acid 51 (60.7 %), %, nalidixic acid 46 (54.8 %), ampicillin 43 (51.2 %) and cotrimoxazole 36 (42.9 %).

Among the Gram-negative isolates, the predominant one was *E.coli* 66(81 %). Of the Gram-negatives, 55 % of all isolates demonstrated resistance to cotrimoxazole 41(61.7 %), followed by amoxicillin-clavulinic acid 32(48.8 %). *E.coli* isolates were sensitive to nitrofurantoin 66 (86.7 %), followed by cefotaxime 57(83.7 %), and gentamicin 54 (81.3 %) (Table 4.5).

The results of antimicrobial susceptibility pattern of the isolates (Table 4.5). A rate of susceptibility of Gram positive isolates ranges from 0-100 %. Majority of Gram positives were sensitive to most antibiotics tested than Gram negatives. All isolates were highly sensitive to nitrofurantoin 36(100 %), Cefotaxime 36(100 %) and amoxicilin-clavulinic acid 36(100 %). Coagulase negative staphylococci which were predominant isolates from Gram positives 25(69.4 %) were sensitive to most antibiotics tested. Their sensitivity patterns of the isolates were found to be nitrofurantoin, Cefotaxime, amoxicilin-clavulinic acid, and ciprofloxacin 100 %, respectively. Their resistance pattern only found in ampicillin 13(52 %) and gentamicin 12 (47 %), respectively.



**Plate 2.1: Measuring of diameters of zone of inhibition using vernier caliper**

**Table 4. 6:** Antimicrobial susceptibility pattern Gram positive uropathogens isolated from urine culture for patients (n=36) at Nakuru Level 5 Hospital, June to December 2013

Bacterial isolates	No. of isolates	Patterns	NIT	CET	AMC	GET	NAL	CIP	AMP	SXT
<i>S. aureus</i>	11	S	8(72.0%)	9(81.0%)	7(63.6%)	3(27.0%)	5(45.5%)	3(27.3%)	5(45.5%)	3(27.3%)
		I	3(27.0%)	2(18.0%)	4(36.4%)	4(36.0%)	2(18.2%)	3(27.3%)	3(27.3%)	3(27.3%)
		R	0(0.0%)	0(0.0%)	0(0.0%)	4(36.0%)	4(36.4%)	5(45.5%)	3(27.3%)	5(45.5%)
			X <sup>2</sup> =21750	df=14	P=0.084					
CNS	25	S	17(69.0%)	20(78.0%)	17(69.6%)	8(30.0%)	3(13.0%)	18(73.0%)	5(21.7%)	4(17.4%)
		I	8(30.0%)	5(22.0%)	8(30.4%)	5(21.0 %.)	7(26.1%	7(27.0%)	7(26.1%)	8(30.4%)
		R	0(0.0%)	0(0.0%)	0(0.0%)	12(47.0%)	15(60.0%)	0(0.0%)	13(52.0%)	13(52.2%)
			X <sup>2</sup> =392500	df=22	P=0.012					
<b>Total no. of isolates</b>	<b>36</b>									
		S+I	36(10.0%)	36(10.0%)	36(10.0%)	20(55.0%)	17(47.0%)	31(86.0 %.)	20(55.0 %.)	13(53.4%)
		R	0(0.0%)	0(0.0%)	0(0.0%)	16(44.0%)	19(53.0%)	5(13.9%)	16(44.0%)	12(46.6%)

AMP = ampicillin, CIP = ciprofloxacin, SXT = co-trimoxazole GET = gentamicin, AMC = amoxicilin + clavulinic acid, NIT= nitrofurantoin, CET= Cefotaxime, NAL= nalidixic acid, CNS= Coagulase negative staphylococci.

Among the total 120 isolates, resistant to  $\geq 2$  drugs were recorded in 108 (90 %) of all uropathogens. Seventy seven (93.9 %) isolates of Gram-negative bacteria and 31(81.6 %) of Gram positive bacteria showed resistance to two or more drugs (Table 4.7).

**Table 4. 7:** Resistance pattern of bacterial isolates to more than two antibiotics of patients (N = 120) at Nakuru Level 5 Hospital, June to December, 2013

<b>Bacterial Isolate</b>	<b>Total (%)</b>	<b>R0</b>	<b>R1</b>	<b>R(&gt; 2)</b>
<b>Gram Negative</b>	84 (70.0%)	5 (6%)	0 (0)	79 (94%)
<i>E. coli</i>	66 (80.5%)	1 (1.2%)	0 (0)	65 (98.5%)
<i>Klebsiella pneumoniae.</i>	12 (14.3%)	3 (3.5%)	0 (0)	8 (66.0%)
<i>Proteusvulgaris</i>	6 (5.9%)	1 (1.2%)	0 (0)	5 (83.3%)
<b>Gram Positive</b>	36 (30%)	7 (19%)	0 (0)	23 (63.8)
CNS	25 (69.4%)	4 (16%)	0 (0)	23 (92%)
<i>S. aureus</i>	11 (30.5%)	3 (27.3%)	0 (0)	8 (72.7%)
<b>Total</b>	<b>120 (100)</b>			

**R0**- No antibiotic resistance, **R1**- Resistance to one, **R2**-Resistance to more than two drugs.

## CHAPTER FIVE

### DISCUSION, CONCLUSIONS AND RECOMMEDATIONS

#### 5.1 Discussion

The prevalence of urinary tract infections among patients in Nakuru Level 5 Hospital was 29.1 %. However, this study is in agreement with other reports which stress that UTI in Kenyatta National Hospital, Nairobi was 26.7 % (Nabbugodi *et al.*, 2015) and in Khartoum North Hospital, Sudan it was 14.0 % (Hamdan *et al.*, 2011), Mwanza North – Western Tanzania 12.1 % (Masinde *et al.*, 2009) and Addis Ababa, Ethiopia was 11.6 % (Kolawole *et al.*, 2009) which were lower rates than that of Nakuru Level 5 Hospital.

