PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF ASYMPTOMATIC BACTERIURIA IN HIV POSITIVE PATIENTS IN ILORIN, KWARA STATE, NIGERIA

BY

BADMUS, AJOKE RAHMAT

(17/27/MM1004)

A THESIS SUBMITTED TO MICROBIOLOGY UNIT, DEPARTMENT OF BIOSCIENCES AND BIOTECHNOLOGY, COLLEGE OF PURE AND APPLIED SCIENCES, KWARA STATE UNIVERSITY, MALETE,

NIGERIA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTERS OF SCIENCE (M.SC) DEGREE IN MICROBIOLOGY

SEPTEMBER, 2019.

CERTIFICATION

I certify that this research project was carried out by **AJOKE RAHMAT BADMUS** with matriculation number **17/27/MMI004** under my supervision and that it is a fair reflection of the student's input.

---------- ----------

Prof. O. Adedayo **Date**

(Supervisor)

---------- ----------

Dr. S. Awe **Date**

(Head of unit, Microbiology)

---------- ----------

Prof. S.K. Subair **Date**

(Dean of P.G. School)

---------- ----------

Prof. A.B. Olayemi **Date**

(External Examiner)

DEDICATION

This project is dedicated to Almighty Allaah, the most merciful for His endless mercy on me.

ACKNOWLEDGEMENTS

All praises and adoration is due to Almighty Allaah for bestowing me with beneficial knowledge.

My gratitude goes to my supervisor Prof. O. Adedayo for his encouragement and advice on how best to put this project together.

My utmost appreciation goes to my inestimable parents.

To my siblings, you are all appreciated.

My love to all my invaluable friends and colleagues.

To all I say JAZAKUMULLAHU KHAYRAN.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

This study investigated the prevalence and antimicrobial susceptibility patterns of bacterial isolates of urinary tract infections among HIV-positive patients. A cross-sectional study was conducted among 300 randomly selected HIV-positive patients within Ilorin metropolis, Kwara State from January to March 2019. Clean-catch midstream urine samples were collected aseptically and examined microbiologically using CLED agar and Blood agar and identified using morphological appearance, Gram reactions and Biochemical tests. Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion technique. The overall prevalence of urinary tract infection was 22.3%, Individuals within the age of 21–40 years and female gender had the highest percentage. *Staphylococcus aureus* 28 (41.8%), *Escherichia coli* 17 (25.4%) and *Klebsiella pneumoniae* 12 (17.9%), were the predominant causes of urinary tract infection and were the most common isolates. Multidrug resistance was observed in 40.8% of the isolates. *S. aureus* was resistant to Augmentin (64.3%), Ciprofloxacin (64.3%), Nalidixic acid (71.4%) and Ceftriaxone (53.6%); *E. coli* was resistant to Augmentin (70.6%), Ciprofloxacin (52.9%), Nalidixic acid (64.7%) and Ceftriaxone (53.6%); but *K. pneumoniae* was resistant to Ciprofloxacin (58.3%), Nalidixic acid (75.0%) and Augmentin (50.0%). The isolated extended spectrum beta-lactamase (ESBL) and Methicillin-Resistant *Staphylococcus aureus* (MRSA) were subjected to a second line of antibiotics. The isolation of high multiple drug resistant bacteria highlight the growing challenge of Urinary tract infections that may be problematic. Consequently, Health professionals may be advised to improve on the current empirical antimicrobial therapy for UTIs among HIV positive patients. Collective actions to help mitigate the spread of resistance are urgently needed not only in the study area but globally to curtail the transmission of diseases.

Keyword: CLED – Cystine lactose electrolyte deficient agar

CHAPTER ONE

INTRODUCTION

Globally, an estimated 34 million people are living with human immunodeficiency virus (HIV) with a high (1.9 million) number of newly infected people in Sub-Saharan Africa. Annually, an estimated 1.8 million people are dying of HIV/AIDS related diseases. In people living with HIV/AIDS, almost every part of the genitourinary system is affected with different diseases (Debalke *et al.,* 2014).

