ISOLATION OF UROPATHOGENIC BACTERIA AND THEIR ANTIMICROBIAL
SUSCEPTIBILITY PATTERN IN URINE SAMPLES OF PATIENTS WITH
SUSPECTED URINARY TRACT INFECTION IN EASTERN REGIONAL
HOSPITAL, KOFORIDUA

BY

BABA KUNTA FOFANA

ID: 1050489

A THESIS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL
FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF
MASTER OF PHILOSOPHY DEGREE IN APPLIED EPIDEMIOLOGY AND
DISEASE CONTROL

JULY, 2016
DECLARATION

I hereby declare that the work presented in this thesis is my own original research undertaken in the department of Epidemiology and Disease Control, School of Public Health, University of Ghana, Legon, under the supervision of Dr. Bismark Y. Sarfo, and that no part of this work has been presented for another degree in this University or elsewhere. All references have been duly acknowledged.

DATE-------------------------------------------
Baba Kunta Fofana
(CANDIDATE)

DATE-------------------------------------------
Dr. Bismark Y. Sarfo
(ACADEMIC SUPERVISOR)
DEDICATION

This work is gratefully and affectionately dedicated to my late father Karamo Fofana, my mother Nyima Manneh, my wife Mariama Balajo and my children.
ACKNOWLEDGEMENT

I gratefully acknowledged Dr. Bismark Y. Sarfo of department of Epidemiology, School of Public Health, University of Ghana for his invaluable contributions of support and supervision. My appreciations also go to Medical Director, and Head of Laboratory department, Eastern Regional hospital, Koforidua, for their approval for this research work to be carried out at this health facility. To all the biomedical scientists at the microbiology department who in many ways supported me throughout this research, debts of gratitude I owe.

My deepest appreciations also go to West Africa Health Organization (WAHO) for fully sponsoring my two years MPhil in Applied Epidemiology and Disease Control, and to the director, coordinator and the entire staff of Ghana Field Epidemiology and Laboratory Training Programme for their support and guidance throughout the course.

Finally, am very much grateful to the Almighty Allah for the strengths He gave me throughout this two years.
ABSTRACT

Background: Urinary tract infection (UTI) is a common bacterial infection known to affect the different parts of the urinary tract and the occurrence is found in both females and males. Distribution and susceptibility of UTI-aetiologic agents change according to time and place. To ensure appropriate treatment, knowledge of these aetiologic agents and their antibiotic susceptibility pattern is mandatory. However, the extensive use of antimicrobial agents have invariably resulted in the development of antibiotic resistance, which, in recent years, has become a major problem worldwide.

Objective: The main objective of this study was to determine the prevalence and antibiotic susceptibility pattern of Uropathogenic bacteria in urine samples of patients with suspected UTI at the Eastern Regional Hospital, Koforidua - Ghana.

Study design: A cross sectional study was conducted among patients who attend Eastern Regional Hospital, Koforidua from December 2015 to March 2016.

Methods: Four hundred and twenty-two study participants with suspected UTI and those admitted in the wards with signs and symptoms of UTI were enrolled. Anyone aged one (1) day and above without being on antibiotic therapy, in labour, or delivered within 48 hours, and not from outside Eastern Region as pointed out by the attending clinician, was conveniently enrolled. Socio-demographic data were extracted from patients’ cards and/or from the Health Administrative Management System (HAMS). Organisms were identified and antibiotic susceptibility was performed on all the bacterial isolates using Kirby-Bauer’s disc diffusion method. Zones of inhibition were measured with caliper.
after 18 to 24 hours of incubation. Measured zone inhibitions were compared with zone
diameter interpretative chart.

Results: A total of 22.5% (95/422) uropathogens were isolated from the study subjects
attending the Eastern Regional Hospital, Koforidua. The bacterial isolates included E. coli 37.89%, Klebseilla spp., 34.74%, Pseudomonas aeruginosa 2.11%, Staphylococcus aureus 2.11% and Citrobacter spp., 2.11%. The majority of isolates, 78.95% (75/95),
were from females in whom the highest percentage of isolates (100%) was seen in the
age group 31 to 40 years whiles for males it was seen in age group 0 to 10 years
(44.44%). All the bacteria showed high sensitivity to Amikacin and Nitrofurantoin. They
showed resistance to Augmentin, Ampicillin, Co-trimoxazole and Gentamycin.

Conclusion: This study shows that Escherichia coli is the main organism causing UTI in
this catchment area. In addition, the study illustrates that females are more susceptible to
UTI than males. Furthermore, the study demonstrated resistance of the isolates to
Augmentin, Ampicillin, Tetracycline and Co-trimoxazole, and sensitive to Amikacin and
Nitrofurantoin. This demonstrates that Amikacin and Nitrofurantoin are clear alternative
first line drugs against UTI causing organisms as clinicians await culture and sensitivity
results from the laboratory.
TABLE OF CONTENT

DECLARATION ............................................................................................................................. ii
DEDICATION .............................................................................................................................. iii
ACKNOWLEDGEMENT .............................................................................................................. iv
ABSTRACT ..................................................................................................................................... v
TABLE OF CONTENT ................................................................................................................. vii
LIST OF TABLES ........................................................................................................................ x
LIST OF FIGURES ....................................................................................................................... xi
LIST OF ABBREVIATIONS ......................................................................................................... xii
CHAPTER ONE ............................................................................................................................ 1
INTRODUCTION .......................................................................................................................... 1
  1.1 Background. .......................................................................................................................... 1
  1.2 Problem Statement. .............................................................................................................. 3
  1.3 Justification. ........................................................................................................................ 4
  1.4 Objectives. .......................................................................................................................... 5
  1.5 Specific objectives. .............................................................................................................. 5

Conceptual framework ................................................................................................................. 6
  Narrative of the Conceptual framework. .................................................................................. 7

CHAPTER TWO .......................................................................................................................... 10

Literature Review ........................................................................................................................ 10
  2.1 Epidemiology of UTI. ......................................................................................................... 10
    2.1.1 Emergence of antimicrobial resistance and their impact on healthcare delivery......... 11
    2.1.2 Prevalence of Urinary Tract Infection in West Africa and Ghana. ......................... 12
  2.1.2 Aetiology ......................................................................................................................... 13
    2.1.2.1 Bacterial UTI. ........................................................................................................... 13
    2.1.2.2 Fungal & Viral UTI. ................................................................................................ 15
    2.2 Modes of bacterial entry. .................................................................................................. 16
      2.2.1 The ascending route. ................................................................................................ 16
      2.2.2 Haematogenous route ................................................................................................. 16
    2.3 Pathogenesis of UTI ........................................................................................................ 16
      2.3.1 Bladder emptying ..................................................................................................... 17
2.3 Clinical Manifestations of Urinary tract infections ........................................ 18
  2.3.1 Asymptomatic bacteriuria ........................................................................ 18
  2.3.2 Cystitis ...................................................................................................... 19
  2.3.3 Acute pyelonephritis ................................................................................. 20
  2.3 Diagnosis of Urinary Tract Infection .......................................................... 20
  2.3.1 Laboratory detection of Urinary Tract Infection ........................................ 21
  2.3.2 Dipstick Urinalysis .................................................................................. 21
    2.3.2.1 Parameters determined by Dipstick ......................................................... 21
    2.3.2.2 Haematuria .......................................................................................... 22
    2.3.2.3 Proteinuria ............................................................................................ 22
    2.3.2.4 Nitrite .................................................................................................... 23
    2.3.2.5 Leukocyte Esterase .............................................................................. 23
  2.3.3 Treatment .................................................................................................. 26
  3.3.4 Prevention of UTI ................................................................................... 27

CHAPTER THREE ........................................................................................................ 28
Materials and methods ......................................................................................... 28
  3.1 Study area ..................................................................................................... 28
  3.2 Study design .................................................................................................. 30
  3.4 Sample size calculation ............................................................................... 30
  3.6 Sample collection .......................................................................................... 31
  3.7 Criteria ........................................................................................................... 32
    3.7.1 Inclusion criteria ..................................................................................... 32
    3.7.2 Exclusion criteria .................................................................................... 33
  3.8 Sample processing ......................................................................................... 33
    3.8.1 Urine dipstick .......................................................................................... 33
    3.8.2 Microbiological culture .......................................................................... 33
    3.8.3 Identification of pure bacterial isolates .................................................... 34
    3.8.4 Antimicrobial Susceptibility Testing ........................................................ 34
    3.8.5 Statistical analysis .................................................................................. 35
    3.8.6 Ethical clearance ..................................................................................... 36

CHAPTER 4 ................................................................................................................ 37
RESULTS ..................................................................................................................... 37
  4.0 Socio-demographic characteristics of study participants ......................... 37
LIST OF TABLES

Table 1 Causes of false positive and false negative results of urine dipstick test .................. 24
Table 2 Socio-demographic characteristics of study participants ........................................... 39
Table 3. Factors associated with bacterial culture positive ..................................................... 42
Table 4. Frequency of microbial isolates among sexes and isolates per total obtained (n=95) ................................................................................................................................. 49
Table 5. Overall sensitivity and resistant patterns of bacterial isolates ................................. 52
LIST OF FIGURES

Figure 1 CONCEPTUAL FRAME WORK ........................................................... 6
Figure 2 Distribution of health facilities ....................................................... 29
Figure 3 Percentage distribution of isolates by age group ............................... 45
Figure 4 Percentage distribution of isolates by Age group and Sex .................... 47
Figure 5 Overall Percentage Resistance of Bacteria isolates to Antibiotics .............. 53
Figure 6 Percentage Resistance of Gram negative bacteria to commonly used antibiotics .... 54
LIST OF ABBREVIATIONS

ATCC    American Type Culture Collections
CFU     Colony Forming Unit
CLED    Cysteine Lactose Electrolyte-Deficient
GHS-ERC Ghana Health Service - Ethical Review Committee
LAP     Lower Abdominal Pain
M.PHIL  Master of Philosophy
OPD     Out Patient Department
RHK     Regional Hospital, Koforidua
μg      Microgram
UTI     Urinary Tract Infection
W.H.O   World Health Organization
CHAPTER ONE

INTRODUCTION

1.1 Background.

Urinary tract infection (UTI) is defined as the bacterial invasion of the urinary tract. It is primarily caused by *Escherichia coli* (*E.coli*), accounting for 75% of bacterial UTI cases. Other bacteria such as *Klebsiella pneumoniae*, *Proteus mirabilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Enterococcus faecalis* have been reported as causative agents by studies in Ghana, Nigeria and other parts of the world (Antwi *et al.*, 2008; Boye *et al.*, 2012; Gyansa-Lutterodt *et al.*, 2014). UTI occurs as a result of significant microbial growth in the urinary tract, especially between the urethra and the kidneys. Thus, the classification of UTI is based on the site of infection. Infection of the bladder is known as cystitis, and infection of the kidneys is called pyelonephritis. It can also be classified clinically as either complicated or uncomplicated depending on the extent of infection (Bennett *et al.*, 2014). Complicated urinary tract infection is known to occur in men and women at any point of their lives and usually has the tendency to produce severe outcomes resulting in death in serious situations. They are highly intricate and are difficult to treat. Complicated urinary tract infections can lead to structural abnormalities that blight the capability of the urinary tract to flush out the urine hence bacteria are provided with better scope for growth (Fihn, 2003). On the contrary, uncomplicated UTI is a consequence of bacterial infection and the prevalence is higher in female than in male. It is not as fatal as complicated UTI (Fihn, 2003).

