PREVALENCE OF BACTERIURIA AND ANTIBIOTIC SENSITIVITY PROFILE OF BACTERIAL ISOLATES AMONG SEXUALLY ACTIVE NON-PREGNANT WOMEN ATTENDING THIKA LEVEL 5 HOSPITAL

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P150/31030/2015

A Thesis Submitted in Partial Fulfillment of the Requirements for the Award of the Degree of Master of Science (Infectious Diseases) in the School of Medicine of Kenyatta University

JUNE, 2019

DECLARATION

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

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DEDICATION

I dedicate this thesis to my supervisors Dr. Margaret Muturi and Dr. Nelson Menza.

ACKNOWLEDGEMENT

I thank the Almighty God for granting me the strength and courage to begin and complete this study. I sincerely thank my supervisors: Dr. Margaret W. Muturi and Dr. Nelson C. Menza from the department of Medical Laboratory Science, Kenyatta University for their unconditional constant guidance in writing this thesis. I would like also to thank Kenyatta University for giving me an opportunity to study and Thika level 5 Hospital for permitting me to undertake the study in their premises. To my lovely husband Mr. Cornelius Makau, my daughter Tracy Musangi and my parents Mr. /Mrs. Musili Munyao, I thank you for your moral support in every step. God bless you.

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LIST OF ABBREVIATIONS AND ACRONYMS

µg	Microgram
ANOVA	Analysis of variance
CFU	Colony Forming Units
CI	Confidence Interval
CLED	Cystine Lactose Electrolyte Deficient
CLSI	Clinical Laboratory Standard Institute
ML	Milliliters
MSU	Midstream urine
SIGN	Scottish Intercollegiate Guidelines Network
SPSS	Statistical Package for Social Sciences
U.V	Ultra violet
UPEC	Uropathogenic *E.coli*
UTI	Urinary tract infection
W.H.O	World Health Organization

ABSTRACT

Bacteriuria is commonly found in women and about thirty three percent (33%) of adult women have encountered an incidence of symptomatic cystitis at least once in their life time. In case the uropathogens are unidentified and management undertaken, bacteriuria may cause more dangerous and adversed complications, including nephritis and renal failure. Majority of studies done in Kenya have focused on pregnant women with no documented information available on the sexually active non pregnant women population despite a heightened risk of bacteriuria in this age group. The objectives of this study were to determine the occurrence of bacteriuria, to isolate bacterial pathogens associated with the bacteriuria, to determine antibiotic susceptibility profile on the isolated bacterial pathogens in sexually active non pregnant women attending Thika level 5 Hospital, Kiambu County, Kenya. The study employed a cross sectional design and random sampling technique. Three hundred and eighty four (384) mid-stream urine samples were collected from sexually active non pregnant women aged 18 to 48years attending Thika level 5 hospital. The urine samples were analyzed using biochemical methods. Microscopy was done to observe the presence of motile bacteria and pus cells. The positive samples were cultured on Cystine Lactose Electrolyte Deficient (CLED) media. The isolated bacterial pathogens were identified by standard bacteriological methods. Antibiotic susceptibility testing was done on the antibiotics recommended for bacteriuria by the Ministry of Health. Demographic characteristics were analyzed using simple percentages among related variables and presented using pie chart and tables. The occurrence of bacteriuria was calculated using the formulae of Le and Boen *et al*. (1995). The bacterial isolates and antibiotic susceptibility tests were analyzed using Pearson's Chi-square test at a confidence interval of 95%. Out of 384 urine samples tested, 311(81%) samples had significant growth (P=0.001) and 73(19%) had no growth (P=0.056). The frequency of the study population was high within age group 18-27 years with (46.88%).The distribution of bacteriuria within age groups was highest in age group 18-27 years with (45.31%, P= 0.001). This study showed decrease of bacteriuria with increasing age (P=0.001). *Escherichia coli* was the most isolated bacterial pathogen (41.5%). *Escherichia coli* and *Staphylococcus saprophyticus* pathogens were isolated in all age groups. Among the ten antibiotics tested, bacterial isolates were sensitive to Cefuroxime at 93.2% (χ^2=29.809, P=0.001) and resistant to ceftazidime at 89.7% (χ^2=62.791, P=0.001*).* The most sensitive bacterial isolate was *P. mirabilis* and the most resistant was *P. aeruginosa.* This study recommended that, since antibiotic resistance among the uropathogens is evolving problem, a routine surveillance to monitor the etiologic agents of bacteriuria and the resistance pattern should be carried out timely to choose the most effective empirical treatment by the physicians. Data obtained from this study is important in appropriate management and treatment of bacteriuria. This will help in proper prescription of the appropriate antibiotic for the treatment of bacteriuria.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of Study

Bacteriuria is the presence of bacteria in urine. It is a common contagion infection among women due to their physiology (Todar, 2012). Bacteriuria in most cases is responsible for urinary tract infections which is fourth most healthcare linked infection in hospitals (August and Rosa, 2012). Bacteriuria may be either asymptomatic or symptomatic. Asymptomatic infection is one which shows no signs of infection while in symptomatic, signs are present. The signs and symptoms of bacteriuria are dysuria, frequency, urgent urination, suprapubic pain, hematuria and lower abdominal pain. In both conditions, a considerable count of bacteria in the urine will be found.

Asymptomatic bacteriuria in sexually active non-pregnant women population in most cases may fail to detect urinary tract infection at initial and reversible stages (Osungunna and Adeyemi, 2016). This is because most women may not feel any urge to look for medical attention. It has been suggested that significant asymptomatic bacteriuria signifies an early clinical history of urinary tract infection which may result to acute infection, chronic infections or even death from kidney malfunction (Mnif *et al.,* 2013). Though the infection may be insignificant in the early stages the patient may develop symptoms as the infection progresses (Mnif *et al.,* 2013). Bacteriuria is fourth most healthcare associated infection with likely over 93,300 bacteriuria in hospitals.

An estimated 150 million individuals are affected by bacteriuria, symptomatically or asymptomatically yearly on a global basis. It has been predicted that every year, at least 13,000 deaths are correlated with bacteriuria (August and Rosa, 2012).

The possible pathogens causing bacteriuria are gram positive bacteria including *Staphylococcus saprophyticus*, and Haemolytic *Streptococcus*. The gram negative bacteria are like *Escherichia coli, Proteus species, Pseudomonas aeruginosa, Klebsiella strains, Salmonella typhi* and *Salmonella paratyphi* (Todar, 2012). Though, as with many community acquired infections, bacterial resistance to antibiotics cause bacteriuria complications which is alarming and a serious health concern in the treatment of bacteriuria (Gupta and Stamm, 2010; Ouma *et al.,* 2012). Antibiotic resistance has become a great concern worldwide to the public health particularly on *E. coli*, the major causative agent of bacteriuria (Chakupurakal *et al*., 2010).

In Kenya, there is no published study on sexually active non-pregnant women that identifies the uropathogenic bacteria and their antibiotic sensitivity profile though studies in Uganda indicated a high resistance to antibiotics commonly used in management of bacteriuria on this group including gentamycin, cefuroxime, nitrofurantoin and norfloxacin (Mwaka *et al*., 2011). Therefore this study intended to determine the current prevalence of bacteriuria, the causative agents and antibiotic sensitivity profile on the isolated bacteria to the most commonly prescribed antibiotics for treatment on sexually active non- pregnant women.

1.2 Statement of the Problem

Bacteriuria is amongst the widespread bacterial infections in medical practice and result for substantial death and increased healthcare expenses. A wide range of uropathogens, predominantly gram negative bacteria investigated at Aga Khan University hospital, Nairobi showed a high rate of resistance to commonly used antibiotics including gentamycin, nitrofurantoin, ampicillin and levofloxacin (Okinda and Revathi, 2012). Most research done globally on UTI are often on nosocomial and/or in institutional settings, particularly in individuals with alterations of the urinary tract either structural or functional (often associated with urinary catheterization) or with other infections like schistosomiasis (Magak *et al*., 2015; Tillekeratne *et al*., 2014). Majority of studies done in Kenya have focused on pregnant women with no documented information available on the sexually active non pregnant women population despite a heightened risk of UTI in this age group. Therefore the study determined the prevalence of bacteriuria, the causative agents and antibiotic sensitivity profile of the isolated bacteria to the most commonly prescribed antibiotics for treatment in sexually active non pregnant women.

1.3 Justification of the study

Antibiotic remedy is the principal medication for bacteriuria, with the prime target being the elimination of the bacterial multiplication in the urinary tract through secure and cost-effective antibiotic use. Bacterial uropathogen resistance to the commonly used antibiotics impairs the effective management of ever increasing range of bacteriuria. Bacteriuria requires intervention across all government sectors and society since it is a

serious threat to global public health. The health care for resistant infection is costly compared to non- resistant infections due to prolonged illness, additional investigations and use of high costly antibiotics (WHO, 2016). There is no information on the prevalence of bacteriuria on sexually active non-pregnant women in Kenya. Lack of this information and the pathogens that cause the infection hinders intervention towards management of bacteriuria in sexually active non-pregnant women. Data obtained from this study is important in appropriate management and treatment of bacteriuria. This will help in proper prescription of the appropriate antibiotic for treatment of bacteriuria.

1.4 Research Questions

i. What is the occurrence of bacteriuria in sexually active non pregnant women?
ii. What are the bacteria causing bacteriuria in sexually active non pregnant women?
iii. What is the antibiotic susceptibility profile for the isolated bacteria?

1.5 Null Hypotheses

Ho1: There is no bacteriuria in sexually active non-pregnant women attending Thika Level five Hospital.

Ho2: Bacteria causing bacteriuria among sexually active non pregnant women at Thika level five Hospital are not resistant to the antibiotics prescribed for treatment.

1.6 Objectives

1.6.1 General Objective

To determine the occurrence of bacteriuria and antibiotic sensitivity profile of bacteria isolated from sexually active non-pregnant women attending Thika level 5 Hospital.

1.6.2 Specific Objectives

i. To determine the occurrence of bacteriuria in sexually active non-pregnant women attending Thika level 5 Hospital.
ii. To isolate the bacteriuria uropathogens in sexually active non-pregnant women attending Thika level 5 Hospital.
iii. To determine the antibiotic sensitivity profile of the isolated bacteria in sexually active non-pregnant women attending Thika level 5 Hospital.

1.7 Significance of the Study

Data obtained from this study is important in appropriate management and treatment of bacteriuria. This will help in proper prescription of the appropriate antibiotic for bacteriuria treatment.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Bacteriuria

Bacteriuria is the commonest infection in sexually active non-pregnant women between the ages of 18-48years (Stamm *et al.,* 2001). In healthy women the prevalence of bacteriuria increases with age. It happens more regularly in developing countries among the low-social financially viable population including Kenya. This infection is persistently associated with varying prevalent by age, sex, sexual activity and urinary tract abnormalities (Nicolle, 2003; Colgan *et al.,* 2006). Bacteriuria has been defined as the state in which bacteria actively multiply in the urine from urine formation in the kidney up to urethral meatus. This is the second frequent health problem in human (Stamm *et al.,* 2001). Studies propose that up to 95% of bacteriuria develop by an ascending route from urethra and progresses upward to the bladder (Chakupurakal *et al*., 2010). Symptomatic bacteriuria can either be complicated or uncomplicated depending on patient anatomical or physical abnormality that triggers urinary tract infection Nicolle (2008).

Bacteriuria infection is caused by both gram positive and gram negative uropathogens. Owing to the wide range of spectrum of UTI, the pathogens causing the infection have been found to develop resistance to the available antibiotics (WHO, 2016). Administration of the broad spectrum antibiotics lead to antibiotic resistance, worsening management of the UTI (Munar and Singh, 2007).

2.2 Epidemiology of Bacteriuria

Bacteriuria infections are considered to be the most frequent urinary tract infection. According to the 1997 National Ambulatory Medical Care Survey and National Hospital Ambulatory Medical Care Survey, UTI accounted for virtually seven million office visits and one million emergency department visits, resulting in 100,000 hospitalizations (Foxman, 2002). The lifetime risk for bacteriuria in women is extreme (greater than 50%). Between 1988 and 1994 the prevalence of bacteriuria principally in lifetime was approximated to be 53,067/100,000 women worldwide (Griebling, 2005). Nonetheless, it is problematic to exactly evaluate the incidence of bacteriuria, in view of the fact that they are not reported infections in Kenya and in Africa. This state is further complicated by the fact that perfect and accurate diagnosis depends on both the presence of symptoms and a positive urine culture, even though in nearly all outpatient backgrounds this diagnosis is mainly made without the benefit of urine culture.

Women are significantly more susceptible to bacteriuria than men (Nicolle, 2005). Virtually 1 in 3 women will have had no less than one incident of bacteriuria necessitating antibiotic treatment by the age of 24 years (Foxman, 2002). Approximately half of all women will encounter one bacteriuria incidence in their lifetime. Special individual populations at increased menace of bacteriuria include infants, pregnant women, the elderly, patients with spinal cord injuries and/or catheters, patients with diabetes or multiple sclerosis, patients with acquired immunodeficiency disease syndrome/human immunodeficiency virus, and patients with underlying urologic

abnormalities. Catheter-associated bacteriuria is the most common nosocomial infection, accounting for >1 million medical cases in hospitals and nursing institutions. The jeopardy of bacteriuria increases with prolonged periods of catheterization (Nicolle, 2008).