The prevalence rate of UTI in females was 62.1 % higher than in males (37.9 %) in this study. This high prevalence of UTI in females is comparable to prevalence rates of 64 % in females and 36 % reported in Kenya by Kebira *et al.* (2006). This could be due to the tendency of men buying antibiotics without prescription in chemists, local shops, supermarkets and from street vendors (Sosa *et al.*, 2012). This was lower than the prevalence reported from Isfahani, Province, Iran (71 % female and 29 % male), in Mubi General Hospital and Yola-Nigeria (74 % female and 36 % male). The high prevalence of infection in females (62.1 %) reported in this study is due to short urethra in females which may predispose them to ascending infection. Most women normally clean perineum area backward from the anus to the vulva instead of forward from vulva to the anus that can cause urinary tract infection. This practice keeps bacteria from getting into the urethra after a bowel movement. Sexual activity moves microorganisms from bowel to vaginal cavity and then urethral opening thus

increasing the chances of prevalence of UTI in female patients from organisms that are normal flora of perianal and vaginal regions. There is a need to have high standard of cleanliness in females which will help in reducing the incidence of UTI. The presence of antimicrobial substances in prostatic fluid in males and longer urethra make them less prone to UTIs (Khoshkht *et al.*, 2013; Tula and Iyoh, 2014). There was no significant difference between patient's level of education and UTI ( $P=0.523$ ). This is because they were equally infected. This agrees with studies carried out in Tanzania and Sudan (Masinde *et al.*, 2009; Namdam *et al.*, 2011). The prevalence of UTI in patients with previous history of infection was significantly higher than of those without previous history ( $p=0.004$ ). The results agreed with studies carried out in Pakistan due to the presence of multidrug resistant microorganisms from those who had a previous history of UTI ((Sabir *et al.*, 2002; Amin *et al.*, 2009). This might be due to presence of resistance strains from those who had previous history of urinary tract infection. The prevalence of UTI among the patients with previous history of catheterization was significantly higher than those without history of previous catheterization ( $P=0.0504$ ). These findings were in agreement with previous report in Gonder (Mengistu *et al.*, 2002) and was associated with predisposing factors such long duration of catheterization and contamination of the urinary system during inserting of caterers (Amin *et al.*, 2009).

### **5.1.2 Isolation and Identification of Bacterial Pathogens**

The prevalence of Gram-negative bacteria was 68.3 % (82) while Gram-positive isolates 31.7 % (38) which was similar to rates 75 % and 25 %, respectively of isolation of Gram-negative and Gram-positive bacteria reported in Kenyatta National Hospital, Kenya. The same rates of isolation of Gram-negative and Gram-positive

bacteria of 60 % and 40 % were reported in Tirkur Anbessa Specialized Hospital Addis Abba, Ethiopia (Assefa *et al.*, 2008). Comparable rates of 61.9 % and 38.1 % reported in Tanzania (Sabrina *et al.*, 2010). This could be associated with moisture and watery environment of the mucosal surface of the patients which helps in the invasion of bacteria to the uroepithelial cells. The initial attachments of microorganisms onto urinary tract tissues allow their replication and tissue invasion resulting into bladder infection and pyelonephritis in patients (Amin *et al.*, 2009).

Among the isolates, *E.coli* was the most predominant organism in Nakuru Level 5 Hospital with total isolation prevalence of 55 %. These findings were more than those reported in other countries such as Yemen, 41.5 % Nigeria, 42.1 %, Khartoum North Hospital, and in Sudan, 42.4 % (Hilbert *et al.*, 2011). These high rates were due to the presence of the normal flora in the rectal and vaginal area. Anatomical and functional changes of females make it difficult to maintain personal hygiene and as result increase the risk of acquiring UTI (Shieve *et al.*, 1986; Masinde *et al.*, 2009). Gram-positive cocci coagulase negative were the second dominant pathogens with total isolation prevalence of 20.8 %. These findings were lower than those reported from Tikur Anbessa Specialized Hospital Addis Ababa, Ethiopia 16 % (Hilbert *et al.*, 2011) and Tanzania 16.7 % (Masinde *et al.*, 2009). Gram-positive cocci coagulase negative were more common in urine samples among the sexually active young women (25-34 years). This is probably due to the fact that they are normal flora of both asymptomatic and patients thus take the advantage of the weak defence mechanisms. These organisms can be spread by hands or transmitted by animate or inanimate objects (Pelcar *et al.*, 2003).