Globally, UTI is a major public health burden leading to increased morbidity and the associated high healthcare cost (T.M. Hooton *et al.*, 1995; Orenstein & Wong, 1999). It is
the second most common infection among patients visiting primary healthcare facilities. In United States, where comprehensive data is available, it is estimated that UTI accounts for seven million hospital visits per year, with millions of those infections leading to visits at the emergency department (Foxman, 2002). The associated cost of health care is enormous accounting for $659 million in direct cost for treating and $936 million in indirect cost, totaling to $1.6 billion annually (Foxman, 2002). Worldwide, it is evident that UTI is more common among young girls, except in neonatal period where boys predominate (Foxman, 2002). Furthermore, it is estimated that about 20% of all women develop UTI during their lifetime (Fihn, 2003).

A number of studies have been conducted in the sub-region that suggested a range of prevalence of UTIs in some selected populations. Reports have indicated that most of these studies were done on febrile and malnourished infants and children below age 12 years, with study sites being primarily hospitals and other health facilities (Wolff & Maclennan). In addition, a few studies were done on male and females adults, including pregnant women. In Kenyatta National Hospital, a cross sectional study on antenatal women presenting with lower abdominal pains showed a prevalence of 26.7% bacterial urinary tract infection (Nabbugodi W. F., 2009).

A demonstrative study conducted at a large Ghanaian hospital in Accra showed that 93% of isolates from urine samples were bacteria species and 7% were yeast (Odonko ST et al., 2011). A retrospective review of urine results of antenatal attendants with asymptomatic UTI at a private hospital in Kumasi indicated a prevalence of 9.9% significant bacterial growth (Ado VN, 2012). A secondary data analysis of urine culture and sensitivity results for 2005 to 2008 at Eastern Regional Hospital, Koforidua, showed
UTI prevalence of 29% with *E. coli* as the most common uropathogens followed by *Klebseilla spp*.

Antibiotics play an important role in minimizing morbidity and mortality associated with infectious diseases. The most common antibiotics used for the treatment of UTI are cefuroxime, amoxicillin/clavulanic acid, trimethoprim/sulpmethoxazole and fluoroquinolones. However, the efficacy of these treatment is affect by bacterial resistant to many of the antibiotics leading to treatment failure, spread of resistant bacterial strains, increased morbidity and ultimately high cost of treatment. The extensive and inappropriate use of antimicrobial agents has invariably resulted in the development of antibiotic resistance which, in recent years, has become a major public health problem worldwide (Gupta et al., 2001; Mordi & Erah, 2006). Furthermore, antibiotic resistance is compounded by the unorthodox treatment of patients with suspected UTI with antibiotics without a valid microbial culture and sensitivity laboratory results. One way to avert this problem is detailed knowledge on the prevalence of bacteria causing UTI and their antibiotic susceptibility pattern. Thus, this study investigated the prevalence and antibiotic susceptibility pattern of Uropathogenic bacteria isolated from urine samples of patients with suspected UTI, in Eastern Regional Hospital, Koforidua.

1.2 Problem Statement.

Bacterial resistant to most antibiotic is a global public health concern leading to treatment failure, high cost of treatment, increased morbidity and mortality. However, this is more of a problem in sub-Saharan Africa, for example Ghana, where bacterial infection is very common, and knowledge on antibiotic resistance pattern is very limited. Most patients with suspected UTI, out of frustration, visit local chemical stores often run by untrained
personnel to buy antibiotics without any prescriptions, leading to antibiotic resistance and treatment failure. Also due to the complexity and time it takes to undertake a bacterial culture and susceptibility laboratory test, doctors mostly start patients with a broad spectrum antibiotics before the lab results are out. Although this is aim at early treatment to alleviate the pain and anguish associated with UTI, it is also a primary contributor to the development of antibiotic resistance. In view of the above limitations, this study envisaged to add to the knowledge base on the bacteria causing UTI and their antibiotic susceptibility pattern, and to assess, if there is any, association between the isolates and socio-demographic characteristics of study participants such as age, sex, marital status and educational background in this catchment area, Eastern Region.

1.3 Justification.

Ghana, as some other Sub-Saharan African countries, has a very high prevalence of bacterial infections, and treatment of these infections is generally very poor due to poor health care delivery facilities and minimal training on proper management of infectious diseases. Most cases of uncomplicated UTIs can be mild and transient, however, sequelae of untreated UTIs can include renal scarring, hypertension, and eventually end-stage renal disease, hence correct and timely diagnosis of UTI is imperative. Antibiotic resistance is a global challenge as it is on increase from one geographical area to another. For instance, the secondary data analyses done on urine culture and sensitivity results at Regional Hospital laboratories in Ghana including Eastern Regional Hospital, Koforidua, show high resistance in commonly used antibiotics against urinary tract infections. Therefore, for effective treatment, clinicians need some baseline information such as the
most prevalent bacteria-causing UTI and their antibiotic susceptibility to guide in the
administration of treatment. This study envisaged to provide that critical information by
investigating the prevalence of local UTI-aetiologic agents in urine samples and their
antibiotic resistant pattern in patients with suspected UTI.

1.4 Objectives.
The overall objective of this study was to determine the prevalence and antibiotic
susceptibility pattern of Uropathogenic bacteria in urine samples of patients with
suspected UTI in Eastern Regional hospital (Koforidua).

1.5 Specific objectives.
- To determine the prevalence of bacteria causing UTI in urine samples of patients
  with suspected UTI in Eastern Regional Hospital, Koforidua.
- To determine the antibiotic susceptibility of bacteria isolated from urine samples
  of patients with suspected UTI.
- To determine the association between the isolated bacteria species and
demographic characteristics of study participants.
- To assess an association between the isolated bacteria species from patients and
  the different hospital units within the Regional Hospital, Koforidua.
Conceptual framework

Diagrammatic

UTI causes:
- LAP
- Dysuria
- Frequency
- Urgency

Risk factors:
- Pregnancy
- Age
- Sexual behavior
- Personal hygiene
- History of UTI

UTI:
- Cystitis
- Pyelonephritis
- Urethritis

Antibiotic susceptibility

Common commensals
- E. coli
- Proteus
- Klebseilla
- Staphylococcus

Antibiotics:
- Ceftriaxone
- Cefuroxime
- Amikacin
- Nitrofurantoin
- Augmentin

*Figure 1 CONCEPTUAL FRAME WORK*
Narrative of the Conceptual framework.

Urinary tract infection is known to be one of the most common infectious disease in both adults and children. In children, they are a common cause of febrile illnesses in their first two years of life, and the majority of urinary tract infections may remain undiagnosed if specific tests like urine culture are not performed. In adults, the infection is common in females than in males.

Urinary tract infections are very serious infections and if untreated, are associated with adverse outcomes to the patient. A number of risk factors are associated with development of UTI. These risk factors may be either modifiable or inherent and are both demographic and obstetric, including, pregnancy, age, sex, sexual behavior, socio-economic status, personal hygiene, medical illnesses and previous history of the infection.

Though prevalence of bacteriuria during pregnancy is similar to that in non-pregnancy, pregnancy enhances the possibility of the infection. UTI occurrence is at its peak during pregnancy which in turn relies on several other factors. In addition to pregnancy, sexual intercourse and family history also play a pivotal role in conferring the infection. During sexual intercourse, bacteria present in the vagina are more easily transferred to the urethra and the urinary tract. This increases the risk of UTI in females.

Age is a known predisposing factor to UTI. For instance, about 3% of prepubertal girls and 1% of prepubertal boys are diagnosed with UTI. In elderly persons, there are many factors that predispose them to UTI including chronic diseases, functional abnormalities, and specific medications.
Though it is uncommon in young men compare to women, UTI becomes progressively more frequent after middle age, and may eventually affect as many as a third of all men. A report by Nicolle, L. E. et al., (1983) stated that after age 70, in men the prevalence of asymptomatic bacteriuria and symptomatic infections in men approach or even excess that in women.

Among this group, asymptomatic bacteriuria is prevalent, though it frequently resolves without treatment and has no long-term sequelae. However, symptomatic UTI among them requires antimicrobial treatment.

Urinary Tract Infection can involve the lower or the upper parts of the urinary tract, each giving different symptoms. Generally, lower urinary tract infections cause symptomatic voiding without constitutional symptoms. Symptoms indicative of upper tract infection or pyelonephritis include fever, chills and flank pains. However, abdominal pain is common to both of them. Despite the high morbidity and mortality associated with untreated UTI, the process of diagnosis and treatment is not standardized. Often patients who present with lower abdominal pains (LAPs) are empirically treated for UTI as presumptive diagnosis.