In non- institutionalized aged populations, bacteriuria is the second most frequent mode of infection, accounting for almost 25% of all infections. There are significant medical and economic implications linked with bacteriuria. In the non obstructed, non pregnant female adult, acute uncomplicated bacteriuria is considered to be a benevolent sickness with no long-term medical consequences. Nevertheless, bacteriuria raises the risk of pyelonephritis, premature delivery, and fetal mortality among pregnant women, and is associated with impaired renal function and end-stage renal disease among pediatric patients (Wagenlehner *et al*., 2008).

Economically, the anticipated annual cost of community-acquired bacteriuria is remarkable, at approximately $1.6 billion. Prescribing outline revealed an increase in the propensity toward using fluoroquinolones as first line treatment remedy for bacteriuria, which was associated with increased fiscal implications. Composite data demonstrated that entirety expenses for the treatment of bateriuria in women in the United States, without outpatient prescriptions expenditure, were estimated to 2.47 billion dollars in 2000 (Griebling, 2005).

2.3 Research done on bacteriuria globally

Bacteriuria is regarded to be the most widespread health concern in individuals (Caljouw *et al*., 2011). Particular special population at higher risk of bacteriuria include infants, expectant women, the old aged, spinal cord injuries or catheterized patients, diabetes individuals, multiple sclerosis patients, acquired immunodeficiency disease syndrome/human immunodeficiency virus patients, and patients with underlying urologic abnormalities. Catheter-associated bacteriuria is the most frequent nosocomial infection, accounting for >1 million cases in hospitals and nursing homes (Marques *et al*., 2012).

2.3.1 Bacteriuria in elderly

In non-institutionalized old aged populations, bacteriuria is primarily the second most form of health concern, accounting for almost 25% of all concerns (Marques *et al*., 2012). Bacteriuria is quite common in elderly populations. A variety of factors predispose older adults to infections. Age-associated changes in adaptive and innate immunity may increase susceptibility to bacteriuria. In community-dwelling older adults, one of the strongest cause of bacteriuria is having a history of bacteriuria (Das R *et al*., 2009). A change in vaginal flora as a result of declining estrogen levels is thought to predispose postmenopausal women to bacteriuria (Marques *et al*., 2012). In men, prostatic hypertrophy causing urinary retention and high postvoid residuals may be associated with developing bacteriuria (Caljouw *et al*., 2011). They tend to affect women more than men; though elderly male patients placed on catheters could also get bacteriuria frequently.

The problem with treating bacteriuria in the elderly is that they are often asymptomatic. As a result, they tend to go unnoticed for a long time leading to various complications.

In normally alert and active elderly people, UTIs typically present themselves in the form of sudden and unexplained delirium or confusion (Detweiler *et al*., 2015). In the community residents' older individuals, the incidence and prevalence of bacteriuria differs with age and gender. The incidence of bacteriuria ranges from 0.07 per person/year in postmenopausal women (Cotter *et al*., 2012) and 0.13 per person/year in individuals aged more than 85 years (Caljouw *et al*., 2011). The prevalence of UTI in one cohort study in women older than 65 years was approximately 16.5% over a 6-month period (Marques *et al.,* 2012).

Another cohort report in women older than 85years established that nearly 30% of women to have reported no less than one bacteriuria incidence within a period of 12 months (Eriksson *et al*., 2010). In men, the yearly occurrence of bacteriuria ranges from 0.05% in men aged 65 to 74 years and is anticipated to rise to 0.08% in men aged 85 years and above (Griebling, 2005). Even though bacteriuria is one of the most principally reported infections in elderly, the meaning of symptomatic bacteriuria differ considerably across the literature, leading to the documented reports on incidences and prevalences of symptomatic bacteriuria in elderly individuals variable.

Escherichia coli (*E. coli*) is the most widespread pathogen isolated from urinary cultures in both community based dwellers and institutionalized aged individuals (Hu *et al*., 2004). Several population based review on postmenopausal women in community dwelling individuals reported *E. coli* to be the most frequent bacteria isolate, accounting for 75% to 82% of bacteriuria among individuals. Other commonly isolated bacterial pathogens include *Klebsiella* species, *Proteus* species and *Enterococcus* species (Marques *et al*., 2012). Bacterial organisms accountable for bacteriuria in long term care residents are similar to those in community populations.

In a cohort study of long-term care dwellers, *E. coli* was established to be the leading organism, accounting for 53.6% of positive urine cultures (Marques *et al*., 2012). On the other hand, other Enterobacteriaceae were also frequent and accounted for 34.8% of the total urine cultures. Specifically these included: *Proteus mirabilis* (14.6%), *Klebsiella aerogenes* (13.9%), and *Providentia stuartii* (3.7%). Gram-positive bacteria including *Enterococcus* and *Staphylococcus* species accounted for 4.5% and 4.1% of bacteriuria infection cases, respectively (Dar *et al*., 2009; Marques *et al*., 2012).

A research study in Uganda and Nigeria on older adults living in 32 long-term care facilities reported *E. coli* to be the most predominant bacteria isolated from urinary cultures, accounting for 69% of positive urine cultures. *Klebsiella aerogenes* was the second frequent pathogen (12%) followed by *Enterococcus faecalis* (8%) (Sundvall *et al*., 2011; Mwaka *et al*., 2011). It is suggested that the postmenopausal situation,

deteriorating incontinence and disability, and increased exposure to antibiotics usage alters the vaginal microbiome of aged women, thus changing the outline of bacterial uropathogens causing UTI in community dwellers and institutionalized individuals (Osungunna and Adeyemi, 2016).

2.3.2 Bacteriuria in infants

Bacteriuria remains as the most predominant bacterial infection in childhood. The cumulative incidences of bacteriuria in children by 6 years of age are 3% to 7% in girls and 1% to 2% in boys. This amounts to 70000 to 180000 children developing bacteriuria annually (Sabeen, 2012). In men, bacteriuria is more frequent during neonatal stage and early infancy though it minimizes afterwards. It is usually associated with anatomical abnormalities and outlet obstruction. Bacteriuria occurs in 0.1% to 0.4% of infant girl and increases to 1.4% during 1-5 years and 0.7% to 2.3% in school aged girls. In boys close to 0.2% of circumcised and 0.7% of uncircumcised boys are at risk which reaches 0.1% to 0.2% during 1-5 years and 0.04 to 0.2% in school age (Bauer and Kogan, 2008).

Asymptomatic bacteriuria occurs at 1% and 3% in infants and preschool age children respectively and in about 1% of older children (Shaikh *et al*., 2008). *Escherichia coli* is responsible for over 80% of paediatrics bacteriuria. Other common Gram negative organisms include *Klebsiella*, *Proteus*, *Enterobacter* and occasionally *Pseudomonas* (Sabeen, 2012). *Proteus mirabilis* is a common pathogen in males and in children with kidney stones. Gram positive pathogens include Group B *Streptococcus* and

Enterococcus in neonates and infants, and *Staphylococcus saprophyticus* in adolescent girls (Bhat *et al*., 2011). Asymptomatic bacteriuria in infants and children should not be treated with antibiotics because it spontaneously resolves over time in most cases. (Shaikh *et al*., 2008).

2.4 Bacteriuria in sexually active non-pregnant women

In bacteriuria, the predominance of bacteriuria are initiated by uropathogenic *E. coli* (UPEC). Which are tremendously frequent infections that preferentially affect women. Further complicating sources, such as catheterization, diabetes, and spinal cord injuries can raise the occurrence and severity of UTIs (Michael *et al*., 2016). The main symptoms of bacteriuria includes; urination necessity, frequency and dysuria are readouts of the autonomic nervous system (ANS) and the preponderance of the causes that lead to complicated bacteriuria have been shown to influence ANS function. Bacteriuria preferentially affect women, who have a 60.4% lifetime risk of developing a UTI (Foxman, 2014). Uncomplicated bacterial cystitis is the most common manifestation of bacteriuria with the majority caused by UPEC (uropathogenic *E. coli*) defined as a subset of extra-intestinal pathogenic *E. coli* with urovirulence (Hooton, 2012).

Bacteriuria is classified into six categories. The first category is an uncomplicated bacteriuria; this is when the urinary tract is normal, both structurally and physiologically, and there is no associated disorder that impairs the host defense mechanisms. The second category is a complicated bacteriuria*;* this is when bacteriuria occurs within an abnormal

urinary tract, such as when there is ureteric obstruction, renal calculi, or vesicoureteric reflux (Mnif *et al*., 2013). The third category, an isolated bacteriuria, is when it is the first episode of bacteriuria, or the episodes are 6 months apart. Isolated bacteriuria affect 25–40% of young females (Dobrindt *et al*., 2016).

The fourth category, an unresolved bacteriuria, is when therapy fails because of bacterial resistance or due to infection by two different bacteria with equally limited susceptibilities (Geerlings *et al*., 2014). The fifth category, reinfection, occurs where there has been no growth after a treated bacteriuria, but then the same organism re-grows two weeks after therapy, or when a different microorganism grows during any period of time (August and Rosa, 2012). This accounts for 95% of recurrent bacteriuria in women. Bacterial persistence happens when therapy is impaired by the accumulation of bacteria in a location that cannot be reached by antibiotics, such as infected stones, urethral diverticula and infected paraurethral glands. The sixth category, relapse, is when the same microorganism causes bacteriuria within two weeks of therapy; however, it is usually difficult to distinguish a reinfection from a relapse (Rama *et al*., 2014).

2.5 Bacteriuria exposure and health consequences

Bacteriuria is frequently in women than in men. Bacteriuria is normally hygienic, but pathogenic bacteria may contaminate and colonize distal urethra from the perianal area hence bacteriuria (Nicolle, 2005). Following Subsequent invasion of the bladder, the bacterial uropathogen may become dominant. About 6-10% of women may demonstrate

asymptomatic bacteriuria. If septicemia happens, severe complications can arise including, shock and rarely death (Mignini *et al.*, 2009; Mohsin *et al*., 2010). In subgroup of individuals, complicated bacteriuria can lead to upper urinary tract infections or urosepsis.

There are several categories of bacteriuria, cystitis and pyelonephritis included depending on host characteristics. Conditions like cystitis and pyelonephritis are characterized by combination of bacteriuria symptoms which includes; urine frequency, urgency and dysuria. These symptoms are associated with ANS control of bladder function. In addition to uncomplicated cystitis and pyelonephritis, numerous other conditions alter bacteriuria by changing the functional and environmental characteristics of the bladder. These complications include Catheter-associated bacteriuria structural abnormalities of the urinary tract, diabetes, neurogenic bladder, pregnancy, and age (Chenoweth *et al*., 2014; Dielubanza *et al*., 2014; Foxman, 2014; Nicolle, 2014).

Further increasing the potential mechanistic diversity of UTI is the wide range of potential bacterial and fungal uropathogens. While UPEC (Uropathogenic *E.coli*) account for 80% of all uncomplicated community acquired bateriuria, the remaining 20% are caused by a range of *Candida* species, Gram-positive and Gram-negative bacteria including *Staphylococcus saprophyticus* and *Klebsiella* species (Hooton, 2012; Kauffman, 2014). Unfortunately, while there are clear mechanistic connections between

the ANS and bacteriuria symptomology, alterations of ANS function have been understudied in relation to bacteriuria risk.

It is well established that in healthy bladders, the sympathetic branch of the ANS suppresses the contraction of bladder detrusor muscle while increasing contraction of bladder neck sphincters, allowing bladder filling and preventing incontinence (Ochodnicky *et al.*, 2013). Conversely, parasympathetic stimulation of pelvic nerves causes the bladder to contract and the bladder neck sphincters to relax, resulting in micturition. Thus, sympathetic stimulation is predominant in bladder filling and parasympathetic stimulation is responsible for voiding (Ochodnicky *et al.*, 2013).

2.6 Pathogens causing bacteriuria

In the study done in Uganda, Mulago Hospital, the bacterial pathogens which were isolated from the 399 urine samples included *E.coli* (58%), *Staphylococcus aureus* (22%), *Enterococci* species, (15%) and *Klebsiella pneumonia* with 5.0% (Mwaka *et al.*, 2011). Other causative pathogens for Urinary tract infections may include *Proteus mirabilis*, *Staphylococcus saprophyticus* and *Staphylococcus epidermidis* (Gupta *et al.*, 2011). *Staphylococcus saprophyticus* and *Escherichia coli* are responsible for approximately 80% of non-hospital acquired bacteriuria mainly in women below fifty years (Ronald, 2003). *Escherichia coli* is a Gram-negative, facultative anaerobe a normal flora of the lower intestine which also harbors *Bacteroides, lactic, methanogens, clostridia* and *Bifidobacteria* (Dobrindt *et al.*, 2016).

Uropathogenic *E. coli* produces alpha and beta hemolysin which lysis epithelial cell lining urinary tract. Among the different serotypes of *E. coli*, 01, 02, 04, 06, 07, 08, 016, 018, 025, and 075 are mostly isolated from individuals with urinary tract infections (Todar, 2012). About 80% of uropathogenic *E. coli* posses P fimbriae used for adherence of uropathogenic *E. coli* to the outer membranes of urothelial cells glycolipids lining of the kidney tubules (Johansen and Naber, 2014). Type 1 fimbriae are another virulence factor which plays an important role in the inter-bacterial adhesion to the uroepithelial cells and biofilm formation. Possession of adhesion protein FimH acts as a major function in the pathogenic procedure of *E. coli* in the colonization.