### 5.1.3 Antimicrobial Susceptibility Pattern of Bacterial Uropathogens.

Susceptibility pattern of Gram-negative bacteria showed that all of the isolates were sensitive to nitrofurantoin (100 %). The rest of isolates were sensitive to ciprofloxacin (79.8 %), cefotaxime (75.3 %), amoxicillin-clavulanic acid (72.8 %) gentamicin (67.6 %), nalidixic acid (65.6 %) cotrimoxazole (46.6 %) and ampicillin (44%). It was in contrary, to a study done at Tikur Ahbessa Specialized Hospital Addis Ababa, Ethiopia (Assefa *et al.*, 2008) which indicated that their susceptibility pattern of Gram-negative bacteria were Gentamicin (93.3 %), Chloramphenical (83.3%), Contrimoxazole (73.3 %) and amoxicillin-clavulanic acid (70 %) were highly resistant. Availability and indiscriminate use of commonly used antibiotics without health care workers prescription lead to an increased multidrug resistance. Due to the increasing multidrug resistance among uropathogens, the health care workers are left with a limited choice of routinely used antibiotics to choose from for the treatment of urinary tract infections (Jaiswal *et al.*, 2013). This can be attributed the fact that bacteria undergo mutation which makes their susceptibility vary from one geographical to the other (Gupta *et al.*, 2001).

Nitrofurantoin was found to be effective (100 %) to both Gram-positive and Gram-negative bacteria this finding agrees with a previous report in Kenya (Mitemo and Kikvi, 2004). It is used as a drug of choice for the treatment of uropathogens. Few of the isolated uropathogens showed resistance to more than two of the commonly used antibiotics. This was in agreement with findings reported in Tikur Anbessa Specialized Hospital Addis Ababa, Ethiopia (Toronko *et al.*, 2009) and could be due to abuse, misuse and underuse of antibiotics (Oladeinde *et al.*, 2011). Prevalence of multidrug resistance in this study was about 85 % of the uropathogens isolated. The

findings of multidrug resistance were similar to the prevalence isolates (85 %) reported by Kimando and Okemo (2010) of Kenyatta university. A lower (74 %) rate was reported in Tikur Anblessa Specialized Hospital Addis Ababa, Ethiopia (Assefa *et al.*, 2008). This resistance rate could be attributed to antibiotic misuse or abuse (Albrich *et al.*, 2004). This could be attributed to few laboratory facilities to efficiently carry out culture and sensitivity which could lower drug resistance. This could be due to inappropriate administration of antibiotics in empirical therapies and lack of correct infection control strategies which cause a shift to increase prevalence of resistance organisms in the community and hospitals (Gupta *et al.*, 2001; Kariuki *et al.*, 2012).

## 5.2 Conclusions

- i) The prevalence of UTI in Nakuru Level 5 Hospital was 29.1 %.
- ii) *E.coli* was the highest among 120 the isolates. Females had a higher (62.1 %) prevalence of UTI than males (37.9 %). Urinary tract infection was associated with the previous infection and with patients who had the history of catheterization.
- iii) All isolates were sensitive to nitrofurantoin, cefotaxime and amoxicillin-clavulanic acid. High resistance was observed in gentamicin, nalidixic acid, ampicillin and cotrimoxazole.
- iv) In the 120 isolates were resistant for more than two antibiotics were recorded in 108 (90 %) isolates. Antibiotic susceptibility patterns of all patients with bacterial uropathogens will reduce multidrug resistance.

### **5.3 Recommendations**

- i) There is a need for continuous surveillance of antibiotic to the currently used antibiotics in management of urinary tract infections covering the entire Nakuru County.
- ii) Nakuru County to enforce policies formulated by pharmacy and poison board to prevent misuse or underuse of antibiotics by giving prescription to only patients with results of culture and sensitivity and therefore treatment UTIs should be based on and sensitivity in order to limit multidrug resistance.
- iii) Continuous follow up to provide an update of laboratory diagnosis of urinary tract infections in order to reduce multidrug resistance bacteria in UTI patients.
- iv) Health care workers should enforce health education to patients in order to adhere to the treatment and thereby reducing drug resistance.
- v) Screening for resistance and identify modes of transmission.

**REFERENCES**

- Aladag, J. and Durak, K. (2009). Investigation of some antibiotics susceptibilities, plasmid profiles and ESBL characteristic of *Klebsiella* system infections. *Applied Science Journal*, 6: 630-636.
- Albrich, W., Monnet, D. and Harbarth, S. (2004). Antibiotic selection and resistance in *Streptococcus pneumonia* and *Streptococcus pyogenes*. *Emerging Infectious Diseases*, 38:363-371.
- Alemu, A. Moges F. Shiferans, Y. Tafess, A., Kassu, A, and Agegn, A. (2012). Bacterial profile and drug susceptibility pattern of urinary tract infection in pregnant Women at University of Gondar Teaching Hospital, Northwest Ethiopia. *International Journal of Pharmaceutical Sciences* 5: 1-15.
- Alliance for the Prudent Use of Antibiotics (APUA) (2011). Final Report: Situation Analysis and Needs Assessment of Antibiotic Resistance in Uganda and Zambia. 1–22.
- Anderson, C., Martin, M. and Hultgren, C. (2004). Host subversion by formation of intracellular bacterial communities in the urinary tract. *Microbes' Infections*, 6: 1094.
- Annapurna, S and Lakshmi, V. (2013). Antibiotic susceptibility pattern and MAR index of Urinary tract isolates. *International Journal of Pharmaceutical Sciences*, 1: 474-478.
- Assefa, A., Asrat, D., Woldeammud, Y., Hiwot, G. and Abdella A. (2009). Melesse drug susceptibility of urinary tract infections in women at Tikur Anbessa specialized hospital Addis Ababa. Ethiopian. *Ethiopian Medical Journal*, 46:227-235.
- Assefa, A., Asrat, D., Woldeammuel, Y., Abdella, A. and Melesse, T. (2008). Bacterial profile and drug susceptibility pattern of urinary tract infection in pregnant women at Tibur Anbessa Specialized Hospital Addis Ababa, Ethiopia. *Ethiopian Medical Journal* 46: 227-235.
- Barbara M., Baird-Parker, T. and Grahame, W. (2000). The microbiological safety and quality of food (II). *American Journal of Pathology*, 45:12-34.
- Bauer W., Kirby, M. and Sherris, C. (1996). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, 45: 493-496.
- Blondeau, J. (2004). Current issue in the management of urinary tract infections: extended-release ciprofloxacin as a novel treatment option. *Drugs*, 64: 611-628.
- Cheesbrough, M. (2002). Medical laboratory manual for tropical countries. *Tropical Health Technology Butterworth London*, 3: 479 - 481.
- Churchill, D., and Gregson, D. (2004). Screening urine samples for significant bacteriuria in the clinical microbiology laboratory. *Clinical Microbiology Newsletter*, 26: 179.