The causative agents of UTI are known but their relative prevalence as well as their antibiotic susceptibility patterns may vary from location to location and time to time, and with the emergence of HIV, the epidemiology of most other diseases have been significantly altered.

The most commonly used antibiotics that have been highly effective are becoming resistant as microbes develop resistance against them. This usually is as a result of their
over use and abuse. It is therefore important to research and keep abreast with the rapidly changing aetiologic agents and antibiotic susceptibility patterns. The high prevalence and the diagnostic dilemma of UTI lead to overuse of antibiotics and development of resistant microbial species.

It is thus, of paramount importance to determine the microbial profiles and their antibiotic sensitivity patterns in cases of empirical treatment.
CHAPTER TWO

Literature Review

2.1 Epidemiology of UTI.

Urinary Tract Infection is caused by bacterial infection mostly due to poor personal hygiene, unprotected sexual intercourse, urinary tract obstruction, long term usage of catheter, urethral reflex, spermicidal contraception, and pregnancy (Antwi et al., 2008). Although everyone is prone to UTI, it is age and sex specific. There are specific subgroups that are at greater risk of contracting UTI. These subgroups include infants, pregnant women, the elderly, and patients with spinal cord injuries and/or catheters (Biering-Sørensen et al., 2001; Ruben et al., 1995; Winberg et al., 1974). Globally, reports have indicated that in 2007, 8.6 million physician visits for UTIs were reported. Of this figure, 84% were by women (T.M. Hooton, 2012). Women are generally more susceptible to UTI than men, mainly attributed to the difference in anatomy of female and male urinary tract (James R Johnson et al., 1995; Uehling et al., 1997). Studies have shown that 25% of women diagnosed with UTI would have a reoccurrence of it within six months after the first infection (Ebie et al., 2001; Kolawole et al., 2010). Also physiological changes, such as hormonal changes during pregnancy, increase the frequency of reoccurrence (Bennett et al., 2014). Furthermore, poor personal hygiene and some cultural practices among women have been cited as major predisposing factors (J.R. Johnson & Stamm, 1989). Cystitis recurs in 25% of healthy women within six months of the first infection, and in 20% of women within 1 year (Fihn, 2003).
Studies have stated that risk factors for UTI may be modifiable or inherent, and differ according to the age of the patient. In the young premenopausal women, UTI is usually related to sexual activity. In the postmenopausal women, however, different at-risk comorbidities are implicated, including vulvar estrogen deficiency and elevated bladder urine stasis (Franco, 2005). Postmenopausal women are likely to have additional predisposing factors such as history of genitourinary surgery, incontinence, presence of a cystocele, and a high post-void residual urine volume (Franco, 2005). Another risk factor such as diabetes mellitus is associated with many unusual uropathogens, and an increased chance of complications such as emphysematous pyelonephritis or cystitis (Foxman, 2002).

Approximately, 1% of children, many of whom demonstrate functional or anatomic abnormalities of the urinary tract, develop infection during the neonatal period (Mårild & Jodal, 1998).

A retrospective population-based study looking at the incidence rate of first-time symptomatic UTI in children less than 6 years, shows that cumulatively, incidence rate was 3 times greater in girls (6.6%) than boys (1.8%) (Mårild & Jodal, 1998).

2.1.1 Emergence of antimicrobial resistance and their impact on healthcare delivery.

One of the greatest achievements in the healthcare delivery of the 20th century was the discovery of antimicrobial agents used to treat human infections. These agents saved many lives from serious microbial infections (Levy & Marshall, 2004). However, a few years after the discovery of antimicrobials, groups of antimicrobial resistant pathogenic bacteria evolved, mainly due to use, misuse, and their abuse (Wilke, 2010, Korczak &
Schoffmann, 2010). The threat of antimicrobial resistance is on increase, more particularly in developing countries (Amabile-Cuevas, 2003).

In Ghana, antimicrobial drugs may be sold with or without prescription by healthcare personnel, thereby resulting in the emergence of antimicrobial resistant bacterial strains (Wolf-Gould et al., 1991). In addition, some patients fail to complete the dose once they feel better. This, in turn, results in selection pressure leading to antimicrobial resistance (Roberts et al., 2008).

2.1.2 Prevalence of Urinary Tract Infection in West Africa and Ghana.

Although, a comprehensive data on UTI prevalence on general population is lacking, there have been a number of studies conducted in the West African Sub-Region that suggest a wide range of prevalence of UTI in some selected populations. According to a report by Mussa-Aisien, 2003, most of these studies were done in febrile and malnourished infants and children below the age of 12 years, with the study sites being primarily hospitals and other health facilities (Wolff & Maclello). Some studies have also been done on male and female adults, including pregnant women. Another report by a study done in Nigeria on febrile infants of 1 to 60 months, reported a UTI prevalence of 9%, with females having a significantly higher prevalence, compared to males (Wolff & Maclello). A study conducted by Babaoye et al., reported a prevalence rate of 43% in children below the age of 10 years (Babaoye et al., 1991).

More still, in Nigeria, an adolescent population study on UTI, gave a 28% incidence with *E. coli* as the causative agent of 52.77% (Aiyegoro et al., 2007).

A 7.3% UTI prevalence rate was reported in a study conducted in Kumasi, Ghana, on pregnant women attending antenatal clinic (Boye et al., 2012). A demonstrative study
conducted at a large Ghanaian hospital in Accra showed that 93% of isolates from urine samples were bacteria species and 7% were yeast (Odonko ST et al., 2011). More still, a retrospective review of urine results of antenatal attendants with asymptomatic UTI at a private hospital in Kumasi, indicated a prevalence of 9.9% significant bacterial growth (Ado VN, 2012). A secondary data analysis of urine culture and sensitivity results from 2005 to 2008 at Eastern Regional Hospital, Koforidua, showed UTI prevalence of 29%, with *E. coli* as the most common uropathogens followed by *Klebseilla spp*.

### 2.1.2 Aetiology

Traditionally, pathogens associated with urinary tract infections are changing many of their features, particularly because of antimicrobial resistance. The etiology of urinary tract infection is also affected by some underlying host factors that complicate it, such as age, personal hygiene, pregnancy, diabetes, spinal cord injury, or catheterization (Antwi *et al.*, 2008). Consequently, complicated urinary tract infection has a more diverse etiology than uncomplicated UTI. Furthermore, it is reported that UTI causing bacteria are more likely to infect individuals with underlying health complications that affect the optimal anatomic, metabolic or immunologic functions than in healthy individuals (Bennett *et al.*, 2014).

#### 2.1.2.1 Bacterial UTI.

The main perpetrators of urinary tract infections are bacteria, most particularly the Gram negative bacteria such as *E. coli, Klebseilla spp.*, *Proteus spp.*, *Pseudomonas, Acinotobacter* and *Serratia* (Shaikh *et al.*, 2008). A few Gram positive bacteria such as *Staphylococci, Enterococci* and *Streptococcus agalactiae* are reported in some cases of
urinary tract infections (Foxman, 2003). Gram-positive organisms are common in the elderly, and polymicrobial infections account for up to 1 in 3 infections in them. In comparison, the most common organisms isolated in children with uncomplicated UTI are Enterobacteriaceae. Etiologic pathogens associated with UTI among patients with diabetes include *Klebsiella spp.*, group *B streptococci*, and *Enterococcus spp.*, as well as *E. coli* (Foxman, 2002). Patients with spinal cord injuries commonly have *E. coli* infections. Other common uropathogens include *Pseudomonas* and *Proteus mirabilis* (Biering-Sørensen et al., 2001). The majority of community-acquired symptomatic UTIs in elderly women are caused by *E. coli* (Nys et al., 2006).

In Karachi - Pakistan, the prevalence of asymptomatic bacteriuria was seen to be 6.2%. *E. coli* and 66.67% *Staphylococcus saprophyticus* resistance to ampicillins, and Sulfonamides, while Enterobacters showed 100% resistance to ampicillins, cephalosporin and nitrofurantoin (Bauer et al., 1966). A hospital based prospective study conducted at the Felege Hiwot Referral Hospital – Ethiopia, showed that the most common isolate was *E. coli* (45.7%) followed by coagulase negative *Staphylococcus* (17.1%) (Wondewesen Tsegaye et al., 2012).

A reported from Imo State University Teaching Hospital, Orlu, Nigeria, stated that *E. coli* accounted for 52.5%, *S. aureus* 33.9%, *P. mirabilis* 8.8%, *Enterococcus spp.* 5.0% and *N. gonorrhea* 1.7%, of UTI cases (Kenechukwu Meszue, et al., 2009).

In Kano - Nigeria, it was reported that UTI was predominantly caused by *Staphylococcus aureus* (67.9%), *Klebsiella species* (7.9 %), and *Pseudomonas* (14.2%) (Adeleke & Asani, 2009).
A study done in Maiduguri – Nigeria, reported a high incidence of UTI in both non-pregnant and pregnant women. The predominant organism in this report was *E.coli* and was found to be highly sensitive to quinolones, while resistant to Co-amoxicillin, Cotrimoxazole and Nalidixic acid (Initiative & W.H.O., 2003). In Kaduna, Nigeria, similar results were observed (Emmerson *et al.*, 1996).

Several studies done in regional hospitals in Ghana: Koforidua, Kumasi and Cape Coast, revealed that *E. coli* and *Klebsiella spp.* are the most dominant bacterial species responsible for UTI (Bosu, W. K. & S. Acquah, 1996; Gyasi-Sarpong *et al.*, 2012).

### 2.1.2.2 Fungal & Viral UTI.

Urinary tract infections due to fungal and viral agents are rare phenomena. However, fungal infections are seen in infants and children who are on long term antibiotics, patients who are immunocompromised, or patients using invasive devices like Intra Venous (IVs) and catheter (Watson, 2004). Fungal infections are predominant in patients with urinary tract anomalies, and is associated with infections after instrumentation such as catherization of the urinary tract (Yildiz *et al.*, 2007). Adenoviruses types 11 and 21, polyomavirus BK, and Herpes simplex viruses are reported to cause viral UTI (Watson, 2004).
2.2 Modes of bacterial entry.