It activates both cellular invasion of *E. coli* and adhesion to mannose-containing glycoprotein (Hadi *et al.,* 2007). Study reviews *in vivo* and *in vitro* reported that the pathogenicity of uropathogenic *E. coli* is principally associated with the mode of colonization and invasion of the bladder epithelium and the capability to form intracellular bacterial communities. FimH enables uropathogenic *E. coli* to escape the host innate immunity by internalization within urothelial cells, mediated by the activation of host signal transduction cascades via protein tyrosine kinases, phosphoinositide-3 kinase, and localized host actin cytoskeletal rearrangements (Mulvey *et al.*, 2000).

Escherichia coli accounts for more than 90% of the more than 7 million cases of cystitis and 250,000 of pyelonephritis estimated to occur in otherwise healthy individuals every year in the United States. UTIs are more common in women, 40% of whom have an

episode in their lifetime, usually when they are sexually active. The reservoir for these infections is the patient's own intestinal *E. coli* normal flora, which contaminate the perianal and urethral area (Kenneth and George, 2004).

In individuals with urinary tract obstruction or instrumentation, environment sources assume some importance. *Staphylococcus saprophyticus* is a main pathogen responsible for urinary tract infection among young women where in a small proportion of women it colonizes the rectum, cervix and urethra (Todar, 2012). *Escherichia coli* is the common bacteria accountable for nosocomial bacteriuria but other Gram negative bacterial pathogens including *Pseudomonas* species *Enterobacter* species *Serratia* species, *Citrobacter* species, and *Klebsiella* urease-producing species, *Proteus* species, *Corynebacterium urealyticum* and *Providencia* species may also cause UTI in this nature of infection (Wagenlehner *et al*., 2009). These pathogens are mainly involved in nosocomial urinary tract infection because of the impermeability of antibiotics into the biofilm formed around and within the infectious stone (Marcus *et al.,* 2008).

2.7 Laboratory diagnosis of bacteriuria

Bacteriuria represents symptoms like dysuria, frequent, urgent urination, lower abdominal pain, suprapubic pain and hematuria. Systematic signs and symptoms are rare or absent. The urine may appear turbid mostly producing unpleasant odour (Franco, 2012). Diagnosis of the infection relies basically on the characteristics of clinical presentations, past medical history, at least three significant urinary cultures performed in

the last twelve months duration in symptomatic individuals and detection of the presence or absence of leucocytes, nitrites or protein in urine (Epp *et al.*, 2010). Bacteriuria is diagnosed using a clean mid-stream voided urine sample. Traditionally, 1000 single isolate bacteria per ml have been used to define significant bacteriuria, with excellent specificity, but a sensitivity of 50% (Fihn, 2003). To diagnose bacteriuria, increasing the colony from 1000 to 10000 bacteria per ml in symptomatic patients improves the sensitivity without significantly compromising the specificity.

Laboratory diagnosis involve; urine dip stick done on midstream urine testing for leucocyte esterase or nitrite test which is a rapid and inexpensive method with a sensitivity of 75% and specificity of 82% (Fihn, 2003). It is a good screening test but women with negative test results and symptoms should still have a urine culture performed because false negative results are common. Culture of the urine sample on CLED media followed by gram stain technique on the colonies obtained after an overnight growth is done. Antibiotic sensitivity testing is done to determine which antibiotic is appropriate for different bacterial species and specific patients.

2.8 Bacteriuria Complications

When treated properly, bacteriuria rarely leads to complications. But left untreated, bacteriuria can have serious consequences. Complications of bacteriuria may include: Recurrent infections, especially in women who experience two or more bacteriuria in a six-month period or four or more within a year. Permanent kidney damage from an acute

or chronic kidney infection (pyelonephritis) due to an untreated bacteriuria. Increased risk in pregnant women of delivering low birth weight or premature infants. Urethral narrowing (stricture) in men from recurrent urethritis, previously seen with gonococcal urethritis. Sepsis, a potentially life-threatening complication of bacteriuria, especially if the bacteria work their way up your urinary tract to your kidneys.

2.9 Antibiotic sensitivity to uropathogens

Most of the antibiotic resistance in bacteriuria is due to misuse of antibiotics which has favoured the emergence and survival of the resistant strains (Hooton, 2012). Antibiotic susceptibility of uropathogens needs regular monitoring. The distribution of antimicrobial susceptibility data of bacteriuria causing microorganism's changes from time to time and from place to place. However, a huge percentage of unrestricted antibiotic use has contributed to the emergence of bacterial infections resistance. As a consequence, the frequency of antibiotics resistance among urinary tract pathogens has been escalating globally (Mirella *et al*., 2016). The high rate of recurrence, coupled with the growing uropathogens resistance to commonly prescribed antibiotics and the capability of these pathogens to persist chronically, enable bacteriuria to be a major troubling healthcare problem (Ferry *et al*., 2004; Totsika *et al*., 2011).

Most of the international guidelines for treatment of community acquired urinary tract infection suggest co-trimoxazole, amoxycillin/ampicillin, norfloxacin as a preferred empirical treatment (Gupta *et al*., 2011). However the therapeutic guidelines propose that

local antibiotics vulnerability paradigm must be considered before choosing and prescribing any antibiotics (SIGN, 2012).

A rate of >20 % resistance in the community is the most accepted rate for empiric decisions as suggested by the infectious diseases society of America. All the studies conducted in various parts of the world have proved the resistance of uropathogens to commonly prescribed antibiotic agents such as amoxycillin/ampicillin and co-trimoxazole (Dash *et al*., 2013; Mwaka *et al*., 2011; Akoachere *et al*., 2012).

The present study also supports that resistance to these antimicrobials have evolved with the resistance rate of 80.3%, 71.4% and 58.4 % respectively for nalidixic acid, amoxycillin and co-trimoxazole. This preliminary finding of high resistance rate for the commonly used antibiotics has huge public health implications. The high resistance might warrant the change of empirical antibiotic treatment of UTI resulting into the change of the national essential drug list and existing guidelines (Foxman, 2014).

Regular antibiotic survey ensures up to date information on the effectiveness of various antibiotics used for the treatment of uropathogens in order to minimize drug resistance which may be as a result of continuous usage of drugs that are not sensitive to some microorganisms (Gupta and Stamm, 2010). A steady increase of resistance pattern to antibiotics has been documented over the past years. The resistance rates of the commonly prescribed antibiotics utilized in the therapeutic treatment of uropathogens

differs considerably in various geographical locations globally (Ouma, 2012). It has been found that the trend of antibiotic sensitivity pattern to various isolates usually change with time and because of that, there is a need of carrying out routine antibiotic sensitivity testing that will be given in the treatment of a particular infection (Okorondu *et al.*,2013).

The World Health Organization (WHO) has called antibiotic resistance an emerging disease (WHO, 2016). Bacteria may be innately resistant or may acquire resistance to antibiotics. The rapid increase of bacterial resistance to antibiotic agents has led to the search for newer and more potent drugs. In many developing countries, antibiotics can be acquired from unrecognized treatment centers and used without medical authorization or supervision. This leads to the inappropriate use of antibiotics and sub optimal dosages and insufficient duration in treatment (Bader *et al*., 2016). Most important, many countries worldwide, lack effective laboratory surveillance, training and facilities for performing standardized antibiotic susceptibility testing of important antibiotic resistance bacterial pathogens. Lack of sufficient information regarding the selection of drugs, incorrect prescription and local markets selling sub- standard or expired drugs increase the resistance of pathogens to antibiotic (Cheesbrough, 2012).

The repercussions of misuse of first line antibiotics commonly used in most developing countries make the management of UTI more difficult. This leads to costly and unaffordable second line antibiotics for treatment of developing resistant pathogens. This

results to prolonged infection with longer periods of infectivity and further spread of resistant strains (Dash *et al*., 2013).

2.10 Management of bacteriuria

Recurrent UTIs are common in women occurring in up to 25-50% within one year of initial infection. Of all women, 3-5% will have multiple recurrences over many years (Fihn, 2003; Hooton, 2012). Management of recurrent bacteriuria should start with a search for known risk factors associated with recurrence. These include frequent intercourse, long- term spermicide use, diaghram use, a new sexual partner, young age at first bacteriuria and maternal history of UTI. Behavioural changes such as using a different form of contraception instead of spermicides should be advised (Fihn, 2003). Patients are encouraged to take plenty of fluids according to the recommended minimum of 1.6 liters per day and micturate more often to clean up bacteria from the urinary bladder (Bader *et al*., 2016).

Retaining urine for prolonged period enhances bacteria growth and colonization of the urinary tract leading to cystitis. Women are also advised on genital cleanliness, ensure proper wiping after urination or coitus and should be done front to back. This minimizes the contamination of *E.coli* from the perianal region to the urethra (Bader *et al*., 2016). Practicing safe and faithful sexual partners also will minimize risk of both UTI and STIs. Women are discouraged on the use of spermicidal contraceptives, diaphragms and vaginal douching (Bader *et al*., 2010).

Antibiotic management is the principal therapeutic measure with main purpose being the elimination and reduction of bacterial progression in the urinary tract through a liable, safe and reasonably priced antibiotics therapy. This is achievable especially when the prescribed antibiotics concentrations retained at adequate dosage levels within a short duration (Gupta and Stamn, 2010). To maintain patient relation and drug adherence the antibiotic prescription dosage should be administered for the shortest time possible to hinder bacterial antibiotic resistance. These drugs should be prescribed as per the antibiotic sensitivity and susceptibility of the infecting bacteria. The MoH and WHO recommends treatment of bacteriuria to be best guided by the results of cultures and antimicrobial susceptibility tests.

Many women with recurrent UTIs are aware of symptoms onset. As the cost of office and hospital emergency room visit continues to increase, patients-initiated therapy has become viable option for treatment. Women are given a prescription for one of the 3 day dosage regimens and instructed to start therapy when symptoms develop. If symptoms do not improve in 48 hours, clinical evaluation should be performed (Cheesbrough, 2012).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was carried out in Thika Level 5 Hospital which is in Kiambu County, Kenya. Kiambu county has a population of 1,766,058 (Census, K. 2009) which is growing rapidly. Thika level 5 hospital is a 265-bed government hospital in Thika town. It provides health services to an average of 20,000 inpatients and 350,000 outpatients annually. It serves patients from the town and slum areas including Kiandutu and Kiganjo around Thika town (Appendix V).

3.2 Study Design

A cross-sectional study design was adopted among the sexually active non-pregnant women aged 18 to 48 attending Thika Level 5 Hospital.

3.3 Study Population

All sexually active non-pregnant women aged 18 to 48 years attending Thika Level 5 Hospital with symptomatic bacteriuria were recruited in the study. Women in the age bracket 18-48 years were recruited in this study as it falls under the reproductive/child bearing age (WHO, 2016).

3.3.1 Inclusion Criteria

i. All those who consented.

ii. Sexually active non-pregnant women aged 18-48 years attending Thika level 5 Hospital with symptoms for bacteriuria.

iii. Sexually active non-pregnant women aged 18-48 years who had not taken antibiotics for the last 14 days.

3.3.2 Exclusion Criteria

i. Sexually active non-pregnant women aged 18-48 years who refused to consent for the study.

ii. Sexually active non-pregnant women aged 18-48 years who had been taking antibiotics for the past 14 days.

iii. All women who were out of the age bracket proposed for the study.

3.4 Sample Size Determination

The sample size was determined by using the Fischer *et al*., 2002 formula with the bacteriuria prevalence of 50%. Prevalence of 50 % was considered since the prevalence of bacteriuria in sexually active non-pregnant women aged 18-48 years is not known in Kenya.

$$N = \frac{Z^2 P (1-P)}{D^2}$$

Where:

N = Minimum sample required

Z = 1.96 standard error

P = Prevalence 50%

D = the desired degree of accuracy at 95% confidence level= 0.05

There by:

$$\mathbf{N} = \frac{1.96^2 \text{ x } 0.5(0.5)}{0.05^2} = 384.$$

Therefore, 384 samples were collected in this study.

3.5 Sampling Technique

The study adopted purposive sampling technique among the sexually active non-pregnant women attending Thika level 5 Hospital with symptomatic bacteriuria. Every second patient was sampled among the patients seen by the clinical officer after examination and referred to the laboratory for diagnosis. This was due to the fact that the estimated number of urinary tract infections at Thika level 5 hospital is 831 (Kebira *et al*., 2009). So this number was divided by the study sample size (384) to get an interval of two.

3.6 Sample Collection

Before urine collection, women were confirmed to be non pregnant after carrying out a pregnancy test. Mid-stream urine samples of about 10-15ml were collected using the

procedure of Reynolds (2011) from the patients. Briefly, the sterile plastic urine bottles with air-tight screw cap tops was labeled with patient's information including, patient's code, age, time of collection and taken to the laboratory microbiology bench. All the samples were analyzed through microscopy, culture, Gram staining technique, biochemical tests and drug sensitivity for the bacteria isolated.

3.7 Laboratory Analysis

Collected midstream urine samples were analyzed biochemically for leucocytes, nitrite and protein. Presence of motile bacteria and pus cells were examined microscopically. Culture procedure was done on the selected urine samples, Gram stain technique was done on the colonies and biochemical tests to identify different bacteria. The identified isolates were used to determine sensitivity profile to prescribed antibiotics using Kirby-Bauer disc diffusion technique for bacteriuria treatment (Brown and Smith, 2014).

3.7.1 Urine Dipstick Test

This test was done immediately or between 1-2 hours after the urine collection using the procedure of Chernecky *et al*., (2008). The test was done using URS-10 dipsticks by Bayer Diagnostic UK limited. This was done by gently pouring the urine sample over the uristrip one after another where all samples presenting with leucocytes, proteins and nitrites were counted as positive for bactriuria and those without, as negative for absence of bactriuria. All the urine samples that were positive for protein, nitrite and leucocytes were subjected to culture procedure.