Human Immunodeficiency Virus (HIV) is a lentivirus that causes immunodeficiency syndrome, a condition in humans in which progressive failure of the immune system allows life threatening opportunistic infections to thrive (Kemajou *et al.,* 2016). Most untreated people infected with HIV-1 eventually develop AIDS (Miguelles and Connors, 2010). These individuals usually die of opportunistic infections or malignancies associated with progressive failure of the immune system. HIV infects vital cells in the human immune system such as helper T cells (specifically CD4+ T cells), macrophages and dendritic cells (Cunningham *et al.,* 2010), The infection leads to low levels of CD4+ T cells through three main mechanisms. First, direct viral killing of the infected cells; second, increased rate of apoptosis in infected cells; and third, killing of infected CD4+ cells by CD8 cytotoxic lymphocytes that recognize infected cells. When CD4+ cells number decline below a critical level (<200), cell mediated immunity is lost and the body becomes progressively more susceptible to opportunistic infections (Kemajou *et al.,* 2016). Urinary tract infection (UTI) is defined as the microbial invasion of any of the tissues of the urinary tract extending from the renal cortex to the urethra meatus. The two types of UTI are lower UTI which is an infection of the lower part of the urinary tract (the bladder and Urethra) and upper UTI which is an infection of the upper part of urinary tract (the kidneys and ureters).

The upper UTI is potentially more serious than the lower one because there is a possibility of kidney damage (Ani and Mgbechi, 2008).

Urinary tract infection (UTI) is one of the significant illnesses that causes burden, it is the most common nosocomial infection and an important source of morbidity as well (Deokar and Badhankar, 2009). In man, the urinary tract is the second commonest site, after the respiratory tract for bacterial infection (Ani *et al.,* 2015). The urinary tract is an anatomical unit and infection of one part could generally spread to other parts (Roberts *et al.,* 1999). When the infection is localized at such single sites as the kidneys, it is referred to as pyelonephritis; to the urethra as urethritis or restricted to the bladder as cystitis and to the prostate as prostitis. It affects both old and young leading to a number of deaths either from acute infection or from chronic renal failure (Katz, 2003).

Specific groups of people are at increased risk of urinary tract infection. Vulnerable populations are women, especially during pregnancy, infants and elderly patients (Ovalle and Levancini, 2001). Also certain conditions may increase susceptibility to infections i.e. spinal cord injuries, urinary catheters, diabetes, multiple sclerosis, immunodeficiency and underlying urologic abnormalities (Foxman, 2003). Urinary tract infection is one of the most common bacterial infection and cause of morbidity and hospitalization in HIV positive individuals (Iweriobor *et al.,* 2012).

HIV disease is associated with a variety of renal syndromes in patients with low CD4+ cell counts, causing neurologic complications which lead to urinary stasis and ultimately infection (Kemajou *et al.,* 2016). Once a patient's $CD4^+$ T cell count falls below 200 cells/mm^3, the individual is then at risk of a variety of opportunistic infections. The infectious organisms may include fungi, protozoa, viruses and bacteria. The most predominant causative organisms are

encapsulated bacteria notably: *Streptococcus pneumoniae* and *Haemophilus influenzae*, but non-typhoidal *Salmonella, Staphylococcus aureus* and *Pseudomonas aeruginosa* have also been implicated (Hidron *et al.,* 2010). Among opportunistic infections, UTI accounts for 60% of AIDS defining illnesses (Kemajou *et al.,* 2016).

Urinary Tract Infection is an infection of the urinary tract comprising kidney, ureters, bladder and urethra. UTIs are of global public health importance and contribute significantly to the cost of providing health care in both economically developed and underdeveloped countries. Urethritis, cystitis, haematuria and pyelonephritis are the infections of the urinary tract caused by bacteria such as *Staphylococcus saprophyticus*, *Schistosoma haematobium*, *Escherichia coli*, *Enterococcus* spp, *Proteus* spp, *Streptococcus* spp, *Klebsiella* spp and *Pseudomonas* spp (Cheesbrough, 2004).