- Ejaz, H., Zafar, A., Anwar, N., Cheema, I. and Shehzad, H. (2006). Prevalence of Bacteriuria in UTIs among children at Iran. *Iranian Journal of Clinical Diseases and Tropical Medicine Research Center*, 3:149-153.
- El-Sweih, N., Jamal, W. and Rotimi, V. (2008). Spectrum and antibiotic resistance of uropathogens isolated from hospital and community patients with urinary tract infections in two large hospitals in Kuwait. *Medicine, Principles Practice*, 14 401-407.
- Fisher, A., Laing, J., Stoeckel, J. and Townsend, J. (1998). Handbook for family planning operations research design. (2<sup>nd</sup> Ed.) New York
- Forouzan, M. Zhra, A. and Amir, B. (2013). Prevalence and antimicrobial susceptibility patterns of uropathogens among patients referring to Valieasr laboratory in Najafabad, Isfahan, Iran. *Middle-East Journal of Scientific Research*, 13:85-90.
- Foxman, B. (2010). Epidemiology of urinary tract infection. *American Journal of Medicine*, 7: 653-660.
- Global Antibiotic Resistance Partnership (GARP)-Kenya Working Group, (2011). Situation Analysis and Recommendations: *Antibiotic Use and Resistance in Kenya*. 21–71.
- Graham, J. and Galloway, A. (2001). The laboratory diagnosis of urinary tract infection. The laboratory diagnosis of urinary tract infection. *Journal of Clinical pathology*, 54: 911-919.
- Gupta K., Hooton T. and Stamm, W. (2001). Increasing antimicrobial resistance and management of uncomplicated community acquired urinary tract infections. *Annual International Internal Medicine*, 135:41-50.
- Gupta, V., Yadav, A., Josh, R. (2002). Antibiotic resistance pattern in uropathogens. *Indian Medical Journal of Microbiology*, 20:96-98.
- Hilbert, W. (2011). Antibiotic resistance in urinary tract infections: Current issues and future solutions. *Femeris Women's Health Research Centre, Medical Diagnostic Laboratories, A Division of Genesis Biotechnology Group, Hamilton NJ United States of America*.
- Ibraheam I.A Saleh I.A Kadhim H.J, Zainab H.J, Al-Mahdi Z.K. (2016) Molecular diagnostic of *Escherichia coli* among urinary tract infections' patients using polymerase chain reaction (PCR). *Pakistan Journal of Biotechnology* 13: 275 – 278.
- Ikeagwu, I., Amadi, E. and Amad, I. (2008). Antibiotic sensitivity pattern of *Staphylococcus aureus* in Abakaliki, Nigeria. *Pakistan Journal Medical Science*, 24:231 – 235.
- Kalantar, E. Motlagh, M. Lornejad, H. and Reshadmanesh, N. (2008). Prevalence of urinary tract pathogens and antimicrobial susceptibility patterns in children at hospital in Iran. *Journal Medical Microbiology*, 3: 149-153.