3.7.1.1 Protein strip test

This test was done to detect presence and levels of protein in urine. This test was done immediately after urine collection. Briefly, the urine samples were gently poured on a urine strip and changes of colour on the protein band recorded according the procedure of Ames-bayer. A dipstick protein band is impregnated with a tetrabromophenol blue indicator. In presence of protein, there is a colour change of the indicator from light yellow to green-blue depending on the amount of protein present. Results were interpreted semi-quantitatively as negative, trace, + 0.3g/l, ++ 1g/l, +++ 3g/l. In this case, samples presenting with protein ranging from 0.3g/l or more were counted as positive for UTI.

3.7.1.2 Nitrite strip test

This test is used to differentiate urinary pathogens that are able to reduce nitrate that is normally present in urine to nitrite. The urine samples were tested immediately after collection. Briefly, the urine samples were gently poured on the uristrips one after the other where the colour change on the nitrite band were recorded within a minute using the procedure of Spicer (2000). The reactive strips detects nitrite by using the Griess reaction in which the nitrite test band is impregnated with sulphanilamide buffered into acidic pH which reacts with nitrite to form a diazonium compound. The diazonium compound then couples with 1 N-ethylenediamine producing a red azo dye changing the colour of nitrite test band from white to pink.

3.7.1.3 Leucocyte strip test

This test detects presence of leucocyte esterase which is present in azurophilic granules of granulocytes and monocytes. Briefly, the urine samples were gently poured on the uristrips one after the other where the color change was recorded after two minutes using the procedure of Greenwood *et al.,* (1997). This test reveals the presence of leucocyte esterase which is specific for polymorphoneuclear neutrophils. The leucocyte esterase catalyses the hydrolysis of derivatized pyrazole amino acid ester to liberate a derivatized hydroxyl pyrazole which reacts with diazonium salt to produce a beige-pink to purple colour. The intensity of the purple colour semi quantitatively determines the levels of leucocyte present.

3.7.2 Bacterial Culture Method

All the 384 midstream urine samples were mixed properly by turning gently the specimen container up and down. Briefly, a 0.002 ml loopful of urine sample was inoculated onto Cystine Lactose Electrolyte Deficient (CLED) agar using a standard calibrated sterile wire loop. Streak method was employed to inoculate each urine sample onto the CLED agar plate using the procedure of Cheesbrough (2012). The plates were labeled corresponding to the urine sample reference number and then incubated aerobically at 35-37°C overnight. This helped in isolation of pure growth plates and those with colonies $\geq 10^5$ were regarded as significant growth and picked for both biochemical test and drug sensitivity testing after the incubation time.

3.7.2.1 Characteristics of Bacterial colonies

The urine samples were cultured on CLED agar and incubated overnight. Briefly, following the overnight incubation, the culture plates were examined in a good light. They were viewed from above to describe the appearance of the colonies in terms of their shapes, sizes, margins, opacity and their surface appearance. The culture plates were also viewed from the sides to describe the elevation of the colonies. The colonies were touched using a wire loop to determine their consistency. The reaction of the colonies on CLED agar was determined by colour appearance. These were done using the procedure of Bergey *et al.*, (2012).

3.7.3 Gram Staining Technique

Gram staining technique is a differential staining used to classify and categorize bacteria in specimens or cultures by their Gram reactions (Gram positives or Gram negatives) and morphology (rods, cocci, vibrios, spirilla and spirochaetes. The test was carried out using the procedure of Gram (1884). Smears were made from pure colonies after overnight growth on grease free slides. These were air dried and covered with crystal violet stain for one minute and rinsed with clean water, it was then covered with lugos iodine for one minute and rinsed. The smear was decolorized with acetone-alcohol for thirty seconds and then counterstained with neutral red for two minutes, rinsed and air dried. The stained smears were examined microscopically under oil immersion objective (100×) (Gram, 1884).

3.7.4 Biochemical Test

Biochemical test was done on pure culture plates and those with colonies $\geq 10^5$CFU/ml for identification of the bacteria present using the procedure of (Bachoon *et al*., 2008). Minimum value of $\geq 10^5$ CFU/ml was used to denote bacteriuria. Bacteria identification tests were performed following laboratory standard operating procedures. These biochemical tests include; Indole, Citrate utilization, Urease and Oxidase.

3.7.4.1 Indole Test

The test was to differentiate Gram negative rods particularly *E. coli.* The test organism was cultured in tryptophan broth and incubated at 37°C for 48 hours. One milliliter of kovacs reagent was then added and mixed gently and the mixture was allowed to stand for few minutes. The results were read by observing a cherry red layer at the top (Bachoon *et al*., 2008).

3.7.4.2 Citrate Utilization Test

This test is based on the ability of the organism to use citrate as its only source of carbon, it is used to differentiate enterobacteria (Bergey *et al.,* (2012). The test organism was inoculated in sterile physiological saline in a small test tube, citrate tablet was then added and incubated overnight at 37°C. After the incubation, the mixture was examined for a red color indicated positive citrate test while yellow-orange color indicated negative test for citrate.

3.7.4.3 Urease Test

This test is used to differentiate enterobacteria by testing for urease enzyme activity. Organisms producing urease enzyme break down the urea to give ammonia and carbon dioxide which makes the medium alkaline changing the color of indicator phenol red to pink-red (Brown and Smith 2014). The test organism was cultured in Christensen's urea broth in bijou bottle and incubated at 37°C for 12 hours. After the incubation, the pink color indicated urease positive while its absence indicated negative test for urease.

3.7.4.4 Oxidase Test

This test is used in identification of organisms which produce enzyme cytochrome oxidase. When the organism is oxidase producing, the phenylenediamine in the reagent will be oxidized to a deep purple color. The oxidase impregnated strip was moistened with a drop of sterile water then the colony of the test organism rubbed using a piece of a glass rod on the strip. The results were examined macroscopically for purple color within 20 seconds where absence of purple color indicated oxidase negative. This was by the procedure of (Bachoon *et al*., 2008).

3.7.5 Antibiotic Sensitivity Testing

The test was carried out on the identified bacterial isolates using sensitivity media Mueller-Hinton agar by modified disc diffusion technique of Kirby-Bauer. This was based on the current Clinical Laboratory Standards Institute (CLSI) guideline for BSAC Disc Diffusion Method on Antimicrobial Susceptibility Testing (Tim *et al*., 2009).

The antibiotics tested were Amikacin (30µg), Cefalexin (30 µg), Gentamycin (10µg), Norfloxacin (10µg), Nitrofurantoin (300µg), Cefuroxime (30µg), Nalidixic acid (30µg), Amoxicillin-clavulanic acid (20/10µg), Ceftazidime (30µg) and Ofloxacin (2µg) which are commonly prescribed for the treatment of bacteriuria at Thika Level 5 Hospital.

One to five colonies from every patient culture plate was picked with a standard wire loop. These colonies were emulsified into sterile 5 mls saline solution in a test tube. The picked colonies were mixed uniformly with the saline by stirring with the wire loop. The saline turbidity was attuned to correspond with the standard McFarland 0.5 suspension. Using a sterile cotton bud, a sample of the mixture was picked and the excess saline removed by pressing the swab against the test tube, this was gently inoculated on the surface of the Mueller-Hinton agar (Murray *et al*., 2010). Discs Impregnated with antibiotics were placed on the surface of Mueller-Hinton agar using sterile forceps, at a minimum distant of 10 mm apart between antibiotic discs, 15 mm from the edge of the plate and a maximum of 5 discs per plate was used as per the Clinical Laboratory Standards Institute, 2009. The inoculated culture plates were incubated aerobically at 37°C overnight.

Quality control was performed using the CLSI recommended quality control strains *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. The diameter of zones of inhibition around each disc was measured using a ruler and interpreted using

CLSI guidelines. The outcome reactions were reported as sensitive or resistant for each antibiotic used according to CLSI standards (Table 3.1).

Table 3.1: Zone breakpoints for bacterial pathogens

Antibiotic	Disc potency(µg)	zone diameter(mm) *S. saprophyticus*		Zone diameter(mm) enterobactericeae		zone diameter(mm) *P. aeruginosa*	
		S≥	R≤	S≥	R≤	S≥	R≤
Cefuroxime	30	23	14	23	14	20	14
Amoxiclav	30	18	13	18	13	18	13
Nitrofurantoin	300	17	14	17	14	18	15
Nalidixic acid	30	19	13	19	13	19	13
Cefalexin	30	18	14	18	14	18	16
Nofloxacin	10	17	12	17	12	17	12
Ofloxacin	2	18	14	16	12	16	12
Ceftazidime	30	18	14	21	17	18	14
Amikacin	30	25	21	17	14	17	14
Gentamycin	10	15	12	15	12	15	12

S=sensitive R=resistant **(Source: CLSI, 2009).**

3.8 Ethical Considerations

Ethical approval was sought from Kenyatta University Ethics and Research Committee. The study objectives were explained to the participants and allowed to ask questions. After the women agreed to participate, they signed a consent form (**Appendix I**) and urine samples were collected where laboratory coding was used to identify the

participants. The interviews and examinations were conducted in a private setting within the clinic and the information/results obtained were privately kept. This study benefitted future patients with bacteriuria through appropriate drug administration. The study had no risks.

3.9 Data Analysis

The data was analyzed using Pearson's Chi-square (χ^2) test at 95% confidence interval (CI). Demographic data was analyzed using percentages and frequencies where the ages were stratified in 3 groups with difference of 10 years. Chi square test was conducted to find out the significance difference between the isolated bacterial pathogen, isolated bacterial pathogen related to different age groups and statistical comparisons for sensitivity pattern. The occurrence of bacteriuria was calculated using the formula of Le and Boen *et al., (*1995). The relationships between the occurrences of bacteriuria in the various age groups on the non-pregnant women was considered as statistically significant at P= 0.05 for all test at 95% level of confidence interval. Significant differences occurring between age groups and isolated pathogens were analyzed using Chi square. The data was presented using tables, bar graph and pie chart. The analysis of the results was done using Statistical Packages for Social Science software version 23.

CHAPTER FOUR

4.0 RESULTS

4.1 Demographic characteristics of the study population among sexually active non pregnant women attending Thika Level 5 Hospital

A total of 384 urine samples were tested from sexually active non pregnant women aged 18 to 48 years and their ages were stratified into different age groups. The percentage distribution of the participants among the different age groups were as follows; 46.88% of women were 18-27 years of age, 32.81% were in the ages of 28-37 years while the least number of women participants 20.31% were in the ages 38-48 years (Figure 4.1).

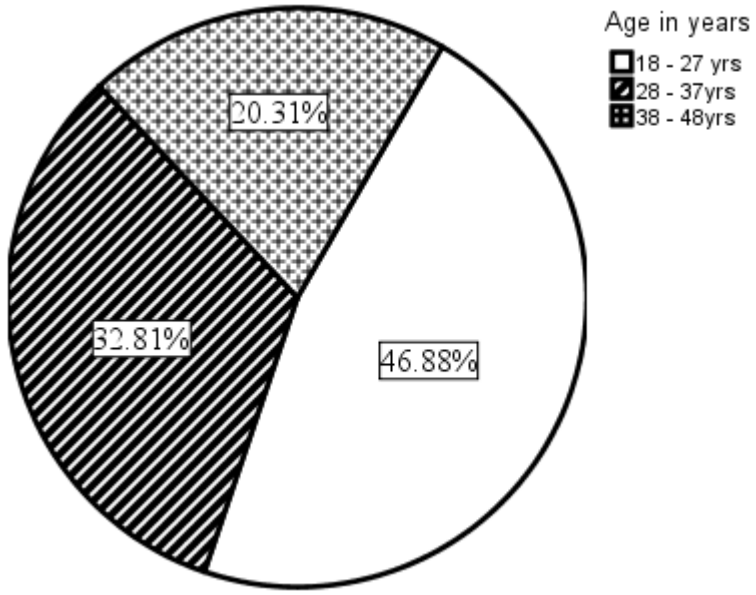

Figure 4.1: Demographic characteristic of the study population among sexually active non pregnant women attending Thika Level 5 Hospital

The frequency of sexually active non pregnant women participants significantly decreased with ages. The difference in number of participants in these different age groups; 18-27, 28-37 and 38-48 years was statistically significant ($F=17.9$, $df_1=2$, $df_2=381$, $P=0.001$).

4.2 Occurrence of bacteriuria among sexually active non pregnant women attending Thika Level 5 Hospital

Out of the three hundred and eighty four (384) patient samples, three hundred and eleven, 311(81%) samples tested had significant bacterial growth and 73(19%) urine samples had no significant bacterial growth (Table 4.1).

Table 4.1: **Occurrence of bacteriuria among sexually active non pregnant women attending Thika Level 5 Hospital**

Status	No. of non pregnant women (N)	Percentage (%)
Bacterial growth	311	81
No bacterial growth	73	19
Total (N)	384	100

Significant bacterial growth obtained was 81%

The number of samples with significant bacterial growth in relation to the total number of participants in the study was calculated to give the percentage occurrence of bacteriuria among sexually active non pregnant women attending Thika Level Hospital.

Using the formula $\frac{O}{P}x100\%$. Where: O = The number of individual with the infection. P=Total number of individuals in the population involved in the study at the study period.