Some of the key factors predisposing to urinary tract infection have been attributed to poor personal hygiene and urinary tract abnormalities. The causative agents for urinary tract infection vary from place to place and they also vary in their susceptibility and resistance patterns. UTIs are caused by different microbial pathogens. The most common pathogenic organisms of UTI are *Escherichia coli, Staphylococcus saprophyticus, S. aureus, Proteus sp., Klebsiella pneumoniae, Pseudomonas aeruginosa*, and enterococci. Treatment of UTI cases is often empirical and therapy is based on information determined from the antimicrobial resistance pattern of the urinary pathogens. In spite of the availability and use of the antimicrobial drugs, UTIs caused by bacteria have been showing increasing trends in recent years. Much of the increase has been related to emerging antibiotic resistance among urinary tract pathogens. Increasing multidrug resistance in bacterial uropathogens is an important and evolving public health challenge. The

prevalence of antimicrobial resistance in urinary pathogens is increasing worldwide (Ekwealor *et al*., 2016).

In the laboratory, bacterial infection of the urinary tract is said to exist when a significant number of bacteria, usually greater than 10^5 cells per milliliter of urine are detected in properly collected mid-stream "clean catch" urine or from catheter specimen urine.

Resistance of urinary tract pathogens to commonly prescribed antibiotics has increased worldwide. There are also reports of change in the resistance pattern over the last decade leading to serious therapeutic challenges. Since the distribution of these pathogens and their susceptibility to antibiotics varies regionally, and treatment for UTI is usually empirical, it is mandatory that there is an adequate knowledge of the epidemiological characteristics of the pathogens involved and their antibiotic susceptibility patterns. This will help to achieve good therapeutic outcomes and prevent the emergence of drug-resistant bacteria strains (Timothy *et al*., 2014).

1.1 Statement of Problems

UTI is an important clinical problem with people living with HIV but it is under investigated. Currently the main therapy for bacterial infections is the use of synthetic antibiotics. However, the misuse and over use of antibiotics lead to emergence of drug resistant strain (Olowe *et al.,* 2015). Resistance to commonly prescribed antibiotics for UTI is an expanding global problem both in developed as well as developing countries, due to the indiscriminate use of antibiotics (Rashmi *et al.,* 2013).These multidrug resistant (MDR) pathogens are relentlessly multiplying in HIV patients and thus, becoming an important circulating source of infections.

1.2 Justification of the Study

There are reports of change in the resistance pattern over the last decade leading to serious therapeutic challenges and the distribution of the urinary tracts pathogens and their susceptibility to antibiotics varies with underline conditions like HIV infections (Samuel *et al.,* 2012). Increasing multidrug resistance in bacterial uropathogens is an important and evolving public health challenge. Since UTI has large impact on the socio-economy and emergence of bacterial resistance, periodic surveillance of antibiotic susceptibility may be helpful (Rashmi *et al.,* 2013).

1.3 Significance of the Study

Treatment for UTI is usually empirical; it is mandatory that there is an adequate knowledge of the epidemiological characteristics of the pathogens involved and their antibiotic susceptibility patterns. This may help to achieve good therapeutic outcomes particularly in HIV patients and curtail the emergence and spread of drug-resistant bacteria strains.

1.4 Aim of the research

The aim of this work is to determine the prevalence and antibiotic susceptibility pattern of asymptomatic bacteriuria in HIV positive patients in Ilorin metropolis, Kwara State.

1.5 Specific Objectives

- To examine the urine samples of HIV positive patients macroscopically and microscopically
- To isolate, quantify and characterize bacteria from urine samples of HIV positive patients
- To determine the antibiotic susceptibility of the isolates from HIV positive patients

- To screen for extended spectrum beta-lactamase (ESBL) and subject the ESBL producers to a second line of antibiotics
- To screen for Methicillin-Resistant *Staphylococcus aureus* (MRSA) and subject the positive strains to a second line of antibiotics.

CHAPTER TWO

LITERATURE REVIEW

Urinary tract infections (UTIs) are a serious health problem affecting millions of people each year. Infections of the urinary tract are the second most common type of infection in the body. Catheterization of the urinary tract is the most common factor, which predisposes the host to these infections. Catheter associated UTI (CAUTI) is responsible for 40% of nosocomial infections, making it the most common cause of nosocomial infection. CAUTI accounts for more than 1 million cases in hospitals and nursing homes annually and often involve uropathogens other than *Escherichia coli*. While the epidemiology and pathogenic mechanisms of uropathogenic *Escherichia coli* have been extensively studied, little is known about the pathogenesis of UTIs caused by other organisms.