- Kariuki, S., Revanthi, G. and Kariuki, N. (2012). Invasive multidrug resistant non-typhoidal *Salmonella* infections in Africa: zoonotic or anthropologic transmission. *Journal of Medical Microbiology*, 55: 585-591.
- Karlowsky, J., Kelly, L., Thornsberry, C., Mark E., Jones. E. and Sahm D. (2002). Trends in antimicrobial resistance among urinary tract infection isolates of *Escherichia coli* from female outpatients in the United States. *Journal of American Society for Microbiology*, 46: 2540–2545.
- Kimando, J. M., Okemo, P. O. and Njagi E. N. M. (2010) Resistance to Antibiotics in Urinopathogenic Bacteria Isolated In Patients Attending Kenyatta University Health Clinic, Nairobi. *East African Medical Journal* 87:115-119.
- Khoshbakht, R., Salimi, A., Aski, A.S. and Keshavarzi, H. (2013). Antibiotic susceptibility of bacterial strains isolated from urinary tract infections Karaj, Iran. *Jundishapur Journal of Microbiology* 6:86-90.
- Kolawole, A., Kolawole, O., Kandaki, O., Babatunde, S., Durowadw, K. and Kolalowole, C. (2009). Prevalence of urinary tract infections (UTIs) among patients attending Dalhatu Araf Specialist Hospital, Lafia, Nasarawa state, Nigeria. *International Journal of Medicine and Medical Sciences* 1: 163-167.
- Komala M. and Sampath Kumar S. (2013). Urinary tract infection: causes, symptoms, diagnosis and its management *Indian Journal of Research in Pharmacy and Biotechnology* 1: 226 -233.
- Kothari, A. and Sangar, V. (2008). Antibiotic resistance in pathogens causing community acquired urinary tract infections in India. *Multicenter Study. Journal of Infections in Developing Countries*, 2:354-8.
- Kumar, Y., Sood, S., Sharma, A. and Mani, K. (2013). Antibigram and characterization of Resistance makers among *E.coli* isolates from urinary tract infection. *Journal of Infection Diseases in Cities* 7:513-519.
- Land, T. (1992). Combating with counterfeit drugs. *International journal of Tuberculosis and Lung disease*, 10:192.
- Lane, M. and Mobley, H. (2007). Role of P-fimbriae mediated adherence in pyelonephritis and persistence of uropathogenic *E.coli* in the mammalian kidney. *Kidney International*, 72:19-25.
- Malachy, E., Ifeanyi Ezeobi I., Amalukwe G., Ugwu B., Okezie U., and Stanley C. (2016). Antimicrobial evaluation of bacterial isolates from urine specimen of patients with complaints of urinary tract infections in Awka, Nigeria. *International Journal of Microbiology*, 6: 1-6.
- Manikandan, C. and Amsath, A. (2013). Antibiotic susceptibility pattern of *Klebsiella pneumoniae* isolated from of microbiology. *International Journal of Microbiology* 2: 330 – 337.

- Manikandan, S., Sapandians, G., Sing, M. and Kuraguru, A. (2011). Antimicrobial susceptibility pattern of urinary tract infections; transmission and risk factors, incidence and costs. *Infections, Disease Clinics of North America*, 17; 227-241.
- Mehta, M. Bhardwaj, S. and Sharma, J. (2013). Screening of urinary isolates for the prevalence and antimicrobial susceptibility of enterobacteria other than *E. coli*. *International Journal of Life science & Pharmacology Research*, 3: 100-104.
- Mengistu, G., Moges, A., Genetu, A., (2002). Antibiotic sensitive of common bacterial pathogens in urinary tract infection in Gondar Hospital, Ethiopia. *East African Medical Journal* 79:140-142.
- Mirzarazi, M., Rezatofghi, S., Pourmahdi, M. and Mohajeri, M. (2013). Antibiotic resistance of isolated Aram Negatiove Bacteria from urinary tract infections (UTIs) in Isfahan. *Judishapur Journal of Microbiology*, 6:1-5.
- Mitta R. Aggarwal S. Sharma S. Chhibber, Harjai K (2009). Urinary tract infections caused by *Pseudomonas aeruginosa*: A Minireview. *Journal of Infection and Public Health*, 2:101 – 111.
- Momoh, A. Orhue, P., Iodnije, O. Oaikhena, A. (2011). The Antibiogram types of *Esherichia coli* isolated from suspected Urinary tract infections samples. *Journal of Microbiology* 3: 57-65.
- Muder, R., Brennen, C., Rihns, J. Wagener, M., Obman, A. Stout, J. and Yu, L. (2006). Isolation of *Staphylococcus aureus* from urinary tract. Association of isolation with Symptomatic Staphylococcal bacteremia. *Journal of Clinical Infectious Disease* 42, 46-50.
- Mwaka, A., Mayanja-Kizza, H., Kigonya, E., Kaddu-Mulindwa, D. (2011). Bacteriuria among adult non-pregnant women attending Mulago hospital assessment centre in Uganda. *African Health Sciences* 11: 182–189.
- National committee for clinical laboratory standards (NCCLS). (2005). Performance standards for antimicrobial susceptibility testing. Ninth informational supplementary. *National Committee for Clinical Laboratory standards* 23:234-236.
- Nicolle, L., Bradley, S., and Colgan, R. (2005). Infectious disease society of America guidelines for the diagnosis and treatment of asymptomatic bacteriuria in adults. *Journal of Clinical Infectious Disease*, 30:643.
- Ogbukagu, C. M., Anakwenze, V. N., Ekwealor, C. C., Ezemba, C. C. and Ekwealor, I. A. (2016). Incidence of Urinary Tract Infections (UTI) amongst Patients Attending Primary Health Centres in Anambra State. *Advances in Microbiology*, 6(07), 537.
- Okeke, I., Aboderin, O., Byarugaba, D., Kayode, K. and Opintan. J. (2007). Growing of multi-resistant. *Journal of Emerging Infectious Diseases* 5, 481-493.
- Oladeinde, B., Omoregie, R., Olley, M. and Anunibe, J. (2011). Urinary Tract in a Rural Community of Nigeria. *North America Journal of Medical Science*, 3:75-77.