Bacteria gain access through the genitourinary tract in two main ways: ascending and haematogenous spread.

2.2.1 The ascending route.

Most cases of pyelonephritis are due to the bacteria ascending from the bladder through ureter and into the renal parenchyma (Tonagho and Mcaninch, 2004). A report by Foxman, showed that most urinary tract infection cases are caused by bacteria ascending from the perineum (Foxman, 2003).

2.2.2 Haematogenous route.

This form of bacteria entering the genitourinary tract occurs in neonates and immunocompromised patients (Dulczac and Kirk, 2005). Because in the first 8 to 12 weeks of life, urinary tract infection may be secondary to haematogenous source, the diagnosis of UTI in young children is very important as it is considered a marker for urinary tract abnormalities in the new born (Schlager, 2001). *Candida species*, *Mycobacterium tuberculosis* and *Staphylococcus aureus* are common pathogens that travel through the blood to infect the urinary tract (Tonagho and Mcaninch, 2004).

2.3. Pathogenesis of UTI.

Most of the urinary tract infections are ascending in origin and are due to bacteria in the gastrointestinal tract that have colonized the periurethrum area (Handley et al., 2002). Just after birth, the periurethrum, that includes the distal urethra, becomes colonized with
both aerobic and anaerobic bacteria. These organisms act as defense barriers against colonization by potential pathogens. Disturbance of the normal periurethral flora, such as it may occur due to use of some birth control agents, or treatment of upper respiratory tract infections with broad spectrum antibiotics, may predispose colonization of the periurethral area with potential uropathogens (Lidfelt et al., 2001). Colonization of periurethral area with uropathogens play a significant role in the pathogenesis of recurrent urinary tract infections in adults (Galleti et al., 2006). These flora are normal inhabitants of the distal urethra (Quigley, 2009). Urine is a sterile fluid within the proximal urethra, the urinary bladder. For urinary tract infection to occur, the uropathogens must gain access to the urinary bladder and proliferate (Mysorekar et al., 2012). Bacteria found in the distal urethra usually gain access to the bladder due to turbulence urine flow during normal voiding dysfunction, or as a result use of instruments. Whatever the case, normal voiding ends up in essentially complete flush out of contaminating bacteria. Thus, colonization of the urinary bladder does not occur unless the bladder defense mechanisms are affected or a virulent strains of bacteria gained an entrance to the bladder (Barnett and Stephens, 1997).

2.3.1 Bladder emptying.

Lack of normal bladder emptying leads to bacterial proliferation in the bladder, which results in the risk of UTI (Mulvey et al., 2000). The urinary tract system is a closed system which is lined with sterile mucosa, composed of epithelium, transitional cells (mysorekar et al., 2012). Constant flow of urine from the kidneys to the bladder with intermittent complete emptying of the bladder via the urethra is known to be the main defense mechanism against UTI. The effect of this washout usually clears off the urinary
tract with pathogens (Dromigny et al., 2002). The urine in itself has some specific antimicrobial characteristics such as low urine pH, polymorphs and Tamm-Horsfall glycoproteins which prevent bacteria from adhering to the bladder mucosal wall (Toth et al., 2003). There is an occurrence of UTI with the introduction of pathogens into this space when there is an association with adherence to the mucosa of the urinary tract. Inadequate washout effect of voiding leads to microbial colonization (Toth et al., 2003). This colonization process is usually followed by microbial proliferation and inflammatory response.

2.3 Clinical Manifestations of Urinary tract infections.

There are varying clinical manifestations of urinary tract infection including asymptomatic bacteriuria, cystitis and acute pyelonephritis.

2.3.1 Asymptomatic bacteriuria.

Bacteriuria (the presence of bacteria in urine) is a clinical feature that is associated with all types of UTI. The condition is often accompanied by no symptoms, hence referred to as asymptomatic bacteriuria. A diagnosis of asymptomatic bacteriuria requires $\geq 10^5$ CFU/ML from a mid-stream, clean catch urine, or for at least 100 CFU/ML for a catheterized specimen (Initiative & W.H.O., 2003). However, more recent studies have suggested that lower levels of bacteriuria ($10^2$ to $10^4$ CFU/ML) should be indicative of UTI, but this raises the question of how and when to follow up on low colony counts (Wilson, M.L. and L. Gaido. 2004, Fihn, 2003).
Hooton et al., 2000 reported that asymptomatic bacteriuria prevalence in healthy young adults was found to be 4 to 6% with an increase to 20% in ambulatory elderly adults (T.M Hooton et al., 2000). It is a condition characterized by a positive urine culture test without the individual experiencing symptoms (e.g. fever, abdominal pain etc.) commonly associated with infection. Studies conducted by Patterson and Andrriole, 1987; Lucas and Cunningham, 1993, have indicated that asymptomatic bacteriuria is also exacerbated by diabetes mellitus in female subjects, as a result of glycosuria (Cunningham & Lucas, 1994).

Pregnancy predisposes women to asymptomatic bacteriuria, as they experience a decrease in immuno-competence. This coupled with risk factors, such as sexual intercourse, which permits both commensal and non-commensal bacteria to colonize and multiply in the urogenital tract (Stamm & Raz, 1999). Infectious Disease Society of America guidelines indicates that untreated bacteriuria in pregnancy has been associated with prematurity, and low birth weight (T.M. Hooton et al., 1996).

2.3.2 Cystitis.

Acute cystitis or uncomplicated UTI is usually defined as symptomatic infection of the urinary bladder in healthy person with a normal genitourinary tract. Its symptoms include polyuria (frequent urination), burning sensation, straining, urinary urgency, and dysuria (pain when passing urine)(Medina-Bombardo et al., 2003). Also, patients may experience haematuria (blood in urine), suprapubic pain or tenderness, and a change in the odour of the urine (Medina-Bombardo et al., 2003).
Furthermore, cystitis is classified based on the etiologic agent and therapeutic approach (Vasudevan, 2014). However, fever and flank pain is not associated with it and it cannot cause kidney damage.

2.3.3 Acute pyelonephritis.

This condition is commonly referred to as upper urinary tract infection. It affects the kidneys. A report from a certain finding shows that pyelonephritis is a progressed result of urinary tract infection due to the ascending of bacteria that colonized the urethra and the bladder (Morgan, 2004).

It was illustrated that the ability of uropathogens to cause upper urinary tract infections has been attributed to bacteria adhesion, virulence factors and their motility, coupled with the host’s anatomic, humoral and genetic factors (Kaper et al., 2004). Acute pyelonephritis is commonly associated with symptoms such as flank and abdominal pains, fevers and chills. However, children may express non-specific symptoms, such as poor feeding, irritability and jaundice in newborns.

2.3 Diagnosis of Urinary Tract Infection.

Urinary tract infection according to Mcloughlin and Joseph (2003), is a combination of clinical features and the presence of uropathogens in urine. Presence of more than 100000 colony forming units per milliliter of single bacteria in urine culture is considered urinary tract infection. The clinical features of UTI include both specific and nonspecific signs and symptoms. Proper diagnosis and treatment of urinary tract infection is important to
limit its associated morbidity, mortality, and to avoid unnecessary and prolonged use of antibiotics (Tonagho and Mcaninch, 2004).

Diagnosis of UTI in some sub-groups like in young children and infants is difficult because clinical presentation of UTI in this age group is often non-specific clinical signs such as fever, irritability and vomiting, which are equally seen in other childhood viral illnesses (NICE, 2012). UTI diagnosis relies on both laboratory investigation and clinical signs and symptoms.

2.3.1 Laboratory detection of Urinary Tract Infection.

Two major laboratory diagnostic methods are commonly employed to detect the presence of uropathogens. These are dipstick urinalysis and microbiological culturing which is currently accepted as ‘gold standard’. However, Polymerized Chain Reaction (PCR) method is also a known powerful tool for diagnosis.

2.3.2 Dipstick Urinalysis.

Although, the urine dipstick is known to be a standard method of diagnosing acute cystitis, there is much disagreement about its utility and role. However, it is rapid, affordable, and easy to conduct for the detection of urinary tract infections.

2.3.2.1 Parameters determined by Dipstick.

Despite the disagreement about its utility and role, some parameters determined in dipstick urinalysis give good indication of infections in the urinary tract. These are haematuria, proteinuria, nitrites and leukocyte esterase.
2.3.2.2 Haematuria.

According to the American Urological Association, presence of more than two red blood cells in the urine of a patient under a high powered light microscope field, could be an indication of urinary tract infections (Mariani et al., 1989). This parameter is measured by detecting the peroxidase activity of haemoglobin or myoglobin, with the aid of an indicator which is oxidized to change colour (Ahmed & Lee, 1997). However, there are other known causes of haematuria such as tumors, urinary shistosomiasis, calculi and exercise-induced haematuria, which may give a false positive result for UTI (Brendler & Gerber, 1998).

2.3.2.3 Proteinuria.

Normal urinary protein excretion is less than 150 mg/day, with majority consisting of secreted proteins such as Tamm-Horsfall protein. Although the glomerulus normally secretes some urinary proteins, such as albumin and globulin, excretion above this is indicative of renal disease. This causes the glomerulus to be porous and unable to reabsorb these proteins or the proteins overwhelming the cells’ ability to reabsorb, reported by Sheets and Lyman, 1986 (Brendler & Gerber, 1998).

Reports have indicated that the dipstick method is able to yield positive results for protein concentrations as low as 5-10 mg/dL with reliable sensitivities and specificities greater than 99% (Woolhandler et al., 1989). However, postural proteinuria, which is a benign condition from prolonged standing could give a false positive for UTI.
2.3.2.4 Nitrite.

As indicated earlier, the result of urinalysis identifies and verifies a number of parameters, and nitrite is one of them. Nitrite in urine is a condition that is different from nitrate in urine. For individuals with UTI, they may have the option marked as ‘present’ or ‘+’ for presence of it. In most of the cases, the presence and detection of nitrites in urine is an indicative of possible presence of a certain type of bacteria that has the ability to convert non-ionic nitrates to nitrite (Woolhandler et al., 1989). Bacteria that have the ability to convert nitrates into nitrites are of the gram negative variety. A vaginal contamination of the sample of urine, exposure of the dipstick to the atmospheric air, contamination of the urine sample with phenazopyridine, a chemical generally used as analgesic, can yield false positive results for nitrite in urine.