Percentage occurrence =$\frac{311}{384}$ x $100\% = 81\%$

4.2.1 Distribution of bacteriuria based on age groups among sexually active nonpregnant women attending Thika Level 5 Hospital

Among the 384 urine samples analysed, five bacterial isolates were identified. The percentage distribution of bacteria isolates among the stratified age groups were as follows; in age group 18-27 years, bacterial isolates were 45.31 %, age group 28-37 years bacterial isolates were 25.26 % and the least number of bacterial isolates with 10.42 % were from age group 38-48 years. The study noted that the percentage of bacterial isolates significantly decreased with increase in age P=0.015. However, the percentage of samples with no growth showed a significant increase with increase in age (P= 0.001). Age group 38-48 years recorded the highest number of samples with no bacterial growth with 9.90 %, this was followed by age group 28-37 years with 7.55 %. The least number of samples with no growth were in age group 18-27 years with 1.56 % (Table 4.2).

Table 4.2: Distribution of bacteriuria based on age groups among sexually active nonpregnant women attending Thika Level 5 Hospital

	Age in years		
	18 - 27 yrs	28 - 37yrs	38 - 48yrs
Bacteriuria infection	45.31%	25.26%	10.42%
No bacteriuria infection	1.56%	7.55%	9.90%
Total	46.87%	32.81%	20.32%

Age group 18-27 had the highest bacteriuria percentage while age group 38-48 had the lowest bacteriuria occurrence.

4.3 Bacterial pathogens isolated and identified from urine samples

Among the 311 urine samples with bacterial growth, five bacterial pathogens were isolated and identified by their biochemical reactions and appearance of their colonies characteristics on CLED agar. Two hundred and sixteen, 216 (69.5%) of the bacterial isolates were gram negative while 95 (30.5%) of bacterial isolates were gram positive. The bacterial isolates were; *Escherichia coli, Staphylococcus saprophyticus, Proteus mirabilis, Pseudomonas aeruginosa* and *Klebsiella aerogenes*. The results showed *Escherichia coli* as the most frequent isolated bacterial pathogen with 129 (41.48%, χ^2 =147.73, P value 0.001) causing bacteriuria among sexually active non pregnant women attending Thika level 5 Hospital. The second most frequent pathogen was *Staphylococcus saprophyticus* with 95 (30.55%), the third most bacterial isolate was *Proteus mirabilis* with 43 (13.83%), *Pseudomonas aeruginosa* was fourth with 27 (8.68%) and *Klebsiella aerogenes* was the least with 17 (5.47%) of the bacterial isolates (Figure 4.2).

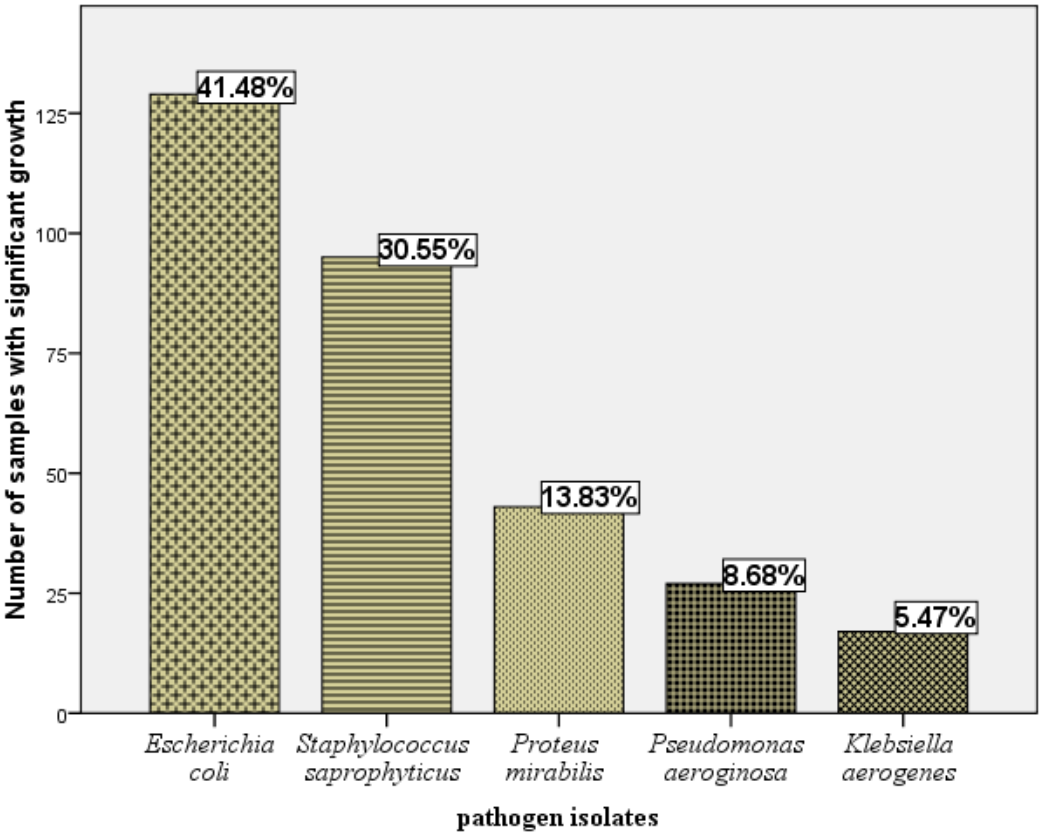

Figure 4.2: Distribution of isolated and identified bacterial pathogen among sexually active nonpregnant women attending Thika Level 5 Hospital
Escherichia coli was the highly isolated *pathogen* with (41.48%, P=0.001).

4.3 (a) Biochemical test

Biochemical reactions for the isolated bacteria were tabulated as shown in table 4.3.

Table 4.3: Biochemical test results

Bacterial pathogen Isolated	Biochemical test results				
	Urea	**Cit**	**Ind**	**Ox**	**Coag**
Escherichia coli	-	-	+	-	
Staphylococcus saprophyticus	-	-	-	-	-
Proteus mirabilis	+	+	-	-	
Pseudomonas aeruginosa	-	-	-	+	
Klebsiella aerogenes	+	+	-	-	

(Source: Cheesbrough, 2012).

Key: + =Positive reaction, - =Negative reaction, **Urea** = urease, **Cit** = Citrate Utilization test,**Ind** = Indole test, **Ox** = Oxidase test and Coag = Coagulase test.

4.3 (b) Appearance of isolated pathogens on CLED agar and Gram staining reaction

The morphology and appearance of colonies of isolated bacterial pathogens on CLED agar and gram staining reaction were tabulated. An example of the isolated bacterial pathogen appearance on CLED agar is as shown in figure 4.3.

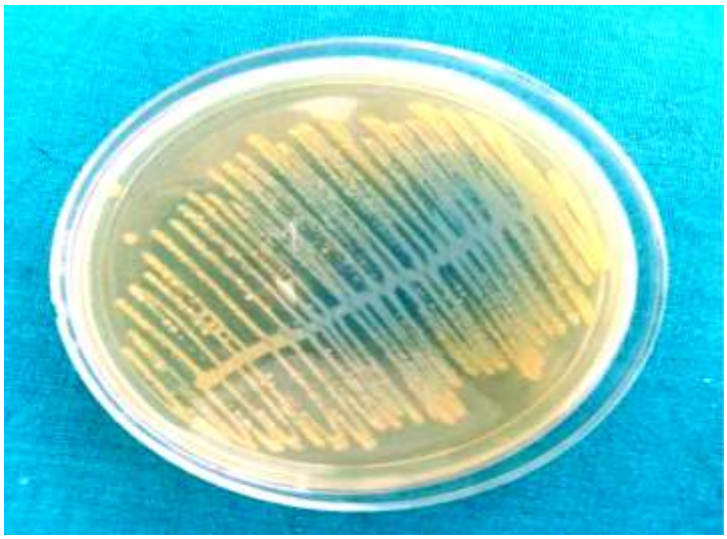

Figure 4.3: Colonies of Escherichia coli on CLED Agar.

The isolated bacterial pathogen characteristics on CLED Agar, morphology and Gram staining reactions were tabulated as shown in table 4.4.

Table 4.4: Chacteristics on morphology and appearance of isolated pathogen colonies on CLED Agar

Isolated bacterial pathogen	Appearance of colony characteristics on CLED agar	Morphology and Gram staining reaction
Escherichia coli	Yellow opaque colonies often with slightly deeper coloured centre	Gram negative rods
Staphylococcus saprophyticus	Yellow to white tiny colonies of uniform colour	Gram positive cocci
Proteus mirabilis	Translucent blue grey colonies	Gram negative rods
Pseundomonas aeruginosa	Green colonies with rough periphery and a characteristic pigmentaion	Gram negative rods
Klebsiella aerogenes	Large muciod yellow-white colonies	Gram negative rods

4.3.1 Distribution of isolated and identified bacterial pathogens based on age groups among sexually active nonpregnant women attending Thika Level 5 Hospital

The results showed that most of the bacterial pathogens were isolated from sexually active non pregnant women at age group 18-27 years with a total of 55.95 % isolates. The age group 28-37 years had 31.19% isolates and the least number of isolates 12.86% were in the age group 38-48 years (Table 4.5). This study noted that the number of bacterial isolates was decreasing with increase in ages. The difference in number of bacterial isolates among the three age groups was statistically significant (P=0.001). These results showed that the association between age group and the pathogens isolated was significant (χ^2=56.236, df=8, P=0.001). However, the difference in number of bacterial isolates between the age group with most bacterial isolates and the least number of bacterial isolate was statistically significant (P=0.001).

Escherichia coli and *Staphylococcus saprophyticus* were the only bacterial pathogens isolated in all age groups with statistically significant difference in all age groups P =0.002 and P=0.001 respectively. All bacterial pathogens were isolated in age groups 18-27 years (P =0.001). The data showed that *K. aerogenes* and *Proteus mirabilis* were not isolated in age group 38-48 years, *P. aeruginosa* was not isolated in age group 28-37 years. The data showed statistical significant in the distribution of bacterial isolates within and between the age groups (P=0.011) as shown in Table 4.5. Age group 18-27 years isolated similar number of *E. coli* and *S. saprophyticus* at (18.65%). Isolation of

Pseundomonas aeruginosa in age group 18-22 years and 38-48 years the difference was statistically insignificant (P=0.059).

Table 4.5: Distribution of isolated and identified bacterial pathogens based on age groups among sexually active nonpregnant women attending Thika Level 5 Hospital

	Age in years			
Bacterial pathogens	18 - 27 yrs	28 - 37yrs	38 - 48yrs	p value
Escherichia coli	18.65%	17.04%	5.79%	0.002
Staphylococcus saprophyticus	18.65%	9.00%	2.89%	0.001
Klebsiella aerogenes	4.50%	0.96%	0.00%	0.034
Pseudomonas aeruginosa	4.50%	0.00%	4.18%	0.059
Proteus mirabilis	9.65%	4.18%	0.00%	0.028
P value	0.001	0.048	0.120	
Total	55.95%	31.19%	12.86%	

All isolates were present in age group 18-27 years.

4.4 Antibiotics sensitivity profile of the isolated uropathogens among sexually active nonpregant women attending Thika Level 5 Hospital

Ten antibiotics commonly recommended by Ministry of Health for treatment of UTI were tested. These included; Cefuroxime (30µg), Amikacin (30 µg), Gentamycin (10 µg), Ofloxacon (2µg), Ceftazidime (30µg), Norfloxacin (10µg), Cefalexin (30µg), Nalidixic acid (30µg), Nitrofurantoin (300µg) and Amoxyclav (30µg). It was observed that all the isolated bacterial pathogens were sensitive to Gentamycin at 83% and Cefalexin at

78.1%. Although Cefuroxime showed to be the most effective antibiotic with 93.2%, *Pseudomonas aeruginosa* isolate was resistant to it.

Antibiotics which showed effectiveness to most of the isolated bacterial pathogens were Cefuroxime 93.2% (χ^2=29.809, P=0.001), Amikacin 84.6% (χ^2=34.680, P=0.001), Gentamycin 83% (χ^2=52.937, P=0.001) and Cefalexin 78.1% (χ^2=43.379, P=0.001) as shown in Table 4.6.

Table 4.6: Antibiotic sensitivity profile to isolated bacterial pathogens among sexually active nonpregant women attending Thika Level 5 Hospital

Antibiotic Tested	Susceptibility outcome	*E.coli*	*S. saprophyticus*	*K.aerogenes*	*P. aerugino sa*	*P.mirabilis*	%of overall susceptibility	Chi-square (χ^2)	P value
Cefuroxime	Sensitive	40.2%	30.5%	5.5%	3.2%	13.8%	93.2%	29.809	0.001
	Resistant	1.3%	0.0%	0.0%	5.5%	0.0%	6.8%	15.235	0.001
Amoxyclav	Sensitive	7.7%	0.0%	2.3%	2.3%	9.2%	21.5%	10.440	0.107
	Resistant	33.9%	30.5%	3.2%	6.4%	4.5%	78.5%	54.297	0.001
Nitrofurantoin	Sensitive	26.0%	2.2%	0.0%	1.0%	4.2%	33.4%	26.647	0.001
	Resistant	15.4%	28.3%	5.5%	7.8%	9.6%	66.6%	1.015	0.001
Nalidixic acid	Sensitive	6.1%	0.0%	0.0%	4.8%	2.6%	13.5%	15.976	0.003
	Resistant	35.4%	30.5%	5.5%	3.9%	11.2%	86.5%	27.741	0.001
Cefalexin	Sensitive	27.0%	29.5%	5.5%	7.4%	8.7%	78.1%	43.379	0.001
	Resistant	14.5%	1.0%	0.0%	1.3%	5.1%	21.9%	19.984	0.003
Norfloxacin	Sensitive	10.3%	3.5%	0.0%	2.9%	7.1%	23.8%	35.096	0.001
	Resistant	31.2%	27.0%	5.5%	5.7%	6.8%	76.2%	56.309	0.001
Ofloxacin	Sensitive	10.6%	11.6%	1.9%	6.1%	3.2%	33.4%	42.155	0.001
	Resistant	30.9%	19.0%	3.5%	2.6%	10.6%	66.6%	26.982	0.001
Ceftazidime	Sensitive	8.3%	0.0%	0.0%	1.0%	1.0%	10.3%	4.431	0.351
	Resistant	33.1%	30.5%	5.5%	7.7%	12.9%	89.7%	62.791	0.001
Amikacin	Sensitive	36.7%	29.8%	5.5%	4.2%	8.4%	84.6%	34.680	0.001
	Resistant	4.8%	0.6%	0.0%	4.5%	5.5%	15.4%	36.978	0.001
Gentamycin	Sensitive	32.5%	29.6%	2.9%	5.5%	12.5%	83.0%	52.937	0.001
	Resistant	9.0%	1.0%	2.6%	3.2%	1.2%	17.0%	27.984	0.001

Bacterial isolates were most sensitive to cefuroxime at 93.2%.