Urinary tract infections (UTIs) are one of the most common bacterial infections affecting humans throughout their life span (Chang and Shortliffe, 2006). UTIs account for more than 8 million visits to physician's offices, 1.5 million emmergency room visits, and 300,000 hospital admissions in the United States annually (Foxman, 2003). UTIs are the second most common infection of any organ system and the most common urological disease in the world (Litwin *et al.,* 2005).

These infections are more common in females than in men. Incidence in women in the age of 20—40 years ranges from 25 to 30% whereas in older women above 60 years of age it ranges from 4 to 43% (Jarvis and Martone, 1992). Urinary tract infections can be classified as uncomplicated or complicated (Mittal *et al.,* 2004). The recognized predisposing factors in complicated UTIs are anatomic defects, vesicouretic reflux (VUR), obstruction, surgery,

metabolic diseases like diabetes mellitus and generalized immunosuppression especially in patients of organ transplant (Bonadio *et al.,* 1999).

A complete urinalysis includes physical, chemical, and microscopic examinations. Midstream clean collection is acceptable in most situations, but the specimen should be examined within two hours of collection. Cloudy urine often is a result of precipitated phosphate crystals in alkaline urine, but pyuria also can be the cause. A strong odor may be the result of a concentrated specimen rather than a urinary tract infection. Dipstick urinalysis is convenient, but false-positive and false-negative results can occur. Specific gravity provides a reliable assessment of the patient's hydration status. Microhematuria has a range of causes, from benign to life threatening. Glomerular, renal, and urologic causes of microhematuria often can be differentiated by other elements of the urinalysis. Although transient proteinuria typically is a benign condition, persistent proteinuria requires further work-up. Uncomplicated urinary tract infections diagnosed by positive leukocyte esterase and nitrite tests can be treated without culture (Lifshitz and Kramer, 2000).

Urinalysis is invaluable in the diagnosis of urologic conditions such as calculi, urinary tract infection (UTI) and malignancy. It also can alert the physician to the presence of systemic disease affecting the kidneys. Although urinalysis is not recommended as a routine screening tool except in women who may be pregnant, physicians should know how to interpret urinalysis results correctly.

2.1 Methods of Performing Urinalysis

2.1.1 Specimen Collection

A midstream clean-catch technique usually is adequate in men and women. Although prior cleansing of the external genitalia often is recommended in women, it has no proven benefit. In fact, a study conducted by Lifshitz and Kramer (2000) found that contamination rates were similar in specimens obtained with and without prior cleansing (32 versus 29 percent). Urine must be refrigerated if it cannot be examined promptly; delays of more than two hours between collection and examination often cause unreliable results (Rabinovitch, 2001).

2.1.2 Physical Properties: Color and Odor

Foods, medications, metabolic products, and infection can cause abnormal urine colors (Hanno *et al.,* 2001). Cloudy urine often is a result of precipitated phosphate crystals in alkaline urine, but pyuria (urine containing white blood cells or pus) can also be the cause.

The normal odor of urine is described as urinoid; this odor can be strong in concentrated specimens but does not imply infection. Diabetic ketoacidosis can cause urine to have a fruity or sweet odor, and alkaline fermentation can cause an ammoniacal odor after prolonged bladder retention. Persons with UTIs often have urine with a pungent odor. Other causes of abnormal odors include gastrointestinal-bladder fistulas (associated with a fecal smell), cystine decomposition (associated with a sulfuric smell), and medications and diet (e.g., asparagus).

Table 2.1: Common Causes of Abnormal Urine Coloration

Color	Pathologic causes	Food and drug causes
Cloudy	Phosphaturia, pyuria, chyluria, lipiduria, hyperoxaluria	Diet high in purine-rich foods (hyperuricosuria)
Brown	Bile pigments, myoglobin	Levodopa (Larodopa), metronidazole (Flagyl), nitrofurantoin (Furadantin), some antimalarial agents
Brownish-black	Bile pigments, melanin, methemoglobin	Cascara, levodopa, methyldopa (Aldomet), senna
Green or blue	Pseudomonal UTI, biliverdin	Amitriptyline (Elavil), indigo carmine, IV cimetidine (Tagamet), IV promethazine (Phenergan), methylene blue, triamterene (Dyrenium)
Orange	Bile pigments	Phenothiazines, phenazopyridine (Pyridium)
Red	Hematuria, hemoglobinuria, myoglobinuria, porphyria	Beets, blackberries, rhubarb Phenolphthalein, rifampin (Rifadin)
Yellow	Concentrated urine	Carrots, Cascara

UTI = urinary tract infection; IV = intravenous.