- Omulo, S., Thumbi, SM., Njenga, MK., and Call, DR. (2015). A review of 40 years of enteric antimicrobial resistance research in Eastern Africa: what can be done better? *Antimicrobial Resistance and Infection Control* 4:1
- Onifade, A., Anibijuwon, I. and Azariah, E. (2011). Urinary tract infection in apparently healthy individuals in Ile-Ife, Nigeria: Detection of predominant microorganisms and antibiotics susceptibility profile. *African Journal of Microbiology Research* 5:3233-3236.
- Onuoha, S. and Fatokun, K. (2014). Prevalence and antimicrobial susceptibility pattern of urinary tract infection among pregnant women in Afikpo, Ebonyi State, Nigeria. *American Journal of life Sciences* 2:46-52.
- Otajevwo, F. (2013). Urinary tract infection among symptomatic outpatients visiting a Tertiary Hospital Based in Midwestern Nigeria. *Global Journal of Health Science* 5: 187-199.
- Parham, N., Pollard, S., Chaudhuri, R. (2005). Prevalence of pathogenicity isolate  $\beta$ -lactamase genes among the extraintestinal clinical isolate of *E.coli*. *Journal of Clinical Microbiology* 43; 2425.
- Patel, S. Taviad, P., Sinha M., Javadekar T., Vipul, P. and Chaudhari, V. (2012). Urinary Tract Infections (UTI) Among Patients at G.G.Hospital & Medical College, Jamnagar: *National Journal of Community Medicine* 3:1138-1141.
- Pecoul, B., Chirac, P., Trouiller, P. and Pine, J. (1999). Access to essential drugs in poor countries; a lost battle? *Journal of American Medical Association* 281:361-367.
- Perfetto, F., Keating, K., Merchants, H. and Nicholas, B. (2004). Acute uncomplicated UTI and *E. coli* resistance; implication for first line empirical antibiotic therapy. *Journal on Management Care of Pharmacy*. 10: 17-25.
- Piranfar, V. and Erfani, M. (2014). Incidence and antibiotic susceptibility pattern of most common pathogens causing urinary tract infections in Tehran, Iran, 2012-2013. *International Journal of Pathology* 2: 1-4.
- Prakash, D. and Saxen, R. (2013). Distribution and antimicrobial Susceptibility Pattern of Bacterial pathogens causing UTI in Urban Community of Meerut City. *Journal of Microbiology* 10:13-18.
- Radyowijati, A. and Haak, H. (2003). Improving antibiotic use in low-income countries; An overview of evidence on determination. *Social Science Medicine*, 57: 733-744.
- Reyes, H., Guiscafre, H. and Munoz, O. (1997). Update on antibacterial resistance in low income countries; Factors favouring the emergence of resistance. *Journal of Clinical Epidemiology*, 50; 1297-1304.
- Riaz, S., Falsal M., and Hasnain, S. (2011). Antibiotic susceptibility pattern and Multiple Antibiotic Resistance (MAR) calculation of Extended Spectrum  $\beta$ - Lactamase

- (ESBC) producing *E. coli*. And *Klebsiella Spp.* in Pakistan. *African Journal of Biotechnology* 33: 6325-6331.
- Saints, B. and Chenoweth, C. (2003). Biofilm and Catheter – associated urinary tract infections. *Infectious Diseases Clinical North America*, 17:411-432.
- Samuel S.O, Salami T.A.T, Adewuyi G.M, Babatope E, and Ekozien M.I. (2012). Prevalence of Urinary Tract Infections among a cohort of HIV Positive Patients accessing care in a rural health centre in Nigeria. *Journal of Microbiology and Biotechnology Research*, 2:507-510.
- Sarathbabu, R. Rajkumari, N and Ramanim, T (2013). Characterization of coagulase negative Staphylococci isolated from urine pus sputum and blood Samples. *International Journal of Pharmaceutical Science Invention* 2: 37-46.
- Schaeffer, A. (2002). The expanding role of fluoroquinolones. *American Journal of Medicine* 113. *Supplementary*. 1: 455-545.
- Shears, P. (2001). Antibiotic resistance in the tropics: Epidemiology and surveillance of antimicrobial resistance in the tropics. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 95:127–30.
- Shilpi, T., Hug, S. and Khatun, M. (2012). Pattern of bacteria causing urinary tract infection of inpatients and outpatients department at Anwar Khan Modern Medical College Hospital: *Answer Khan Modern Medical College Hospital* 3:13-15.
- Sire, J., Nabett, P. and Calude, J. (2007). Antimicrobial resistance in outpatient *E.coli* urinary isolate in Dakar, Senegal. *Journal of Infectious Disease in developing countries*, 1:263-268.
- Tambekar, D., Dhanorkar, D., Gulhane,S., Khandelwal, V. and Dudhane, M. (2006). Antibacterial susceptibility of some urinary tract pathogens to commonly used antibiotics. *African Journal of Biotechnology* 5:1562-1565.
- Tula M. Y. and Iyoha O. (2014). Distribution and Antibiotic Susceptibility Pattern of Bacterial Pathogens Causing Urinary Tract Infection in Mubi General Hospital, Yola-Nigeria. *American Journal of Life Sciences* 2:46-52.
- Vila, J. and Pal, T. (2010). Update on antibacterial resistance in low income countries; Factors favouring the emergence of resistance. *The Open Infection Diseases Journal*, 4:38-54
- Wamalwa J, Omolo J, and Makokha A. Prevalence and risk factors for urinary tract infections among pregnant women Phidelis. *East African Medical Journal* 87:115-119.
- WHO (2000). Guidelines on standard operating procedures for microbiology. Regional Office for South-East Asia, New Delhi.
- Wilson, M. and Gaido, L. (2004). Laboratory diagnosis of urinary tract infections in adult patients. *Clinical Infectious Diseases*, 38:1150-1158.