2.3.2.5 Leukocyte Esterase.

A positive detection of this enzyme, leukocyte esterase, in urinalysis indicates the presence of white blood cells. Normally this is suggestive of an inflammation of some sort, most often as a result of UTI. A simple urinalysis using dipstick can detect the presence of this enzyme (Panesar, 2013).

However, a test for leukocyte esterase sometimes produces negative results but later follow-up indicates a disease condition such as UTI. This could be because the laboratory technician did not allow enough time before reading the dipstick. It could also be due to a higher than normal specific gravity of the urine, presence of glucose or ketone in urine, urinary protein, drugs or supplements (Panesar, 2013).
**Table 1 Causes of false positive and false negative results of urine dipstick test.**

<table>
<thead>
<tr>
<th>Dipstick</th>
<th>false positive</th>
<th>false negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dehydration, exercise,</td>
<td>Capropril (Capoten) elevated specific gravity, pH&lt;5.1,</td>
</tr>
<tr>
<td>Blood</td>
<td>haemoglobinuria,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>menstrual blood,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>myoglobinuria</td>
<td>proteinuria</td>
</tr>
<tr>
<td></td>
<td>alkaline or concentrated urine, phenazopyridine,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>quaternary ammonia</td>
<td>acidic or dillute urine, primary</td>
</tr>
<tr>
<td></td>
<td>protein compounds</td>
<td>protein is not albumin</td>
</tr>
<tr>
<td></td>
<td>elevated specific gravity, elevated Urobinogen levels, nitrate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Contamination, exposure of dipstick to air,</td>
<td>reductase –negative bacteria, pH &lt;</td>
</tr>
<tr>
<td>Nitrate</td>
<td>phenazopyridine.</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Elevated specific gravity, glycosuria, ketonuria, proteinuria,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>some oxidizing drugs (cephalexin, nitrofurantoin, tetracycline,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>gentamicin), vitamin C</td>
<td></td>
</tr>
<tr>
<td>leukocyte esterase</td>
<td>contamination</td>
<td></td>
</tr>
</tbody>
</table>
2.3.2 Microbiological Culture.

Cultivation of pathogenic microorganisms in specific media has remained the ‘gold standard’ for detecting infections (Zorc et al., 2005). A pure culture, one containing a single kind of microbe, is required in order to study concepts such as growth characteristics, pathogenicity, metabolism, and antibiotic susceptibility. The predominant Gram-negative bacteria that are associated with UTI, grow on specific solid media at specific pH, temperatures and time. This gives rise to formation of colonies originating from a single cell, or changes in the colour and/or production of gases in liquid media, due to metabolic activities. This indicates presence and probable number of the microorganism(s).

Currently, there are three main dilution methods that are commonly employed in microbiology laboratories for the cultivation of bacteria. These are the streak plate, the spread plate, and the pour plate techniques.

In the streak plate technique, a calibrated loop is used to streak the mixed sample many times over the surface of a solid culture medium in a Petri disc. This method of streaking the loop repeatedly over the agar surface, theoretically cause the bacteria to fall off the loop one by one and ultimately to be distributed over the agar surface, where each cell develops into a colony. The streak plate is the most common isolation technique in used today.

The spread plate can be used for quantitative work (colony counts) if the inoculum is a measured volume delivered by pipette. A few drops of broth culture added onto solid surface (agar) and a spreader is used on it, making sure the entire agar surface is covered.
In the pour plate method, a small amount of inoculum from broth culture is added by pipette to a molten, cooled agar medium in a test tube, distributed evenly throughout the medium, thoroughly mixed and then poured into a petri dish to solidify. The isolated cells give rise to individual colonies growing in the agar itself. The technique is a little tricky. If the melted agar is too hot, the organisms will be killed, if it is too cool, there will be a lump in the petri dish.

2.3.3 Treatment.
Treatment is best guided by the results of culture and antimicrobial susceptibility tests. Antimicrobial drugs have played a significant role in reducing morbidity and mortality associated with infectious diseases in humans (Bosu & Acquah, 1996). In management of UTI, the commonly used antibiotics include cefuroxime, Amoxicillin/clavulanic acid, trimethoprim/sulpmethoxazole and fluoroquinolones (Bosu & Acquah, 1996; Franco, 2005). However, treatment of UTI has recently become difficult due to the emergence of resistant bacterial strains (e.g. E.coli) to the commonly used antibacterial drugs (Jha & Bapat, 2004). The report by Kenechukwu Menzue indicated that the isolated organisms from urine of the UTI patients were more susceptible to the quinolones compared to penicillins and aminoglycosides (Holmberg et al., 1987).

Also at Port Harcourt - Nigeria, a study indicated that most uropathogens isolated were resistant to tetracycline, ampicillin and Cotrimoxazole but showed susceptibility to Nitrofurantoin, Gentamycin and Nalidixic Acid (Holmberg et al., 1987). Resistant to antimicrobial drugs has significant public health consequences and contributes to increase cost of healthcare in improvised nations such as those in the Sub-Saharan Africa.
3.3.4 Prevention of UTI.

There are a number of preventive methods against urinary tract infections. In cases of recurrent cystitis, there are various behavioural strategies that are useful. Some of these useful strategies may be reduction of frequency of sexual intercourse, if possible, as this is a strong risk factor for UTI, urination without delay, wiping from back after defaecation (for females), urinating as soon as possible after intercourse and drinking plenty of fluid; avoidance of spermicide and condoms with spermicides, which alter the vaginal flora.
CHAPTER THREE

Materials and methods.

3.1 Study area.

The study was carried out in the Microbiology (bacteriology) department of the Eastern Regional Hospital, Koforidua, Ghana. The Eastern Region is the sixth largest region in Ghana. It lies between latitudes 6 and 7 degrees North and longitude 1.30 degree West, 0.30 degree East. It shares boundaries with five other regions: Greater Accra, Volta, Brong Ahafo, Ashanti and Central regions.

The vegetation is tropical and the rainfall pattern is the double maxima with dry and wet seasons. It has a total population of 2,921,437 (2015) representing 10.7% of Ghana’s total population. The population is the 3rd highest after Ashanti and Greater Accra regions. Its population is made up of 49% males and 51% females, giving a sex ratio of 96.8 males to 100 females. The Regional Hospital is the biggest public hospital in the region. It caters for persons in and around Region’s Administrative capital, Koforidua. The hospital also handles referral cases from all the districts within the region. Out of 26 districts and municipalities, 18 have at least one hospital. The figure below illustrates the health facilities in the region.
Distribution of hospitals in the Eastern Region of Ghana, 2015

Figure 2 Distribution of health facilities.
3.2 Study design.
A cross sectional study design was used among 422 study participants attending clinics/wards in the Eastern Regional Hospital, Koforidau – Ghana. The study involves collecting data from study participants’ folders/cards/HAMS.

3.3 Study population.
The study was conducted among 422 participants attending clinics/wards in Eastern Regional Hospital, Koforidua – Ghana. Study participants were anyone aged one (1) day old and above without been on antibiotic therapy, in labour or delivered within 24 hours, and not from Eastern Region. Data about their socio-demographics were collected from their forms/cards and/or Health Administration Management System (HAMS).

3.4 Sample size calculation.
Assuming the prevalence of UTI is 50%, the minimum sample size therefore was obtained using the formula below. 50% is used because of paucity of data on UTI in Ghana.

Using the $n = z^2 \times p \times (1-p)/d^2$ formula (Cochran, W. G. 1977) where $n$ is the desired sample size, $z$ the normal deviation = 1.96 that corresponds to 95% confidence interval, $p$ = proportion of the target population with desired characteristics, 0.5= 50% (due to the paucity of data on UTI in Ghana) and $d$ = degrees of freedom, 0.05.

$n = 1.96^2 \times 0.5 \times (0.5)/0.0025$

$= 384$
Thus a sample size of 422 (sample size plus its 10%) participants was taken to increase the representativeness of the sample, minimize sampling errors, increase generalizability of the result and cater for any attrition. There was no challenge obtaining the desired sample size because there were enough urine samples received from study subjects who met the desired criteria.

3.5 Sampling method.

The study used purposive sampling method. This sampling technique was used to select participants who on account of suspected UTI use the services of the Regional Hospital, Koforidua - Ghana. Applying strictly the inclusion and exclusion criteria, subjects were chosen to be included in the study. Prior to the start of the study, the clinicians and nurses in the various wards were met and the study protocol was explained to them. The nurses conveniently selected suspected patients pointed by the attending clinician and who met the inclusion criteria, demonstrated to them how to collect the urine sample. The selection into the study was done until the desired sample size was obtained. No questionnaires were administered.

3.6 Sample collection.

Sample collection for this study was conducted in collaboration with nurses at the clinics/wards where patients visit for medical service. The nurses demonstrated to the
suspected patients who met the inclusion criteria, as pointed by the attending clinician, how to collect clean catch midstream urine into a 20ml calibrated sterile screw-capped, labelled universal container given to them. It was explained to them how to carefully cleanse the periurethrum before voiding and allowing the initial part of the stream to flush the urethra before collecting a specimen for examination. The labelled specimens were transported to the laboratory, and processed within an hour. Midstream urine was used because the first few milliliters of urine passed will contain skin flora from the urethral orifice and will yield potentially misleading results. The optimal timing for specimen collection was the 1st voiding of urine in the morning as it is the most concentrated and suitable sample for culture and sensitivity.

3.7 Criteria.

3.7.1 Inclusion criteria.

- Patients with signs and symptoms of Urinary Tract Infection as indicated by the attending clinician.
- Any patient with asymptomatic Urinary Tract Infection suspected by the attending clinicians.
3.7.2 Exclusion criteria.

- Any patient, pointed out by the attending clinician, already on antibiotic treatment for any other reason.
- Pregnant women in labour and those who delivered within 24 hours.
- Any patient from other region(s).