2.1.3 Specific Gravity

Urinary specific gravity (USG) correlates with urine osmolality and gives important insight into the patient's hydration status. It also reflects the concentrating ability of the kidneys. Normal USG can range from 1.003 to 1.030; a value of less than 1.010 indicates relative hydration, and a value greater than 1.020 indicates relative dehydration (Kavouras, 2002). Increased USG is associated with glycosuria and the syndrome of inappropriate antidiuretic hormone; decreased USG is associated with diuretic use, diabetes insipidus, adrenal insufficiency, aldosteronism, and impaired renal function (Kavouras, 2002). In patients with intrinsic renal insufficiency, USG is fixed at 1.010—the specific gravity of the glomerular filtrate.

2.1.4 Urinary pH

Urinary pH can range from 4.5 to 8 but normally is slightly acidic (i.e., 5.5 to 6.5) because of metabolic activity. Ingestion of proteins and acidic fruits (e.g., cranberries) can cause acidic urine, and diets high in citrate can cause alkaline urine (Kiel and Moskowitz, 1987). Urinary pH generally reflects the serum pH, except in patients with renal tubular acidosis (RTA). The inability to acidify urine to a pH of less than 5.5 despite an overnight fast and administration of an acid load is the hallmark of renal tubular acidosis. In type I (distal) renal tubular acidosis, the serum is acidic but the urine is alkaline, secondary to an inability to secrete protons into the urine. Type II (proximal) RTA is characterized by an inability to reabsorb bicarbonate. This situation initially results in alkaline urine, but as the filtered load of bicarbonate decreases, the urine becomes more acidic.

Determination of urinary pH is useful in the diagnosis and management of UTIs and calculi. Alkaline urine in a patient with a UTI suggests the presence of a urea-splitting organism, which may be associated with magnesium-ammonium phosphate crystals and can form staghorn calculi. Uric acid calculi are associated with acidic urine.

2.1.5 Hematuria

According to the American Urological Association, the presence of three or more red blood cells (RBCs) per high-powered field (HPF) in two of three urine samples is the generally accepted definition of hematuria (Brendler, 1998). The dipstick test for blood detects the peroxidase activity of erythrocytes. However, myoglobin and hemoglobin also will catalyze this reaction, so a positive test result may indicate hematuria, myoglobinuria, or hemoglobinuria. Visualization of intact erythrocytes on microscopic examination of the urinary sediment can distinguish hematuria from other conditions. Microscopic examination also may detect RBC casts or dysmorphic RBCs. Hematuria is divided into glomerular, renal (i.e. nonglomerular), and urologic etiologies (Ahmed and Lee, 1997).

2.1.5.1 Glomerular Hematuria

Glomerular hematuria typically is associated with significant proteinuria, erythrocyte casts, and dysmorphic RBCs. However, 20 percent of patients with biopsy-proven glomerulonephritis present with hematuria alone (Fassetti *et al.,* 1982). IgA nephropathy (i.e. Berger's disease) is the most common cause of glomerular hematuria.

2.1.5.2 Renal (Nonglomerular) Hematuria

Nonglomerular hematuria is secondary to tubulointerstitial, renovascular, or metabolic disorders. Like glomerular hematuria, it often is associated with significant proteinuria; however, there are no associated dysmorphic RBCs or erythrocyte casts. Further evaluation of patients with glomerular and nonglomerular hematuria should include determination of renal function and 24-hour urinary protein or spot urinary protein-creatinine ratio.

2.1.5.3 Urologic Hematuria

Urologic causes of hematuria include tumors, calculi, and infections. Urologic hematuria is distinguished from other etiologies by the absence of proteinuria, dysmorphic RBCs, and erythrocyte casts. Even significant hematuria will not elevate the protein concentration to the 2+ to 3+ range on the dipstick test (Brendler, 1998). Up to 20 percent of patients with gross hematuria have urinary tract malignancy; a full work-up with cystoscopy and upper-tract imaging is indicated in patients with this condition (Sutton, 1990). In patients with asymptomatic microscopic hematuria (without proteinuria or pyuria), 5 to 22 percent have serious urologic disease and 0.5 to 5 percent have a genitourinary malignancy (Khan *et al.,* 2002).