The sensitivity of the isolated bacterial pathogens to different antibiotics used gave different results depending on the antibiotic used and the bacterial isolate in question. *Pseudomonas aeruginosa* was the only bacterial isolate sensitive to Nalidixic acid and ofloxacin but it was resistant to cefuroxime. *Proteus mirabilis* was the only bacterial isolate sensitive to Amoxyclav (χ^2=8.91, P=0.03) and norfloxacin. *Escherichia coli* was the only bacterial isolate sensitive to nitrofurantoin (χ^2=34.62, P=0.03). The overall antibiotic sensitivity profile pattern showed all bacterial isolates were sensitive to gentamycin and all resistant to ceftazidime. Most of the isolated bacteria showed to be resistant to many antibiotics as follows; Nalidixic acid 86.5% (χ^2=27.741, P=0.001), Amoxyclav 78.5% (χ^2=54.297, P=0.001), Norfloxacin 76.2% (χ^2=56.309, P= 0.001), Nitrofurantoin, 66.6% and Ofloxacin, 66.6% resistance (Table 4.7).

All bacterial pathogen isolated showed overall resistance of 89.7% (χ^2=62.791, P=0.001) to Ceftazidime. Four of the isolated bacterial pathogens showed resistant to five tested antibiotics. These includes;- *Escherichia coli*, *P. mirabilis*, *K. aerogenes* and *S. saprophyticus* had an overall resistant of 86.5% to nalidixic Acid, *E. coli*, *K. aerogenes*, *S. saprophyticus* and *P. aeruginosa* had an overall resistant of 78.5% to Amoxyclav, *E. coli*, *K. aerogenes*, *S. saprophyticus* and *P. aeruginosa* had an overall resistant of 76.2% to Norfloxacin, *S. saprophyticus, P. aeruginosa, P. mirabilis* and *K. aerogenes* had an overall resistant of 66.6% to Nitrofurantoin and *E. coli*, *P. mirabilis*, *K. aerogenes* and *S. saprophyticus* had an overall resistant of 66.6% to Ofloxacin. Some of the isolated bacterial pathogens were 100% sensitive to some of the tested antibiotic. *Proteus*

mirabilis, *K.aerogenes* and *S.saprophyticus* were 100% sensitive (χ^2=15.25, P=0.001) to Cefuroxime, *K. aerogenes* was sensitive to Amikacin and Cefalexin P=0.001.

Klebsiella aerogenes was resistance to Nitrofurantoin, Nalidixic acid, Norfloxacin and Ceftazidime while *Staphylococcus saprophyticus* was resistant to Ceftazidime, Amoxyclav and Nalidixic acid, (χ^2=2.32, P=0.95). *Staphylococcus saprophyticus* and *Klebsiella pneumonia* were 100% resistant to Nalidixic acid and Ceftazidime. *Pseudomonas aeruginosa* had a unique sensitivity pattern whereby it was sensitive to Nalidixic acid, Ofloxacin, Cefalexein and Gentamycin while it was resistant to Cefuroxime 63.0% (χ^2=5.130, P=0.163) and Amikacin 51.9% (χ^2=4.569, P=0.206) which other bacterial pathogens isolated were highly sensitive (Table 4.7).

Table 4.7: Distribution of Antibiotic susceptibility pattern by the isolated bacterial pathogens among sexually active nonpregant women attending Thika Level 5 Hospital

Antibiotic susceptibility pattern of bacterial isolates					
	pathogen isolates				
Tested antibiotic sensitivity pattern	***E. coli***	***S. saprophyticus***	***K. aerogenes***	***P. aeroginosa***	***P. mirabilis***
Cefuroxime	S	S	S	R	S
Amoxyclav	R	R	R	R	S
Nitrofurantoin	S	R	R	R	R
Nalidixic acid	R	R	R	S	R
Cefalexin	S	S	S	S	S
Norfloxacin	R	R	R	R	S
Ofloxacin	R	R	R	S	R
Ceftazidime	R	R	R	R	R
Amikacin	S	S	S	R	S
Gentamycin	S	S	S	S	S

S=sensitive **R=Resistant**

All isolated bacterial pathogens were sensitive to Gentamycin and Cefalexin while all bacterial pathogens isolated were resistant to Ceftazidime.

4.4.1 Antibiotics susceptibility profile pattern based on age groups among sexually active nonpregant women attending Thika Level 5 Hospital

The antibiotic susceptibility of the tested ten antibiotics was analyzed based on the stratified age groups. Isolated bacterial pathogens showed Cefuroxime to be the most effective antibiotic with an overall bacterial sensitivity rate of 93.2%. Age group 18-27 years comprised the bigger proportion that had bacterial pathogens sensitive to cefuroxime (53.4%).This result showed a statistically significant association between sensitivity of cefuroxime and age in years (χ^2=87.206, P=0.001).

Bacterial isolates had an overall sensitivity rate at 84% on Amikacin which was second after cefuroxime. Age group 18-27 years comprised of 49.2% pathogens sensitive to amikacin (χ^2=78.714, P=0.001). Bacterial isolates had an overall sensitivity rate of 83.0% on Gentamycin. Age group 18-27 years comprised of 48.9% isolated bacterial pathogen sensitive to gentamycin compared to other age groups which was statistically significant at (χ^2=90.154, P=0.001). Bacterial sensitivity rate on Cefalexin was at 78.1% overall with age group 18-27 years comprising of 48.2% bacterial pathogen sensitive to cefalexin (Table 4.8).

Isolated bacterial pathogens were resistant to Ceftazidime with an overall sensitivity rate of 10.3% (Table 4.8). Age group 18-22 years comprised of 6.4% sensitive bacterial pathogen to Ceftazidime. The sensitivity difference of ceftazidime within age groups were statistically insignificant (χ^2=1.392, P=0.498). Age group 18-27 years comprised of

higher proportion of 49.8% resistant bacterial pathogens compared to other age groups. Nalidixic acid was the second most antibiotic which showed isolated bacterial pathogen resistance at 86.5% overall. Age group 18-27 years comprised a higher proportion of pathogens sensitive to nalidixic acid (7.4%). The association between nalidixic acid sensitivity and age in years was significant (χ^2=21.499, P=0.001). Age group 23-27 years recorded the least number of isolated pathogen sensitive to Nalidixic acid (1.6%).

Bacterial isolates had an overall sensitivity of 21.5% on amoxyclav antibiotic. Isolated bacterial pathogen sensitivity to amoxyclav in association with age was significant (χ^2=81.837, P=0.035) with age group 18-27 years comprising of (15.1%) pathogen sensitive to amoxyclav (Table 4.8). Isolates had overall sensitivity of 23.8% on Norfloxacin. Bacteria isolates sensitivity to Norfloxacin in association with age in years was insignificant (χ^2=0.598, P=0.742). bacterial isolates had similar overall sensitivity of 33.4% to Nitrofurantoin and Ofloxacin. Age group 18-22 years had higher isolates sensitive to Nitrofurantoin and Ofloxacin with (17.0%) and (18.6%) respectively. Bacterial isolates sensitivity to Ofloxacin in all age groups statistically was insignificant (χ^2=2.426, P=0.297) as shown in Table 4.8.

Table 4.8: Antibiotics sensitivity profile based on age groups among sexually active nonpregant women attending Thika Level 5 Hospital

		Age in years					
Antibiotic	Antibiotic susceptibility	18-27	28-37	38-48	Overall sensitivity %	Chi-square(χ^2)	P value
Cefuroxime	Sensitive	53.4%	29.9%	10.0%	93.2%	87.206	0.001
	Resistant	2.9%	1.0%	2.9%	6.8%		
Amoxyclav	Sensitive	15.1%	4.5%	1.9%	21.5%	81.837	0.035
	Resistant	41.2%	26.4%	10.9%	78.5%		
Nitrofurantoin	Sensitive	17.0%	10.9%	5.5%	33.4%	76.152	0.001
	Resistant	39.2%	19.9%	7.4%	66.6%		
Nalidixicacid	Sensitive	7.4%	1.6%	4.5%	13.5%	21.499	0.001
	Resistant	48.9%	29.3%	8.4%	86.5%		
Cefalexin	Sensitive	48.2%	17.4%	12.5%	78.1%	114.183	0.001
	Resistant	8.0%	13.5%	.3%	21.9%		
Norfloxacin	Sensitive	13.2%	8.0%	2.6%	23.8%	0.598	0.742
	Resistant	43.1%	22.8%	10.3%	76.2%		
Ofloxacin	Sensitive	18.6%	9.3%	5.5%	33.4%	2.426	0.297
	Resistant	37.6%	21.5%	7.4%	66.6%		
Ceftazidime	Sensitive	6.4%	2.3%	1.6%	10.3%	1.392	0.498
	Resistant	49.8%	28.6%	11.3%	89.7%		
Amikacin	Sensitive	49.2%	24.4%	10.9%	84.6%	78.714	0.001
	Resistant	7.1%	6.4%	1.9%	15.4%		
Gentamycin	Sensitive	48.9%	21.9%	12.2%	83.0%	90.154	0.001
	Resistant	7.4%	9.0%	.6%	17.0%		

Age group 18-27 composed of higher proportions of pathogens sensitive to all antibiotics P=0.001.

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Demographic characteristics of the study population

A total of 384 urine samples from sexually active non pregnant women aged 18 to 48 years were analysed in this study. Their ages were stratified into three different age groups. Age group 18-27 years showed the highest number of respondents with 46.88%, followed by age group 28-37 years with 32.81% and age group 38-48 years had the least number of participants with 20.31%. The difference in the percentages of the respondents in age groups 18-27, 28-37 and 38-48 years was statistically significant at 95% CI, P=0.05. Majority of the participants were from age groups 18-27 years with percentage of 46.88%.

This high number of participants in age group 18-27 years could probably be due to the fact that this age group is within the peak reproductive period with participants being sexually active. Poor personal hygiene, having multiple sexual partners could have contributed to high risk of exposure. This finding was in line with the study by Mwaka *et al*., (2011) who reported 210 (56.2%) out of 399 participants were from age group 15-28 years and the study by Singh *et al*., (2016) which reported 54.9% of the participant were from age group 18-35 years. Least number of respondents (20.31%) was reported in age group 38-48 years. The difference in participant's number in age group 18-27 years in this study compared to reports done by Mwaka *et al*., (2011) and Singh *et al*., (2016) in

the similar age brackets could be due to different study design employed, number of samples and the participants entry criteria used. However, geographical location may also affect participants outcome due to different ethnic believes and economic status (Okorondu *et al*., 2013).

The low turnout of participants in age group 38-48 years could have been attributed to improved personnel hygiene which is perceived to improve on increasing age, reduced sexual activity therefore minimal exposure (Osungunna & Adeyemi, 2016). Subsequently, older women are associated with asymptomatic bacteriuria which often resolves without any treatment, and is not associated with morbidity or mortality hence most of them may not seek medical care (Okorondu *et al*., 2013). A similar trend was reported by Mwaka *et al*., (2011) in Mulago Hospital, Uganda. However, the study noted that the number of respondents was decreasing with increasing age.

5.1.2 Occurrence of bacteriuria among sexually active non pregnant women attending Thika Level 5 Hospital

In this study, a prevalence of 81% bacteriuria among sexually active non pregnant women was recorded. The high prevalence of bacteriuria in Thika level 5 Hospital could be associated with the participants engaging in casual sex probably with multiple sexual partners due to high urbanization growth, surrounding slum areas and high population resulting to high risk of exposure. Misuse of antibiotics which lead to destruction of

vaginal normal flora resulting to reduction of vaginal immunity could have also contributed to the increase in the pervasiveness of bacteriuria in this study population.

The prevalence of this study compares well with other studies elsewhere. In a study by Subhashini *et al.,* (2016) the occurrence of bacteriuria was documented to be 84%. In a study by Kalowole *et al.,* (2010) the prevalence was reported to be 60%. In a study by Mwaka *et al.,* (2011) in Mulago Hospital, Uganda reported a prevalence of 17.9%. In a study by Osungunna and Adeyemi, (2016) the prevalence was reported to be 13.0%. The difference in prevalence of bacteriuria in this study and other studies which show low prevalence may be due to the study setting where the source of vaginal bacteria varies significantly among women from different ethnic and geographical backgrounds. Moreover, vaginal potential of Hydrogen (pH) among women was found to vary among different age groups as well (Linhares *et al*., 2010).