3.8 Sample processing.

3.8.1 Urine dipstick:
Urine samples were preliminarily analyzed for UTI by use of DIRUI A10 urine test strips. It was carried out within the first two hours of sample collection. Each strip was dipped in urine sample and analyzed according to the manufacturer’s instructions. A positive test for leukocyte esterase and/or nitrite is usually scored ‘pos’ for UTI using dipstick and ‘neg’ for absence of UTI using dipstick.

3.8.2 Microbiological culture.
Prior to dipping urine dipstick in each urine sample for urinalysis, the urine samples were aseptically plated on dried surface of Cystine Lactose Electrolyte - Deficient (CLED) agar plate. A calibrated loop method was used for the inoculation and isolation of uropathogens. A sterile 4.00 mm nichrome wired calibrated loop was used, which delivered 0.001 ml of urine. Each urine specimen was mixed by rotating the container and then a loopful urine sample was plated on Cysteine Electrolyte-Deficient (CLED) agar. The inoculated plates were incubated at 37°C for 24 hours. After the 24 hours incubation, the number of isolated bacterial colonies were multiplied by 1000 for the
estimation of bacterial load per milliliter of the urine sample. A specimen was considered positive for UTI if an organism was cultivated at a concentration of $\geq 10^5$ cfu/ml.

### 3.8.3 Identification of pure bacterial isolates.

Bacterial isolates were identified based on their biochemical characteristics. Identification of the gram negative bacteria such as *E. coli*, *Klebsiella spp.*, *Proteus mirabilis*, *Morganella morganii*, *Citrobacter spp.*, *Enterococcus faecalis* and *Serratia marcescens* were done using biochemical reagents: indole, citrate, and urea. *Pseudomonas aeruginosa* were identified using cytochrome oxidase reagent tests. For the gram positive bacteria, they were identified using catalase and coagulase test. For further confirmation, smears on slides were prepared from these colonies, stained using Gram’s staining technique and observed under the light microscope.

### 3.8.4 Antimicrobial Susceptibility Testing.

Each bacterial isolate was subjected to antimicrobial susceptibility testing using the standard Kirby Bauer’s disc diffusion method (Bauer *et al.*, 1966). Standard inoculum, adjusted to 0.5 McFarland turbidity, was swabbed on Mueller Hinton agar plate and allowed to soak for 2 to 5 minutes. After 5 minutes, antibiotic discs were placed on the surface of the media and pressed gently using sterile forceps. The swabbed plates with antibiotic discs were then incubated at $37^\circ$C for 24 hours. After the 24 hours, antibiotics were observed for sensitivity or resistance. Zone sizes of the sensitive ones were measured and interpreted using caliper/ruler according to the recommendations of the clinical and laboratory standards (Wikler, 2006). The following standard, commonly used antibiotic discs were used for the isolates: Ciprofloxacin (5μg), Nalidixic acid (30μg),...
Amikacin (30μg), Gentamycin (10μg), Ceftriaxone (30μg), Nitrofurantoin (300μg), Tetracycline (30μg), Co-trimoxazole (25μg), Pipemidic acid (20μg) and Augmentin (30μg).

For quality control purpose, these standard strains of *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923) and *P. aeruginosa* (ATCC 27853) were routinely used in this study.

### 3.8.5 Statistical analysis.

Bio-data and characteristics of the study subjects in terms of predictability and determination of risk factors of UTI generated from this study were entered into Excel spreadsheet (2013). After manual verification and cleaning, the data were analyzed using STATA 14.0. The distribution of study population in relation to each of the socio-demographic variable (age, educational status, marital status, sex) was assessed. The association between UTI and respective age, sex, marital status and educational status was assessed by cross-tabulations and Chi-squared test was used to assess the strength of the respective associations. The culture results from the samples collected were used to calculate prevalence of UTI, characterize the type of microbial growth (isolates) and to test for antimicrobial susceptibility test. The associations of isolate type as well as cases of UTI by age group, sex, and hospital unit respectively were also calculated, using Chi-square test to assess the strength of the association. A p-value of 0.05 was considered as statistically significant for all the test, and at 95% level of confidence interval.
3.8.6 Ethical clearance.

Ethical approval for the study was sought and obtained from the Ethics Committee, Ghana Health Service (ethical approval – ID number: GHS-ERC: 13/10/15). Subsequently, a permission was also sought and obtained from the Medical Director of the Regional Hospital, Koforidua.
CHAPTER 4

RESULTS

Four hundred and twenty-two patients were enrolled in the study. The prevalence of Uropathogenic microorganisms causing the urinary tract infections among study population was 22.5% (95/422), and these were predominantly females (75/95).

4.0 Socio-demographic characteristics of study participants.

4.1 Distribution of UTI suspected cases by educational status.

Educational status of the participants varied from no education to tertiary education, and more were predominantly with no education 68.1%. Table 1.

The Chi-square test for association between significant microbial growth (culture positive) and educational status showed a statistically significant variation (p< 0.05). Table 2.

4.1.1 Marital status of study participants.

Marital status of the study participants were none, single, cohabitation, widowed, divorced and married. The chi-square test of association between significant microbial growth (isolates) and marital status showed statistically significant variation (p<0.05) with more isolates from married participants, 59.0% (56/95) followed by those who were single 35.8% (34/95). Tables 1 and 2 respectively.

4.1.2 Distribution of suspected UTI cases by hospital units.

Of the 422 urine samples screened, the highest number of urine samples came from OPD with 56.6% (239/422) samples. This was followed by Kids ward with 15.9% (67/422)
samples. Neonatal Intensive Care Unit (NICU) and Male Medical wards contributed the least number of samples, 0.2% (1/422) each. Table 1.
Table 2: Socio-demographic characteristics of study participants

<table>
<thead>
<tr>
<th>Variables</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age group (years)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 to 10</td>
<td>103</td>
<td>24.41</td>
</tr>
<tr>
<td>11 to 20</td>
<td>32</td>
<td>7.58</td>
</tr>
<tr>
<td>21 to 30</td>
<td>108</td>
<td>25.59</td>
</tr>
<tr>
<td>31 to 40</td>
<td>84</td>
<td>19.91</td>
</tr>
<tr>
<td>41 to 50</td>
<td>33</td>
<td>7.82</td>
</tr>
<tr>
<td>51 to 60</td>
<td>21</td>
<td>4.98</td>
</tr>
<tr>
<td>&gt;60</td>
<td>41</td>
<td>9.72</td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>211</td>
<td>50.00</td>
</tr>
<tr>
<td>Married</td>
<td>179</td>
<td>42.42</td>
</tr>
<tr>
<td>Widowed</td>
<td>13</td>
<td>3.08</td>
</tr>
<tr>
<td>Divorced</td>
<td>11</td>
<td>2.61</td>
</tr>
<tr>
<td>None</td>
<td>7</td>
<td>1.66</td>
</tr>
<tr>
<td>Co-habitation</td>
<td>1</td>
<td>0.24</td>
</tr>
<tr>
<td>Variables</td>
<td>Number</td>
<td>Percentage</td>
</tr>
<tr>
<td>-----------------</td>
<td>--------</td>
<td>------------</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Akan</td>
<td>221</td>
<td>52.37</td>
</tr>
<tr>
<td>Northerner</td>
<td>59</td>
<td>13.98</td>
</tr>
<tr>
<td>Ga-Adangbe</td>
<td>44</td>
<td>10.43</td>
</tr>
<tr>
<td>Ewe</td>
<td>41</td>
<td>9.72</td>
</tr>
<tr>
<td>Krobo</td>
<td>25</td>
<td>5.92</td>
</tr>
<tr>
<td>Fante</td>
<td>21</td>
<td>4.98</td>
</tr>
<tr>
<td>Akwapim</td>
<td>11</td>
<td>2.60</td>
</tr>
<tr>
<td>Educational status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>287</td>
<td>68.01</td>
</tr>
<tr>
<td>Primary/pupil</td>
<td>32</td>
<td>7.58</td>
</tr>
<tr>
<td>Secondary</td>
<td>78</td>
<td>18.72</td>
</tr>
<tr>
<td>Tertiary</td>
<td>24</td>
<td>5.69</td>
</tr>
</tbody>
</table>
4. 1.3 Distribution of isolates by hospital unit.

Of the 22.5% (95/422) urine samples that yielded significant microbial growth, OPD contributed the highest isolates, 58.0% (55/95), followed by prenatal ward, 10.5% (10/95) Kids ward, 9.5% (9/95), Gynaecology ward 8.4% (8/95) and Antenatal ward 7.4% (7/95). The least numbers of isolates came from casualty, female medical, male medical and male surgical wards, 1.1% (1/95) each, with no isolate from Neonatal Intensive Care Unit and Urology wards. However, there was no statistically significant variation (> 0.05) among the isolates and different hospital units. Table 2.
Table 3. Factors associated with bacterial culture positive.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Participants with bacterial culture positive</th>
<th>Participants without bacterial culture positive</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>56</td>
<td>123</td>
<td>0.02</td>
</tr>
<tr>
<td>Single</td>
<td>34</td>
<td>177</td>
<td></td>
</tr>
<tr>
<td>Cohab</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Widowed</td>
<td>2</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Divorced</td>
<td>2</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>1</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>20</td>
<td>114</td>
<td>0.02</td>
</tr>
<tr>
<td>F</td>
<td>75</td>
<td>213</td>
<td></td>
</tr>
<tr>
<td>Educational level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tertiary</td>
<td>9</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>25</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>8</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>53</td>
<td>234</td>
<td>0.02</td>
</tr>
<tr>
<td>Characteristics</td>
<td>Participants with culture positive</td>
<td>Participants without culture positive</td>
<td>p-value</td>
</tr>
<tr>
<td>------------------</td>
<td>-----------------------------------</td>
<td>---------------------------------------</td>
<td>---------</td>
</tr>
<tr>
<td>Wards</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OPD</td>
<td>58</td>
<td>185</td>
<td>0.13</td>
</tr>
<tr>
<td>Prenatal</td>
<td>10</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Kids</td>
<td>9</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>Gynaecology</td>
<td>8</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Antenatal</td>
<td>7</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>Lying-in</td>
<td>2</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Female medical</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Male medical</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Male surgical</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Casualty</td>
<td>1</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>NICU</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Urology</td>
<td>0</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>
4.1.4 Distribution of isolates by age group.