Exercise-induced hematuria is a relatively common, benign condition that often is associated with long-distance running. Results of repeat urinalysis after 48 to 72 hours should be negative in patients with this condition (Siegel *et al.,* 1979).

2.1.6 Proteinuria

In healthy persons, the glomerular capillary wall is permeable only to substances with a molecular weight of less than 20,000 Daltons. Once filtered, low–molecular-weight proteins are reabsorbed and metabolized by the proximal tubule cells. Normal urinary proteins include albumin, serum globulins, and proteins secreted by the nephron. Proteinuria is defined as urinary protein excretion of more than 150 mg per day (10 to 20 mg per dL) and is the hallmark of renal disease. Micro albuminuria is defined as the excretion of 30 to 150 mg of protein per day and is a sign of early renal disease, particularly in diabetic patients.

The reagent on most dipstick tests is sensitive to albumin but may not detect low concentrations of γ-globulins and Bence Jones proteins. Dipstick tests for trace amounts of protein yield positive results at concentrations of 5 to 10 mg per dL—lower than the threshold for clinically significant proteinuria (Kiel and Monkowitz, 1987). A result of 1+ corresponds to approximately 30 mg of protein per dL and is considered positive; 2+ corresponds to 100 mg per dL, 3+ to 300 mg per dL, and 4+ to 1,000 mg per dL (House and Cattran, 2002). Dipstick urinalysis reliably can predict albuminuria with sensitivities and specificities of greater than 99 percent (Hanno *et al.,* 2001). Asymptomatic proteinuria is associated with significant renal disease in less than 1.5 percent of patients (Von-Bonsdorff, 1981).

Proteinuria can be classified as transient or persistent (Ahmed and Lee, 1997). In transient proteinuria, a temporary change in glomerular hemodynamics causes the protein excess; these conditions follow a benign, self-limited course (Springberg *et al.,* 1982) Orthostatic (postural) proteinuria is a benign condition that can result from prolonged standing; it is confirmed by obtaining a negative urinalysis result after eight hours of recumbency.

Persistent proteinuria is divided into three general categories: glomerular, tubular, and overflow. In glomerular proteinuria, the most common type, albumin is the primary urinary protein. Tubular proteinuria results when malfunctioning tubule cells no longer metabolize or reabsorb normally filtered protein. In this condition, low–molecular-weight proteins predominate over albumin and rarely exceed 2 g per day. In overflow proteinuria, low–molecular-weight proteins overwhelm the ability of the tubules to reabsorb filtered proteins.

Further evaluation of persistent proteinuria usually includes determination of 24-hour urinary protein excretion or spot urinary protein-creatinine ratio, microscopic examination of the urinary sediment, urinary protein electrophoresis, and assessment of renal function (Caroll and Temte, 2000).

2.1.7 Glycosuria

Glucose normally is filtered by the glomerulus, but it is almost completely reabsorbed in the proximal tubule. Glycosuria occurs when the filtered load of glucose exceeds the ability of the tubule to reabsorb it (i.e., 180 to 200 mg per dL). Etiologies include diabetes mellitus, Cushing's syndrome, liver and pancreatic disease and Fanconi's syndrome.

2.1.8 Ketonuria

Ketones, products of body fat metabolism, normally are not found in urine. Dipstick reagents detect acetic acid through a reaction with sodium nitroprusside or nitro-ferricyanide and glycine. Ketonuria most commonly is associated with uncontrolled diabetes, but it also can occur during pregnancy, carbohydrate-free diets, and starvation.

2.1.9 Nitrites

Nitrites normally are not found in urine but result when bacteria reduce urinary nitrates to nitrites. Many gram-negative and some gram-positive organisms are capable of this conversion, and a positive dipstick nitrite test indicates that these organisms are present in significant numbers (i.e., more than 10,000 per mL). This test is specific but not highly sensitive. Thus, a positive result is helpful, but a negative result does not rule out UTI (Kavouras, 2002). The nitrite dipstick reagent is sensitive to air exposure, so containers should be closed immediately after removing a strip. After one week of exposure, one third of strips give false-positive results, and after two weeks, three fourths give false-positive results (Gallagher *et al.,* 1990). Non-nitrate–reducing organisms also may cause false-negative results, and patients who consume a low-nitrate diet may have false-negative results.