5.1.2.1 Distribution of bacteriuria based on age groups

In the 384 urine samples analyzed, a higher frequency of bacteriuria was observed in age group 18-27 years (45.31%), followed by age group 28-37 years (26.26%). This frequency of bacteriuria could have been higher in age group18-27 years compared to others due to the fact that women in this age bracket 18-27 years are at the peak reproductive period hence they are more sexually active and most women may be having multiple sexual partners. Poor personal hygiene could also attribute to high risk of exposure. The observation in this study is consistent with reports of other studies. In India, Dash *et al*., (2013) reported 55.4% in age group 18-37 years, in Cameroon,

Akoachere *et al*., (2012) reported 59.1% in age group 20-39 years. Although, they reported a slightly higher percentage than this study, the differences could be associated with the age group ranges. The susceptibility of young women to acquire UTI has been elucidated on the basis of their anatomical or physical abnormalities and certain behavioral factors (Manges *et al*., 2006; Nicolle, 2008; Mirella *et al.,* 2016).

The study noted that frequency of bacteriuria significantly decreased with increasing age in sexually active non pregnant women. A lower rate of prevalence of bacteriuria was reported within the age groups; 38-48 years (10.42%). Women in this age bracket 38-48 years are nearing their menopause age and have reduced sexual activity hence they might have less exposure (Avis, 2000). They seldomly misuse antibiotics and are believed to have improved personal hygiene (Cheesbrough, 2012). This finding is in line with previous study by Singh *et al.,* (2016) who reported a prevalence rate of 8.9%, within the age groups 38-48 years.

5.1.3 Bacterial pathogens isolated from urine samples

Among the 311 urine samples with significant bacterial growth analyzed, five bacterial pathogens were isolated. The results showed *Escherichia coli* (41.5%) as the most dominant bacterial pathogen causing bacteriuria among sexually active non pregnant women attending Thika Level 5 Hospital. This dominance of *Escherichia coli* in this study could be associated with the pathogen rising from the periurethral areas infected by faecal normal flora because of the urethra proximity to the anus (Hooton, 2012). This

could also be attributed to poor personal hygiene among women especially after toilet use. The predominance of *Escherichia coli* could have also been attributed to its strains which possess a variety of virulence characteristics that facilitate intestinal carriage, persistent in the vagina and then ascension and invasion of the normal urinary tract Chakupurakal *et al*. (2010).

The results in this study are consistent with previous studies by Mwaka *et al.,* (2011) reported *Escherichia coli* with prevalence of 57.5%. Studies by Wanyama, (2002); (Moges *et al.*, 2002); (Singh *et al.*, 2016) documented prevalence rates of *E. coli* between 40-46%. A study by Okinda and Revathi, (2012) in Aga Khan Hospital reported a prevalence of 35% on *E. coli*. This prevalence was lower compared to this study possibly due to study population involved. The second most isolated pathogen was *S. saprophyticus* with 30.5%. Although *S. saprophyticus* is less common than *E. coli*, it is more aggressive. *Staphylococcus saprophyticus* is known to cause UTI in young women (Cheesbrough, 2012). Isolation of *S. saprophyticus* in this study could be associated with the bacterium having the ability for choosy adherence to human urothelium where it causes direct hemagglutination. It produces an extracellular enzyme complex that can inhibit growth of both Gram positive and Gram negative bacteria. Earlier studies in Mulago hospital, Uganda by Mwaka *et al.* (2011) reported a prevalence of *S. saprophyticus* 24.9%. However, this finding is different from a Nepal study by Singh e*t al.* (2016) who reported prevalence of *S. saprophyticus* to be 8.3%. The variation could have been attributed to the study setting and the study participants employed. However,

geographical location and time of the study may be the reason for this huge difference in prevalence (Okorondu *et al*., 2013).

The other bacterial isolates in this study included; *Proteus mirabilis* (13.8%), *Pseudomonas aeruginosa* (8.7%) and *Klebsiella aerogenes* (5.5%). Infections due to these pathogens are associated with community acquired UTI in sexually active non pregnant women. This is in concurrence with previous studies done in Mulago Hospital, Uganda by Ouma (2012) and Wamalwa *et al.*, (2013) who isolated *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Klebsiella aerogenes* at 10%, 5.2% and 7% respectively. More recently, in Tikur Anbessa Specialized Hospital in Addis Ababa, Ethiopia *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Klebsiella aerogenes*, were isolated at rates of 16.9%, 5.5% and 5.3% respectively. These prevalence differences in pathogen isolation in this study compared to other studies could be attributed to geographical locations and the study participants recruited.

5.1.3.1 Distribution of isolated bacterial pathogens based on age groups

The five isolated bacterial pathogens in this study were distributed within the stratified different age groups. A higher frequency of bacteriuria was isolated within age group 18-27 years (55.95%). The second most infected age group was 28-37 years (31.19%) and the least infected age group was 38-48 years with (12.86%). The difference in bacteriuria outcome among these three age groups 18-27, 28-37 and 38-48 years was statistically significant (P=0.001). The high rate of bacterial isolates in age group 18-27 years could

be attributed to the fact that, this age group is in the peak reproductive period and most women are more sexually active. This could also be associated with most women having multiple sexual partners therefore becoming more exposed to bacteriuria (Foxman, 2014). Sex is one of the common mode of transmissions of bacteriuria causes UTIs in women. Some birth control methods like the use of diaphragm and spermicides increases the risks of bacteriuria. The products positioning inside the vagina exerts pressure to the urethra hence high exposure.

The prevalence of bacteriuria in age group 18-27 years (55.95%) in this study is not consistent with reports from other researchers. Singh *et al*., (2016) reported a (30%) prevalence rate within age group 16-30 years in Nepal. Mwaka *et al*., (2011) reported a prevalence rate of (27.2%) in age group 20-35 years in Mulago Hospital, Uganda. The difference in occurrence of bacteriuria between this study and other studies may be due to the geographical location, study participants recruited and the study setting either community based or hospital based study. The isolation rate of pathogens reduced significantly with increasing age (P=0.006) which could be associated with reduced sexual activity and less sexual partners (Kalowole *et al*., 2010).

Lower rates of bacterial pathogens were isolated in age group 38-48 years with (12.86%). These results could be associated with improved personal hygiene. Women in this age group are nearing menopause and majority might have reduced sexual activity hence low risk of exposure. Women in this age bracket are believed to rarely misuse antibiotics

which lead to increased vaginal immunity to infections (Cheesbrough, 2012). These factors probably contributed to the low occurrence rate of bacteriuria in increasing ages. The findings in this study compared well with similar findings by Mwaka *et al*., (2011); Okorondu *et al*., (2013); and Singh *et al*., (2016).

This study noted that age group 18-27 years isolated all the bacterial pathogens unlike other age groups. *Escherichia coli* was highly isolated in age group 18-27 years (18.65%) with relatively similar outcomes in age group 28-37 years (17.04%). *Staphylococcus saprophyticus* was highly isolated in age group 18-27 years (18.65%). In the study by Mwaka et al., (2011) reported *E. coli* and *S saprophyticus* in similar age group to be (16.1%) and (11.9%) this difference could be associated with study population, study settings and recruitment criteria used. *S. saprophyticus* is the second most causative agent of acute UTI in young women same finding was reported in the study by Erikisson *et al.,* (2013). *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Klebsiella aerogenes* were most frequently isolated in age group 18-27 years with 9.65%, 4.5% and 4.5% respectively as shown in Table 4.5.

This study noted that, the isolated bacterial pathogens high prevalence rates were isolated within the ages 18-37 years. This might have been attributed to women in these ages being highly sexually active, they could have been misusing antibiotics in bacteriuria treatment leading to reduction of vaginal normal flora. Low personal hygiene and majority probably might be having multiple sexual partners resulting to high exposure.

These factors might have contributed to high isolation rates of all pathogens in this age bracket. This finding compares well with other studies documented by Osungunna, and Adeyemi, (2016) and Mwaka *et al.,* (2011) who reported similar high occurrence of bacteriuria in this age bracket. Faisal *et al*., (2017) reported a prevalence of 62.5% for age group 18-32years. The reason for high occurrence of bacteriuria in this age bracket could be due to social factors such as: early age of marriage and sexual activity since this is the peak reproductive age hence high risk of exposure (Ronald, 2003).

5.1.4 Antibiotic sensitivity profile of the isolated bacterial pathogens

Ten antibiotics commonly prescribed as first line and second line antibiotics for treatment of urinary tract infection by the Ministry of Health were tested *in vitro* against all isolated bacterial pathogens to determine their susceptibility. The choice of antibiotics in any infection treatment must be individualized on the basis of the patient's allergy history, local practice methods, occurrence of antibiotic resistance, availability, cost of medicine, and patient compliance to treatment (Owen & Lautenbach, 2008). The Isolated bacterial pathogens were most sensitive to Cefuroxime with overall sensitivity rate of 93.2% (P=0.001). The sensitivity rates were 96.9%, 100%, 100% and 100% for *E. coli*, *S. saprophyticus*, *K. aerogenes* and *P. mirabilis* respectively. *Pseudomonus aeruginosa* was the only isolated bacterial pathogen resistant to Cefuroxime with resistance rate of 63.0% (Table 4.6). *Pseudomonus aeruginosa* is resistant to most commonly used antibiotics. Antibiotics that usually show activity against *P. aeruginosa* include Aminoglycosides, Polymyxin, some Penicillins and some Cephalosporins (Cheesbrough, 2012).

Pseudomonus aeruginosa evades antibiotic activities and cause diseases due to its ability to produce a destructive IgA protease antibody that neutralizes or destroys host defense mechanism activity (Cheesbrough, 2012). Cefuroxime is a broad spectrum antibiotic against a wide variety of bacteria. It is administered through intravenous route and no oral administration available. It is well absorbed and expensive. These factors reduce the chances of the antibiotic from losing its potency therefore the sensitivity of Cefuroxime in high against a wide variety of bacterial pathogen. This finding compared well with a study by Singh *et al.* (2016) from western region of Nepal reported sensitivity of (94%).

The second most effective drug was Amikacin with an overall sensitivity rate of (84.6%). The isolated bacterial pathogens which were sensitive to Amikacin includes; *E. coli* (88.4%), *S. saprophyticus* (97.9%), *K. aerogenes* (100%) and *P. mirabilis* (60.5%). The drug showed intrinsic resistance to *P. aeruginosa* with (51.9%). *Pseudomonas aeruginosa* isolates been resistant to Amikacin in this study was also reported in studies by (Mwaka *et al*., 2011; Dash *et al*., 2013; Singh *et al*., 2016; Faisal *et al*., 2017) who found P. aeruginosa resistant to be (59.7%), (52.3%), (54.1%) and (50.9%) respectively. Amikacin is administered parenteral intravenously in I.V fluids so oral administration forms are unavailable. This mode of administration in fact minimizes the chances of drug abuse because over the counter use is minimal and it's not suitable for outpatient treatment. This compares well with a study done by Rama *et al.,* (2014) in Bangladesh reported sensitivity of 86.4%. Gentamycin antibiotic was effective to the isolated bacterial pathogens with an overall sensitivity of (83%). The isolated pathogens

sensitivity rates to Gentamycin includes; *E. coli* (78.3%), *S. saprophyticus* (96.8%), *K. aerogenes* (52.9%), *P. aeruginosa* (63%) and *P. mirabilis* (90.7%). All isolated bacterial pathogens were sensitive to Gentamycin antibiotic (Table 4.7). Gentamycin antibiotic mainly requires parenteral route of administration and therefore, will not be suitable for treating out patients (Osungunna & Adeyemi, 2016).

Amikacin and Gentamycin belong to aminoglycosides group of antibiotics. Mostly aminogycosides are used as second line regimen for UTIs. The mode of administration of Aminogycosides antibiotics mainly is through parenteral route which minimizes their chances of antibiotic abuse therefore the drug potency is maintained. This compares well with other studies by Rama *et al., (*2014) and Singh *et al.,* (2016) who reported Gentamycin sensitivity rate of (78.5%) and (81.2%) respectively.

In this study, all isolated bacterial pathogens were sensitive to Cefalexin with an overall sensitivity of (78.1%). This study noted that all the isolated bacterial pathogens were sensitive to Cefalexin. Cefalexin is a broad spectrum antiobiotic that is active against a wide variety of bacteria. Cefalexin is first generation cephalosporins beta lactam antibiotic mainly administered though oral route. Cefalexin is well absorbed that provide adequate serum and tissue concentration levels with oral administration, cost effective, short duration of treatment and availability of the antibiotic in local markets. These factors could have attributed to the effectiveness of this antibiotic to the isolated bacterial

uropathogen. This study is in consistent with other studies elsewhere Rama *et al.,* (2014) and Mwaka *et al.,* (2011) reported sensitivity of (74.2%) and (80%) respectively.

Bacterial pathogens resistance to antibiotic varies over time and by patient population in different geographical locations (Bader *et al*., 2010). The isolated bacterial pathogens were resistant to Ceftazidime with an overall resistant rate of (89.7%). *Escherichia coli* was (79.8%), *S. saprophyticus* (100%), *K.* aerogenes (100%), *P. aeruginosa* (88.9%) and *P. mirabilis* (93%) resistant to Ceftazidime. Ceftazidime is a third generation Cephalosporins class of antibiobic and isolated bacterial pathogens resistance to it is of medical concern. In the study by (Owens & Lautenbach, 2008) reported resistance rate of (78.3%) to uropathogens. The high resistance of this antibiotic could be associated with repeated use of the antibiotic, prolonged exposure of uropathogens to the antibiotic and low cost making the drug subject to abuse. These factors could have attributed to the reduced antibiotic potency. In the report by (WHO, 2016) cited that uropathogens are developing resistance to many antibiotic used in UTI therapy. A similar trend was reported by Moges *et al.,* (2012) in Ethiopia.