The highest susceptible age group of patients with UTI was 21-30 years (23.2%), followed by 0-10 years and 31-40 years (19%) each and greater than 60 years (15.8%). The lowest was found in age group 11-20 (6.3%), followed by 41-50 years and 51-60 years (8.4%) each. This is depicted in figure 3 below.
Figure 3 Percentage distribution of isolates by age group
4.1.5 Distribution of isolates by age group and sex.

Comparatively, more cases of UTI were seen in females than in males in all age groups. The highest UTI prevalence in females was found in the age group 31-40 years (100%). Apparently, in males, the highest susceptible age group to UTI was 0-10 years (44.4%). The Chi-square test showed statistically significant variation (P<0.05) at 95% confidence interval for the infected and non-infected male and female patients. The figure 2 shows percentage distribution of isolates by age group and sex.
Figure 4 Percentage distribution of isolates by age group and sex.
4. 1.6 Microbial isolates.

Ten different Uropathogenic organisms were identified among the 422 urine cultures that yielded significant microbial growth. These were: Klebsiella, E. coli, Morganella, Enterococcus, Citrobacter, Staphylococcus, Candida, Proteus, Pseudomonas and Serratia. The predominant bacterial isolate was E. coli (37.9%) followed by Klebsiella spp. (34.7%).

Table 4 below summaries the distribution of isolates among sexes.
Table 4. Frequency of microbial isolates among sexes and isolates per total obtained (n=95)

<table>
<thead>
<tr>
<th>Microbial isolates</th>
<th>Male</th>
<th>Females</th>
<th>Isolate per total</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>4 (22.2%)</td>
<td>32 (41.56%)</td>
<td>36 (37.89%)</td>
</tr>
<tr>
<td>Klebseilla spp.</td>
<td>7 (38.9%)</td>
<td>26 (33.77%)</td>
<td>33 (34.74%)</td>
</tr>
<tr>
<td>M. morganii</td>
<td>2 (11.1%)</td>
<td>3 (3.90%)</td>
<td>5 (5.26%)</td>
</tr>
<tr>
<td>Candida spp.</td>
<td>1 (5.6%)</td>
<td>10 (12.99%)</td>
<td>11 (11.58%)</td>
</tr>
<tr>
<td>S. marcescens</td>
<td>1 (5.6%)</td>
<td>0 (0%)</td>
<td>1 (1.05%)</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>1 (5.6%)</td>
<td>0 (0%)</td>
<td>1 (1.05%)</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>0 (0%)</td>
<td>2 (2.60%)</td>
<td>2 (2.11%)</td>
</tr>
<tr>
<td>aeruginosa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citrobacter</td>
<td>1 (5.6%)</td>
<td>1 (1.30%)</td>
<td>2 (2.11%)</td>
</tr>
<tr>
<td>S. aureus</td>
<td>1 (5.6%)</td>
<td>1 (1.30%)</td>
<td>2 (2.11%)</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>0 (0%)</td>
<td>2 (2.60%)</td>
<td>2 (2.11%)</td>
</tr>
<tr>
<td>Total</td>
<td>18 (19.0%)</td>
<td>77 (81.05%)</td>
<td>95 (100%)</td>
</tr>
</tbody>
</table>
Relationship between dipstick positive urine & culture positive results.

<table>
<thead>
<tr>
<th></th>
<th>Culture</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Positive</td>
<td>91</td>
<td>95</td>
</tr>
<tr>
<td>Dipstick Negative</td>
<td>4</td>
<td>232</td>
</tr>
<tr>
<td>Total</td>
<td>95</td>
<td>327</td>
</tr>
</tbody>
</table>

Sensitivity = $\frac{91}{95} \times 100$

= 95.6%

Specificity = $\frac{232}{327} \times 100$

= 70.1%

Of the 95 cultured positive, 97.9% (93/95) were also positive for urine dipstick. Therefore urine dipstick which is fast, is also a reliable screening test for urinary tract infection.
4.1.7 Antibiotic Sensitivity Pattern.

A greater percent of bacteria isolated were observed to be sensitive to Amikacin (98.8%), and Nitrofurantoin (63.1%). High bacterial resistance was observed with Augmentin (98.8%), Ampicillin (97.6%), Co-trimoxazole (95.2%) and Gentamycin (91.7%). Table 5 illustrates the summary of total number of antibiotics used with the sensitivity and resistance patterns of the Uropathogenic bacteria isolated in this study.
Table 5. Overall sensitivity and resistant patterns of bacterial isolates.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Number tested</th>
<th>Sensitive (%)</th>
<th>Resistance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>84</td>
<td>83 (98.8%)</td>
<td>1 (1.2%)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>84</td>
<td>2 (2.4%)</td>
<td>82 (97.6%)</td>
</tr>
<tr>
<td>Augmentin</td>
<td>84</td>
<td>1 (1.2%)</td>
<td>83 (98.8%)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>84</td>
<td>30 (35.7%)</td>
<td>54 (64.3%)</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>84</td>
<td>4 (4.8%)</td>
<td>80 (95.2%)</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>84</td>
<td>25 (29.8%)</td>
<td>59 (70.2%)</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>84</td>
<td>7 (8.3%)</td>
<td>77 (91.7%)</td>
</tr>
<tr>
<td>Nalidixic Acid</td>
<td>84</td>
<td>29 (34.4%)</td>
<td>55 (65.5)</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>84</td>
<td>53 (63.1%)</td>
<td>31 (36.9%)</td>
</tr>
<tr>
<td>Pipemidic acid</td>
<td>80</td>
<td>17 (21.3%)</td>
<td>63 (78.8%)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>78</td>
<td>4 (5.1%)</td>
<td>74 (94.9%)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>6</td>
<td>0(0%)</td>
<td>6 (100%)</td>
</tr>
</tbody>
</table>
Figure 5 Overall Percentage Resistance of Bacteria isolates to Antibiotics.
Figure 6 Percentage Resistance of Gram negative bacteria to commonly used antibiotics.
For the commonly used antibiotics, *E. coli* and *Klebsiella spp.*, isolated in this study showed a high resistance to all antibiotics. However, *E. coli* was found to be sensitive to Amikacin, Nitrofurantoin and Nalidixic acid.
CHAPTER FIVE

DISCUSSION

Urinary Tract Infection is a very common disease, its diagnosis and treatment have important implications for patients’ health, development of antibiotic resistance, and health care cost (T.M. Hooton et al., 1995; Orenstein & Wong, 1999). Surveillance of local UTI’s aetiology as well as of antibiotic susceptibility is deemed useful to guide empirical treatment, as prevalence of Uropathogenic organisms and their characteristics may vary with geographical area and time (Foxman, 2002). There should also be attempts to increase prediction of causative uropathogens through the use of demographic and clinical information.

This study provides valuable information to compare, contrast, and monitor the status of antibiotic resistance among isolated bacteria to enhance empirical therapy. The prevalence of urinary tract infection in this study was found to be 22.5%. Although this rate of prevalence is lower compared with findings obtained in other studies done in Ghana Police Hospital (31.6%), Cape Coast – Ghana (50.4%), Urban Community of Meerut, India (53.82%) (Prakash & Saxena, 2013; Gyansa-Lutterodt et al., 2014)), it was higher with other studies done in tertiary school in Jos - Nigeria that accounted for 4.2% (David, 2013), and in India 10.86%, and17.19% (Kothari & Sagar, 2008; Akram et al., 2007). However, the prevalence rate of UTI in this study correlates with other studies done in University of Jos and in Ibadan - Nigeria which accounted for 22% and 25.6% (Ekweozor & Onyemenen, 1996; Kolawole et al., 2010).
The study revealed a lower prevalence rate compared to other studies done within the country, and the sub-region. This could be because the study lacked the capacity to confirm other causes of UTI. Aside the common bacteria known to cause UTI, microorganisms such as Chlamydia and Mycoplasma which require special culture techniques to isolate and as such could not be determined in this study, may also cause UTI (Uwaezuoke & Ogbulie, 2006). In addition, the difference could also be based on the differences in sanitary conditions and observed personal hygiene.

This study showed a high prevalence of UTI in females (79.9%) than in males (21.1%) which is affirmed by other findings which revealed that the frequency of UTI is greater in females than in males (Abubakar, 2009; James R Johnson et al., 1995; Randrianirina et al., 2007; Uehling et al., 1997). Anatomical differences between males’ and females’ urethra (shorter in females than in males), close proximity of the urethral meatus to the anus, sexual intercourse and lack post coital voiding, incontinence, diaphragm and spermicide use, and estrogen deficiency may be underlining factors for the high prevalence rate in females. It could also be due to use of tub baths. Accordingly, it had been a repeated observation over the years that many women who present with urinary tract infection give a history of taking frequent tub baths (Rinke, 1983). It is plausible that the hot water washes away some of the protective mucosal coating the urethral and vaginal introituses, making the mucosa drier and more susceptible to bacterial colonization (Rinke, 1983).

Generally, the highest occurrence of urinary tract infection recorded in this study was among the age group 21-30 years which correlates with studies done in Nigeria (74.7%)(Omigie et al., 2009) and Kuwait (63.4%)(Dimitrov et al., 2004). It was observed
that females of the age group 31-40 years were found to be more susceptible to urinary tract infection followed by 21-30 years 41-50 years and 11-20 years.

These findings correlate with other reports which showed that females are more prone to urinary tract infections than males during adolescence and adulthood (Akram et al., 2007; Kolawole et al., 2010; Tambekar et al., 2006). A prospective study of risk factors for symptomatic urinary tract infection in young women, indicated that the factors of this increasing incidence of UTI in females in these age groups are associated with high sexual activity, use of diaphragm with spermicide and history of recurrent UTIs (T.M. Hooton et al., 1996). Diaphragm and spermicide use increase risk of urinary tract infection. This is due to partial urethral compression by the rim of the diaphragm. Spermicide contains monoxynol-9 which can cause a chemical irritation to the vaginal and urethral mucosa as well as changes in the normal flora. This in turn predisposes to colonization by coliforms as well as *Staphylococcus saprophyticus* (Rinke, 1983).