2.1.10 Leukocyte Esterase

Leukocyte esterase is produced by neutrophils and may signal pyuria associated with UTI. To detect significant pyuria accurately, 30 seconds to two minutes should be allowed for the dipstick reagent strip to change color, depending in the brand used. Leukocyte casts in the urinary sediment can help localize the area of inflammation to the kidney.

Organisms such as Chlamydia and Ureaplasma urealyticum should be considered in patients with pyuria and negative cultures. Other causes of sterile pyuria include balanitis, urethritis, tuberculosis, bladder tumors, viral infections, nephrolithiasis, foreign bodies, exercise, glomerulonephritis, and corticosteroid and cyclophosphamide (Cytoxan) use.

2.1.11 Bilirubin and Urobilinogen

Urine normally does not contain detectable amounts of bilirubin. Unconjugated bilirubin is water insoluble and cannot pass through the glomerulus; conjugated bilirubin is water soluble and indicates further evaluation for liver dysfunction and biliary obstruction when it is detected in the urine.

Normal urine contains only small amounts of urobilinogen, the end product of conjugated bilirubin after it has passed through the bile ducts and been metabolized in the intestine. Urobilinogen is reabsorbed into the portal circulation, and a small amount eventually is filtered by the glomerulus. Hemolysis and hepatocellular disease can elevate urobilinogen levels, and antibiotic use and bile duct obstruction can decrease urobilinogen levels.

2.1.12 Microscopic Urinalysis

Microscopic examination is an indispensable part of urinalysis; the identification of casts, cells, crystals, and bacteria aids in the diagnosis of a variety of conditions. To prepare a urine specimen for microscopic analysis, a fresh sample of 10 to 15 mL of urine should be centrifuged at 1,500 to 3,000 rpm for five minutes. The supernatant then is decanted and the sediment resuspended in the remaining liquid (Fogazzi and Garigdi, 2001). A single drop is transferred to a clean glass slide, and a cover slip is applied and then viewed under x10 and x40 objective lens.

2.1.13 Bacteriuria

Under high-powered magnification, gram-negative rods, streptococci, and staphylococci can be distinguished by their characteristic appearance.

Gram staining can help guide antibiotic therapy, but it is not indicated in routine outpatient practice. Clean-catch specimens from female patients frequently are contaminated by vaginal flora. In these patients, five bacteria per HPF represents roughly 100,000 colony-forming units (CFU) per mL, the classic diagnostic criterion for asymptomatic bacteriuria and certainly compatible with a UTI. In symptomatic patients, a colony count as low as 100 CFU per mL suggests UTI, and antibiotics should be considered. The presence of bacteria in a properly collected male urine specimen is suggestive of infection, and a culture should be obtained.

2.1.14 Cells

Leukocytes may be seen under low- and high-power magnification. Men normally have fewer than two white blood cells (WBCs) per high-power field (HPF); women normally have fewer than five WBCs per high-power field.

Epithelial cells often are present in the urinary sediment. Squamous epithelial cells are large and irregularly shaped, with a small nucleus and fine granular cytoplasm; their presence suggests contamination. The presence of transitional epithelial cells is normal. These cells are smaller and rounder than squamous cells, and they have larger nuclei. The presence of renal tubule cells indicates significant renal pathology. Erythrocytes are best visualized under high-power magnification. Dysmorphic erythrocytes, which have odd shapes because of their passage through an abnormal glomerulus, suggest glomerular disease.

2.1.15 Casts

Casts in the urinary sediment may be used to localize disease to a specific location in the genitourinary tract (Kavouras, 2002). Casts, which are a coagulum of Tamm-Horsfall

mucoprotein and the trapped contents of tubule lumen, originate from the distal convoluted tubule or collecting duct during periods of urinary concentration or stasis, or when urinary pH is very low. Their cylindrical shape reflects the tubule in which they were formed and is retained when the casts are washed away. The predominant cellular elements determine the type of cast: hyaline, erythrocyte, leukocyte, epithelial, granular, waxy, fatty, or broad.