**APPENDICES**  
**Appendix I: Questionnaire**

I am a student at Kenyatta University undertaking a study on antibiotic susceptibility pattern of bacterial uropathogens isolated from patients in Nakuru Level 5 Hospital, Kenya presenting at Nakuru level 5 Hospital, Nakuru County, Kenya.

Please fill in accurately. The information obtained will be confidential and used for the above stated study.

Study number (to be provided).....

**A) GENERAL INFORMATION**

**Tick where appropriate**

Date of interview.....Code of the interview.....

1. Age

1-14 .....

15-24.....

25-34.....

35-44.....

45-54.....

55 and above.....

2. Gender of the participant      Male.....      Female.....

3. Level of education....Illiterate.....Primary.....Secondary.....Tertiary.....

4. Inpatient.....Outpatient.....

5. History of patient of urinary tract infection (UTI). Yes.....No.....

6. History of catheterization      Yes.....      No.....

7. . Have been on antibiotic treatment for the last one week

Yes..... No.....

8. If yes in 3 above, what was the duration.....

9. Have been diagnosed of a urinary tract before? .. Yes.....No.....

10. If yes when did you last use drugs (Antibiotics)?

## **Appendix II: Informed consent form**

My name is Gachuhi George Tibi. I am a Master's student in the Department of Microbiology, Kenyatta University. I am conducting a study on Antibiotic susceptibility pattern of uropathogens causing urinary tract infections in Nakuru County, Level 5 Hospital.

### **Procedure to be followed**

Participation in this study will require that I ask you some questions and you will be examined by a clinician in order to screen you for urinary tract infection. You will be required to give 10 mL of early morning urine specimen for analysis. I will record your results in your laboratory request form.

You have the right to refuse participation in this study. You will get 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 hospital today or that you will get from any other clinic at any other time. Please remember participation in the study is voluntary. You may ask questions related to the study at any time. You may refuse to respond to any questions and you may also stop the interview at any time. Being interviewed is voluntary.

### **Discomfort and Risks**

Some of the questions you will be asked are on intimate subject and may be embarrassing or make you uncomfortable. If this happens, you may refuse to answer these questions if you so choose. You may also stop the interview at any time. The interview may add approximately half an hour to the time you receive your routine services.

**Benefits**

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

**Confidentiality**

The interviews and examinations will be conducted in a private setting within the hospital. Your name will not be recorded on the questionnaire and will be kept in a locked cabinet for safety. Everything will be kept private.

**Contact information**

If have any questions you may contact Dr John Maingi on 0722 880280 or Dr Kebira on 0715 032643 or the Kenyatta University Review Committee on [kuerc@ku.ac.ke](mailto:kuerc@ku.ac.ke).

**Participant’s 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 entirely voluntary. I understand that my records will be kept private and that I can leave the study in any time. I understand that I will get the same care and medical treatment whether I decide to leave or not and my decision will not change the care I will receive from the hospital today or that I will get from any other clinic at any other time.

Name of participant.....

.....

Signature.....Date.....

Sign.....Date.....

**Investigator's Statement**

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

Name of Interviewer.....

.....

.....

Interviewer Signature

Date

**Appendix III: Minor assent form**

Project Title: Antibiotic susceptibility pattern of bacterial uropathogens causing urinary tract infections in Nakuru Level 5 Hospital, Kenya

We are doing a research study about urinary tract infection. A research study is a way to learn more about people. If you decide that you want to be part of this study, you will be asked to bring 10 ml of early morning urine specimen. You will give a urine specimen in a clean sterile bottle, which will be examined for the presence of bacteria and other indicators of urinary tract infection.

There are some things about this study you should know. The procedure for collection of urine specimen is not invasive, and does not cause pain or harm to you.

Not everyone who takes part in this study will benefit. A benefit means that something good happens to you. We think these benefits might be results obtained from this study will be useful for your treatment and will be communicated through your doctor.

When we are finished with this study we will write a report about what was learned. This report will not include your name or that you were in the study. You do not have to be in this study if you do not want to be. If you decide to stop after we begin, that's okay too. Your parents know about the study too. If you decide you want to begin this study, please sign your name.

I want to be in this research study.

.....

.....

(Sign your name here)

(Date)

## **Appendix IV: Analytical profile index (API)**

### **API 20 E 18-24 Hour Procedure**

In many clinical isolates, identifying procedures are lengthy, tedious and laborious. But, this job has become very simple after the introduction of ready-made kits (e.g. API 20A) and automated analyzers (e.g. Vitek). This chapter deals with API 20 E as a representative identifying procedure.

API 20 system is valuable to laboratory personnel to identify members of the family Enterobacteriaceae and other gram negative bacteria accurately and easily. There are two procedures: (1) 18-24 hour (2) same day. This convenient and economical procedure makes use of dehydrated substrates in micro tubes.

#### **1. Preparation of Bacterial Suspension**

1. Add 5 ml. of 0.85% saline, pH 5.5-7.0 to a sterile test tube.

NOTE: Saline containing preservatives of bacteriostatic agents should NOT be used in preparing the bacterial suspension.