The isolated pathogens in this study showed resistance to Amoxiclav with an overall resistant rate of (78.5%). *Escherichia coli* was (81.4%), *S. saprophyticus* (100%), *K. aerogenes* (58.8%) and *P.aeruginosa* was (74.1%) resistant to Amoxyclav antibiotic. Among the isolated bacterial pathogens *P. mirabilis was* the only pathogen sensitive to Amoxyclav with (67.4%). In most cases, Amoxyclav antibiotics have been used in

management of UTIs infection in pregnant women (Wamalwa *et al*., 2013). The uropathogens resistance to this antibiotic could be associated with the ability of the isolated pathogen to produce beta-lactamase enzyme which destroys the beta lactam ring of penicillin (Amoxiclav) which inactivates the antibiotic. A similar trend was reported by Rama *et al.,* (2014) which showed a resistance of (88.2%).

Bacterial isolates had a resistance rate of (66.6%), (86.5%), (76.2%) and (66.6%) to Nitrofurantoin, Nalidixic acid, Norfloxacin and Ofloxacin respectively. Among the isolated bacterial pathogens only one pathogen were sensitive to these antibiotics. *E. coli* was sensitive to Nitrofurantoin with (62.8%), the reliability and increased susceptibility levels of *E. coli* to Nitrofurantoin could have been attributed to Nitrofurantoin narrow spectrum activity, narrow tissue distribution and restricted contact with bacteria outside urinary tract. A similar result was found by Linhares *et al.,* (2010) for nitrofurantoin with the same susceptibility pattern for *E. coli* but low for non-*E. coli*, although they analyzed male and female patients. Nitrofurantoin is known to have no activity against *Proteus* spp. and *P. aeruginosa*.

This compares well with recent study in India which showed that Nitrofurantoin having the best susceptibility profile against *E. coli.* This finding is contradictory to other studies by (Singh *et al.,* 2016) who reported similar bacterial isolates as highly sensitive to Ofloxacin and Nitrofurantoin drug with sensitivity of (80.1%) and (70.4%) respectively. Mwaka *et al.,* (2011) reported uropathogens sensitivity to Nitrofurantoin to be (90%) in

Mulago Hospital, Uganda. The difference of uropathogens sensitivity rate to Ofloxacin and Nitrofurantoin in this study from (Singh *et al*., 2016) and (Mwaka *et al*., 2011) might be due to over the counter use of antibiotics leading to inappropriate antibiotic use, sub optimal dosages and incomplete dose course.

Pseundomonas aeruginosa was sensitive to Nalidixic acid and Ofloxacin with a sensitivity rate of (55.6%) and (70.4%) respectively. *P. mirabilis* was sensitive to Nofloxacin with (51.2%). The widely available and commonly prescribed antibiotics are Norfloxacin, Nalidixic acid and Ofloxacin which have confirmed a rather low overall *in vitro* sensitivity of 23.8%, 13.5% and 33.4% respectively for all isolated bacterial pathogens. In Africa, earlier study by Ouma *et al*., (2012) and Moges *et al*., (2012) reported very high sensitivity of uropathogens to Norfloxacin, Nalidixic acid and Ofloxacin with (90%) sensitivity rate. However, the isolated pathogens sensitivity to these drugs in this study are 33.4% (P=0.184), 23.8% (P=0.170) and 13.5% (P=0.185) for Ofloxacin, Norfloxacin and Nalidixic acid respectively as opposed to studies by (Ouma *et al*., 2012) and (Moges *et al*., 2012). Ofloxacin, Norfloxacin and Nalidixic acid belongs to quinolone class of antibiotics which have shown uropathogens to have increased antimicrobial resistance (Samia *et al*., 2014). Recently, over the counter use of these drugs most probably has contributed to such reduced level of sensitivity. Resistance of the isolates to some of the antibiotics is not only due to drug abuse, it could be also due to their vulnerable cell wall that is protected by an outer membrane that prevents permeation of the antibiotics (Okorundu *et al*., 2013).

In this study, isolated bacterial pathogens were resistant to antibiotic commonly prescribed in UTIs therapies which in earlier studies were effective against a variety of pathogen. Antibiotic resistance is a serious public health concern particularly in developing countries where there is high level of poverty, illiteracy and poor hygienic practices. High prevalence of fake and spurious antibiotics of questionable quality in circulation that are easily available in the community without prescriptions and low cost makes these drugs subject to abuse. These factors could have attributed to unnecessary use or misuse of these antibiotics contributing to decreased antibiotic effectiveness.

In a study by (Singh *et al*., 2016) reported isolated pathogens sensitivity to Norfloxacin and Ofloxacin to be (85%) and (91%) respectively and isolated pathogen resistant rate to Nalidixic acid was (87.2%). In study by (Rama *et al*., 2014) reported isolated bacterial pathogen sensitivity to Norfloxacin was (40%), Amoxyclav (11.8%), Nalidixic acid (34.6%) and ceftazidime (11.4%).

5.1.4.1 Antibiotics susceptibility profile pattern based on age groups

Ten antibiotics commonly used in treatment of urinary tract infections were tested for their isolated pathogens sensitivity based on the stratified age groups. The isolated bacterial pathogen were sensitive to cefuroxime, Amikacin and Cefalexin with age group 18-27 years constituted a higher proportions of pathogens sensitive to these antibiotics compared to other age groups (P=0.001). Age group 38-48 years had large proportion of pathogen resistant to tested antibiotics commonly prescribed for bacteriuria. This study

demonstrated that efficacy of most antibiotic tested decreased with increasing age while the resistance of isolated bacterial pathogen to tested antibiotics increased with increasing age. There is a statistically significant decline on sensitivity with age (P=0.001). This study showed that with increasing age, less proportion of bacterial isolates were sensitive to majority of antibiotics. This reason could be attributed by greater exposure to antibiotics during life, associated comorbidities and for more invasive procedures leading to increased torelance of pathogen to antibiotic.

This finding correlates well with a study by Singh *et al*., (2016) in western region of Nepal who reported (55.18%) of Pathogens sensitive to tested antibiotics in age group 18-32 years. Isolated bacterial pathogens were sensitive to some antibiotic like norfloxacin, ofloxacin and gentamycin sensitivity similar to all age groups. This study showed that only one isolated pathogen were sensitive to norfloxacin and ofloxacin antibiotic and all isolated bacterial pathogens were sensitive to gentamycin.

5.2 Conclusions

i. Bacteriuria was high (81%) in age group 18-27 years isolating the highest percentage followed by age group 28-37 years.
ii. All the bacterial pathogens were isolated in the age group 18-27 years.
iii. The most common bacterial pathogen responsible for bacteriuria was *Escherichia coli*.

iv. The isolated bacterial pathogens were sensitive to Cefuroxime, Amikacin, Gentamycin and Cefalexin with overall sensitivities of (93.2%), (84.6%), (83.0%) and (78.1%) respectively.

v. The isolated bacterial pathogens were resistant to Ceftazidime, Nalidixic acid, Amoxyclav, Norfloxacin, nitrofurantoin and Ofloxacin with overall resistance rates of (89.7%), (86.5%), (78.5%), (76.2%), (66.6%) and (66.6%) respectively.

5.3 Recommendations

i. Awareness and public training on hygiene is highly recommended in order to reduce bacteriuria occurrence.

ii. The effective antibiotics like cefuroxime, amikacin, gentamycin and cefalexin should be used as first line regimen for UTI treatment policy by the Ministry of Health.

iii. For bacteriuria treatment, culture and antibiotic susceptibility testing should be routinely done as one of the diagnostic techniques before initiating treatment.

5.4 Further studies

i. Surveillance on the antibiotics recommended by the ministry of health for UTI should be done.

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APPENDICES

Appendix I: Consent Form

Informed Consent

My name is Purity Musili, I am a Masters student from Kenyatta University. I have conducted are search study on *"*Occurrence of Bacteriuria and Antibiotic sensitivity profile among sexually active non-pregnant women attending Thika Level five Hospital, Kenya. The information will be used by the Ministry of health to improve access and quality for screening and management of bacteriuria in hospitals as well as in other regions in Kenya.

Procedures followed

Please remember the participation in this study was voluntarily. You could ask questions related to the study at any time.

Discomforts and Risks

There were no risks in this study

Benefits

Participants who tested positive for urinary tract infection were treated using the appropriate antibiotics.

Reward

There were no rewards to be provided after one agreed to participate in this study.

Confidentiality

The interviews and examinations were conducted in a private setting within the clinic. The names of the participants were written on the sample collected or anywhere on the records instead coding method were used. Everything was kept private.

Contact Information

If you have any questions you may contact me on 0717753797 or my supervisor Dr. Margaret Muturi on 0722758523 or Dr. Nelson Menza on 0725011570 or the Kenyatta University Ethical Review Committee Secretariat onchairman.kuerc@ku.ac.ke, secretary.kuerc@ku.ac.ke, secretariat.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 the information I will give will be handled in confidence.

Name of participant

_____ _____

Signature or Thumbprint Date

Investigators statement

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

Name of interviewer signature or thumb print

_____ _____

Appendix II: Ethical Approval

KENYATTA UNIVERSITY
ETHICS REVIEW COMMITTEE

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

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

Our Ref: **KU/ERC/APPROVAL/VOL.1** (37) Date: 13th April 2017

Purity Musili
Kenyatta University,
P.O Box 43844,
Nairobi

Dear Purity,

APPLICATION NUMBER, PKU/626/I710: TITLE "DETERMINATION OF BACTERIURIA AND ANTIBIOTIC SENSITIVITY PROFILE AMONG SEXUALLY ACTIVE NON-PREGNANT WOMEN ATTENDING THIKA LEVEL FIVE HOSPITAL, KENYA

1. **IDENTIFICATION OF PROTOCOL**

The application before the committee is with a research topic application Number, **PKU/626/I710: TITLE** "Determination of Bacteriuria and Antibiotic Sensitivity profile among sexually active non-pregnant women attending Thika Level Five Hospital, Kenya," Received on 8th March 2017 and Approved on11th April 2017

2. **APPLICANT**

Purity Musili

3. **SITE**

Thika Level Five Hospital, Kenya

4. **DECISION**

The committee has considered the research protocol in accordance with the Kenyatta University Research Policy (Section 7.2.1.3) and the Kenyatta University Review Committee Guidelines **AND APPROVED that the research may proceed for a period of ONE year from 13th April, 2017.**

ADVICE/CONDITIONS

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

When replying, kindly quote the application number above.
If you accept the decision reached and advice and conditions given please sign in the space Provided below and return to KU-ERC a copy of the letter.

18 APR 2017

DR. TITUS KAHIGA
CHAIRMAN ETHICS REVIEW COMMITTEE

I ..... accept the advice given and will fulfill the conditions therein.

Signature..... Dated this day of... 18/04 .....2017.

cc.
DVC: Research Innovation and Outreach

Appendix III: Research Permit

THIS IS TO CERTIFY THAT:
MS. PURITY ELIZA MUSILI
of KENYATTA UNIVERSITY, 1219-90200
kitui,has been permitted to conduct
research in *Kiambu County*

on the topic: *DETERMINATION OF BACTERIURIA AND ANTIBIOTIC SENSITIVITY PROFILE AMONG SEXUALLY ACTIVE NON PREGNANT WOMEN ATTENDING THIKA LEVEL FIVE HOSPITAL*

for the period ending:
5th May,2018

Permit No : NACOSTI/P/17/53537/16930
Date Of Issue : 8th May,2017
Fee Recieved :Ksh 1000

Applicant's Signature

Director General
National Commission for Scienc
Technology & Innovation

CONDITIONS

1. You must report to the County Commissioner and the County Education Officer of the area before embarking on your research. Failure to do that may lead to the cancellation of your permit.
2. Government Officer will not be interviewed without prior appointment.
3. No questionnaire will be used unless it has been approved.
4. Excavation, filming and collection of biological specimens are subject to further permission from the relevant Government Ministries.
5. You are required to submit at least two(2) hard copies and one (1) soft copy of your final report.
6. The Government of Kenya reserves the right to modify the conditions of this permit including its cancellation without notice

REPUBLIC OF KENYA

National Commission for Science, Technology and Innovation

RESEACH CLEARANCE PERMIT

Serial No.A 4022

CONDITIONS: see back page

Appendix IV: Research Authorization

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 8710901 Ext. 57530

Our Ref: P150/31030/15

DATE: 10th November 2016

Director General,
National Commission for Science, Technology
& Innovation
P.O Box 36023-00100
NAIROBI

Dear Sir/Madam,

RE: RESEARCH AUTHORIZATION FOR PURITY MUSILI– REG. NO. P150/31030/15.

I write to introduce Ms. Purity Musili who is a Postgraduate Student of this University. She is registered for M.Sc degree programme in the Department of Medical Laboratory Science.

Ms. Musili intends to conduct research for a M.Sc. Proposal entitled, **"Determination of Bacteriuria and Antibiotic Sensitivity Profile among Sexually Active Non Pregnant Women Attending Thika Level 5 Hospital, Kenya".**

Any assistance given will be highly appreciated.

Yours faithfully,

MRS. LUCY N. MBAABU
FOR: DEAN, GRADUATE SCHOOL

Appendix V: Thika Level 5 Hospital Map