Gram negative bacteria, normal flora of the intestinal tract, have been reported to be the predominant aetiologic agents, accounting for more than 85% of all UTI cases (Antwi et al., 2008; Bonadio et al., 2001). This value compares favourably with findings of this study, as the only Gram positive bacteria isolated were *Staphylococcus aureus* (2) and *Enterococcus faecalis* (2) which constituted only 4.2% of the significant microbial growth. *Escherichia coli* (37.89%) was found the most prevalent Gram negative bacteria, followed by *Klebsiella spp.* (34.74%) in this study. Studies conducted in Urban Community of Meerut - India, Abuja and Zaria – Nigeria, showed similar patterns with *E. coli* as the prevalent pathogen followed by *Klebsiella spp.*, (Gyansa-Lutterodt et al., 2014).
Studies done on UTI in other parts of the world, showed that *E. coli* and *Klebsiella* spp. are the commonest uropathogens in UTIs (Akram *et al.*, 2007; Kolawole *et al.*, 2010; Tambekar *et al.*, 2006). High incidence of Gram negative, Enterobacteriaceae, in causing UTI has many factors which are responsible for their attachment to the uroepithelium. Also, they are able to colonize in the urogenital mucosa with adhesins, pili, fimbriae, and P-1 blood group phenotype receptor (Das *et al.*, 2006).

Knowing the antibiotic resistance pattern among uropathogens responsible for UTI is not only important in guiding clinicians to prescribe appropriate antibiotics, but also provides evidence-based recommendations in empirical antibiotic therapy of UTI (Moyo *et al.*, 2010). The results of this study demonstrated high resistance of Gram negative bacteria to Augmentin, Ampicillin, Co-trimoxazole (sulphamethoxazole/trimethoprim), Tetracycline and Gentamycin.

Resistance of *E. coli* to Tetracycline, Augmentin and Co-trimoxazole correlates with other finding (Uwaezuoke & Ogbulie, 2006), though lower than findings from Ghana Police Hospital (Gyansa-Lutterodt *et al.*, 2014).

The tested fluoroquinolones in this study showed resistance to *E. coli* and *Klebsiella*. Ciprofloxacin and Nalidixic acid show resistance to *Klebsiella* spp. (31.25%) each. *E. coli* showed resistance to Ciprofloxacin (25%), though it showed a very low resistance to Nalidixic acid (2.5%). This resistance rate against fluoroquinolones was also reported by some other studies done in Spain, Europe, Iran (Rashedmarandi *et al.*, 2008), and in India (Kothari & Sagar, 2008). This resistance rate might be due to use of antibiotics without restriction. It has been shown in studies that highly prescribing habits of the physicians are the driving factor for the antibiotic resistance for this group of antibiotics. The best
example of this problem is findings by McEwen et al., which indicated that 37% of physicians actually prescribe trimethoprim-sulphamethoxazole (Co-trimoxazole) closely by fluoroquinolones (32%), and the average duration of antibiotic therapy is 8.6 days in United States (McEwen et al.).
CHAPTER SIX

6.1 CONCLUSION.

Despite the range of antibiotics available to manage UTIs, their incidence and recurrence rates still remain high. Effective management is further complicated as the causative uropathogens continue to develop resistance to antibiotics. In this study, Amikacin and Nitrofurantoin were seen to be active against the isolated bacteria. However, there was an overall high antibiotic resistance pattern observed. Most of the isolates have high resistance to the commonly used antibiotics such as Augmentin, Ampicillin, Co-trimoxazole and Gentamycin.

6.2 RECOMMENDATIONS.

There is a need for regular monitoring of the local UTI-aetiologic agents and their antibiotic susceptibility pattern. There is an increasing population of immunocompromised individuals potentially at risk for UTI, therefore a need for research to investigate the implications to the overall epidemiology of UTI.

According to the findings of this study, Amikacin and Nitrofurantoin are recommended first line drugs against UTI whiles clinicians await culture and sensitivity results from the laboratory.
6.3 LIMITATIONS

Due to lack of facility to culture mycoplasma and chlamydia, the study could not provide data on these organisms, though they can cause UTI.
REFERENCES.


Initiative, Treat 3 Million by, & W.H.O. (2003). How will the 3 by 5 Initiative deal with HIV drugs resistance?


Prakash, D., & Saxena, R.S. (2013). Distribution and antimicrobial susceptibility pattern of bacterial pathogens causing urinary tract infection in urban community of Meerut City, India. *ISRN microbiology, 2013*.


APPENDIX I

Media and standard solutions used

Media and standards solutions preparing for culture, Identification and Antibiotic Susceptibility testing.

The following were prepared under an observed aseptic conditions as prescribed by the manufacturer’s instructions using sterile distilled water. Where necessary, the media and solutions were autoclaved at 121°C for 15 psi pressure for 15 minutes. For the agar plates, dehydrated powders were weighed and dissolved in appropriate distilled water according to the manufacturer’s instruction. The prepared mixture were thoroughly mixed to produce a uniform solution, and then autoclaved. After the autoclave, were allowed to cool to about 50 – 55°C then 25ml volumes were dispensed into 90 cm sterile Petri dishes. The Petri dishes were left to set and agar surfaces dried before use. Quality and sterility of the prepared media were ascertained by routinely streaking the agar plates with the quality control strains.
### A. Cysteine Lactose Electrolyte-Deficient (CLED)

**Composition g/l**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactose</td>
<td>10.0</td>
</tr>
<tr>
<td>Beef extract</td>
<td>3.0</td>
</tr>
<tr>
<td>L-cysteine</td>
<td>0.128</td>
</tr>
<tr>
<td>Casein Peptone</td>
<td>4.0</td>
</tr>
<tr>
<td>Gelatin Peptone</td>
<td>4.0</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0</td>
</tr>
<tr>
<td>Bromothymol blue</td>
<td>0.02</td>
</tr>
<tr>
<td>pH</td>
<td>7.3 ± 0.2</td>
</tr>
</tbody>
</table>

**Preparation:**

36.0 g of the medium was suspended in one liter of distilled water. The prepared solution was slowly heated while stirring it frequently to have a uniform solution, and then boiled for a minute. After boiling, the mixture was autoclaved at 121 °C (15lbs. of psi) for 15 minutes. The medium is then allowed to cool at 50 – 55°C before pouring into the 90 ml sterile Petri dishes. After allowing the media to solidify, the Petri dishes were inverted to avoid excess moisture.
B. Mueller Hinton Agar

Composition: g/l

- Beef extract: 2.0
- Acid hydrolyzed casein: 17.5
- Starch: 1.5
- Agar No 1: 17.0

pH 7.3 ± 0.1

Preparation:

38.0 g of the medium was suspended in one liter of distilled water and heated with frequent agitation and boiling for one minute to completely dissolve. The dissolved, uniformed solution was autoclaved for 121 °C (15 lbs. of 15 psi) for 15 minutes. The prepared medium was then dispensed into 90 ml sterile Petri dishes after allowing it to cool at 50 – 55°C. The Petri dishes were then inverted to avoid excess moisture.
C. Urea

Composition g/l

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>1.0</td>
</tr>
<tr>
<td>Peptone</td>
<td>1.0</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.0</td>
</tr>
<tr>
<td>Disodium phosphate</td>
<td>1.2</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>0.8</td>
</tr>
<tr>
<td>Phenol red</td>
<td>0.012</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0</td>
</tr>
</tbody>
</table>

pH 6.8 ± 0.2

Preparation:

2.4 g of the dehydrated powder were weighed and suspended in 95ml of distilled water. The suspension was then brought to boil for complete dissolution. After sterilizing by autoclaving at 121\(^\circ\)C for 15 minutes, it was allowed to cool at 50 \(^\circ\)C and aseptically added 5ml of sterile 40% Urea Solution SR0020. Thoroughly mixed, 10ml amounts were distributed into sterile containers and allowed to set.
D. Citrate

Composition g/l

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>g/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium sulfate</td>
<td>0.2</td>
</tr>
<tr>
<td>Ammonium di-hydrogen phosphate</td>
<td>1.0</td>
</tr>
<tr>
<td>Di-potassium phosphate</td>
<td>1.0</td>
</tr>
<tr>
<td>Sodium citrate (dehydrated)</td>
<td>2.0</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.0</td>
</tr>
<tr>
<td>Bromothymol blue</td>
<td>0.08</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0</td>
</tr>
</tbody>
</table>

pH 6.9

Preparation

Prepared according to the manufacturer’s (OXOID) instructions. The completely dissolved mixture were dispensed into sterile tubes and the autoclaved. After the autoclave, the media were allowed to solidify.
E. Kovac’s indole

Composition

p-dimethylanobenzaldehyde 5g
Amyl alcohol 75ml
Conc. Hydrochloride 25ml

Preparation

The aldehyde was dissolved in the alcohol by warming the mixture gently in a water bath. The mixture was allowed to cool and the acid carefully added and then kept in a brown bottle to protect it from sunlight.

F. McFarland 0.5 Turbidity Standard (per 100ml)

Composition

1ml conc. H\textsubscript{2}SO\textsubscript{4}
0.5g Dihydrate barium chloride (BaCl\textsubscript{2}.H\textsubscript{2}O)

Preparation

1ml of conc. H\textsubscript{2}SO\textsubscript{4} was added to 90 ml of distilled water and mixed well for 1% v/v solution of H\textsubscript{2}SO\textsubscript{4}. 0.5g of Dihydrate barium chloride was dissolved in 50 ml of distilled water for 1% v/v solution of barium chloride. 0.6ml of the prepared barium chloride solution was then added to 99.4ml of the prepared 1% v/v of H\textsubscript{2}SO\textsubscript{4} solution. The solutions were thoroughly mixed and dispensed into tube.
APPENDIX II

Positive and negative biochemical tests

Positive indole test