2. Gently touch the centre of a well-isolated colony (2-3 mm) or larger in diameter) with the tip of a wooden applicator stick. Insert the applicator stick into the tube of saline and, with the tip of the stick at the base tube, rotate the stick in vortex-like action. Recap the tube.

Alternate procedure: with flamed inoculating loop, carefully touch the centre of a well-isolated colony (2-3 mm or larger in diameter) and thoroughly mix the inoculum with the tube with saline.

#### **2. Preparation of Strips:**

1. Set up an incubation tray and lid

2. Record the patient's specimen number on the elongated flap of the tray
3. Dispense 5 ml. of tap water into the incubation tray to provide a humid atmosphere during incubation. A plastic squeeze bottle may be used for this.
4. Remove the API strips from the sealed pouch and place one strip in each incubation tray.

### **3. Inoculation of the Strips**

The API 20 E strips contain 20 micro tubes, each of which consists of a tube and Cupule section.

1. Remove the cap from the tube containing the bacteria suspension and insert a 5 ml. Pasteur pipette.
2. Tilt the API 20 E incubation tray and fill the tube section of the microtubes by placing the pipette tip against the side of the cupule. Note The ADH, LDC, ODC, H<sub>2</sub>S, and URE reactions can be interpreted best if these microtubes are slightly under-filled.
3. Fill both the TUBE and CUPULE section of the CIT VP and GEL tubes
4. After inoculation, completely fill the capsule section of the ADH, LDC, ODC, H<sub>2</sub>S and URE tubes with mineral oil.
5. Using the excess bacterial suspension, inoculate an agar slant or plate (non-selective media such as nutrient agar, blood agar, or tryptic (trypticase) soy agar is suggested) as a purity check and for oxidase testing, serology and /or additional biochemical testing. Incubate the slant or plate for 18-24 hours at 35-37<sup>0</sup>C.

### **4. Incubation of the Strips**

1. After inoculation, place, the plastic lid on the tray and incubate the strip for 18- 24 hours at 35-37<sup>0</sup> C in a non-CO<sub>2</sub> incubator.
2. Weekend incubation; the biochemical reactions of AP1 20 E should be read after 18- 24 hour of incubation. If the strips cannot be read after 24 hours incubation at 35- 37<sup>0</sup>c the strips should be removed from the incubator and stored at 2-8<sup>0</sup>C

(Refrigerator) until the reactions can be read.

### **Reading the Strips**

1. After 18 hours of incubation and before 24 hours incubation, record all reactions not requiring the addition of reagents.
2. If the GLU tube is negative (blue or green), do not add reagents. Consult recommended procedure for the identification of Enterobacteriaceae and other Gram-negative Bacteria (18-24 hour) (See fig 35-1 for further instructions).

## **Appendix V: Inoculating urine with a calibrated loop method**

### **Principle**

The number of microorganisms per milliliter recovered on urine culture can aid in differential diagnosis of UTI. Plastic or wire loops, available commercially, have been calibrated to deliver a known volume of liquid when handled correctly, thus enabling the microbiologist to estimate number of microorganisms in the original specimen based on CFU of growth cultures.

### **Method**

Flame a calibrated wire inoculating loop and allow it to cool without touching any surface. Alternatively aseptically remove a plastic calibrated loop from its package.

Mix the urine thoroughly and remove the top of the container. If urine is in a small-diameter tube, the surface tension will alter the amount of specimen picked by the loop. A quantitative pipette should be considered if the urine cannot be transferred to a larger container.

Insert the loop vertically into the urine to allow urine to adhere to the loop.

Spread the loopful of urine over the surface of the agar plate. A standard quadrant streaking technique is also acceptable.

Without re-flaming, insert the loop vertically into the urine again for transfer of a loopful to a second plate. Repeat for each plate.

Incubate for at least 24 hours at 35° to 37° C in air. Colonies are counted on each plate. The number of CFUs is multiplied by 1000 (if a 0.001, mL was used) or by 100 (if a 0.01-mL was used) to determine the number of microorganisms per milliliter in the original specimen.

Because of antimicrobial treatment or other factors may inhibit initial growth, re-incubate plates with no growth or tiny colonies for additional 24 hours before discarding plates.

To store the inoculating loop, place (handle down) in test tube taped to the wall, rather than flat on the bench, to prevent destroying the calibration.



## Appendix VIII: Research Authorization Letter



### NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

Telephone: +254-20-2213471,  
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P.O. Box 30623-00100  
NAIROBI-KENYA

Ref: No.

Date:

**10<sup>th</sup> February, 2014**

**NACOSTI/P/14/6559/596**

George Tibi Gachuhi  
Kenyatta University  
P.O.Box 43844-00100  
**NAIROBI.**

#### **RE: RESEARCH AUTHORIZATION**

Following your application for authority to carry out research on "*Antibiotic susceptibility pattern of uropathogens isolated from urinary infections at Provincial General Hospital Nakuru, Kenya,*" I am pleased to inform you that you have been authorized to undertake research in **Nakuru County** for a period ending **11<sup>th</sup> April, 2014.**

You are advised to report to **the Medical Superintendent, Provincial General Hospital, Nakuru 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.

**DR. M. K. RUGUTLA, PhD, HSC.**  
**DEPUTY COMMISSION SECRETARY**  
**NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION**

Copy to:

The Medical Superintendent  
Provincial General Hospital.