2.1.16 Crystals

Crystals may be seen in the urinary sediment of healthy patients. Calcium oxalate crystals have a refractile square "envelope" shape that can vary in size. Uric acid crystals are yellow to orange-brown and may be diamond- or barrel-shaped. Triple phosphate crystals may be normal but often are associated with alkaline urine and UTI (typically associated with Proteus species). These crystals are colorless and have a characteristic "coffin lid" appearance. Cystine crystals are colorless, have a hexagonal shape, and are present in acidic urine, which is diagnostic of cystinuria.

2.2 Uncomplicated UTI

Uncomplicated urinary tract infection (UTI) is most common in young, sexually active, nonpregnant, premenopausal women. Gram-negative bacteria are isolated from 75 to 95% of these infections (Hooton, 2012). The remaining proportions of uncomplicated UTI are associated with a variety of organisms, including the Gram-positive bacteria *Staphylococcus saprophyticus, Enterococcus faecalis, Streptococcus agalactiae* (group B Streptococcus), and other less frequently isolated organisms. In demographic groups such as in pregnant women and the elderly, Gram-positive bacteria are found more often as etiologic agents of UTI. Symptoms associated with uncomplicated UTI caused by Gram-positive uropathogens are similar to those

caused by Gram-negative organisms and usually include dysuria, urinary frequency, urinary urgency, and/or suprapubic pain. Fever, chills, costovertebral-angle tenderness, flank pain, and/or nausea are suggestive of upper urinary tract (kidney) involvement.

2.3 Point of care diagnosis of UTI

While the gold standard for UTI diagnosis is bacterial culture of the urine, dipstick urinalysis is commonly used in point-of-care diagnosis. In some clinical settings such as with infants, leukocyte esterase (LE) and pyuria (by dipstick analysis) have a very high sensitivity and specificity for UTI (>90% as defined by the culture of uropathogen from urine with >100,000 colony forming units (CFU) per ml) (Schroeder *et al.,* 2015). However, in contexts such as pregnancy, dipstick analysis using LE, pyuria, or presence of nitrites is less reliable as an indication of UTI per the microbiological definition of 105 CFU/ml cut off (Demilie *et al.,* 2014). While dipstick urinalysis that is positive for LE and/or nitrites in a clean-catch urine sample is consistent with a UTI diagnosis, these tests can miss UTIs that meet the gold standard of bacteriuria diagnosis in relation to adverse outcomes in pregnancy (e.g. the microbiological 105 CFU/ml definition). One likely explanation is that nitrite tests are likely to be negative if the infecting organism does not reduce nitrate, as is the case for most Gram-positive uropathogens including *S. saprophyticus*, *enterococci*, and group B *Streptococcus* (Mehnert-kay, 2005). Given the higher prevalence of Gram-positive bacteria as causes of UTI in certain populations such as the elderly, it is perhaps not surprising that some studies conclude that LE and nitrite are inadequate for UTI screening in this setting (Arinzon *et al.,* 2009). In short, while dipstick urinalyses can help to quickly identify UTI caused by Gram-negative bacteria, they are less useful for infections involving Gram-positive uropathogens and perform poorly in ruling out these infections with certainty.

2.4 Complicated UTI

Complicated UTI is defined as cystitis or pyelonephritis that occurs in individuals with predisposing anatomic, metabolic, or functional risk factors that make UTI more difficult to treat. Complicated UTIs often occur in nosocomial and/or institutional settings, particularly in individuals with structural or functional alterations of the urinary tract (often associated with urinary catheterization), or other underlying renal, metabolic, or immunological disorders (Wagenlehner and Naber, 2012); these populations are at greater risk of Gram-positive and polymicrobial UTI (Edward and Baker, 2005). Another less frequently recognized anatomic risk factor for UTI is Female Genital Cutting (FGM). A recent meta-analysis of five comparative studies showed that women who had experienced FGM were at 3-times higher risk of UTI compared to those who were uncut (Berg *et al.,* 2014).

2.5 Catheter associated UTI

Catheter-associated UTI (CAUTI) account for 40% of all nosocomial infections (Maki and Tambyah, 2001) and are the most common complication of indwelling urinary catheters (Jain *et al.,* 2005). Catheter-associated bacteria are thought to be derived largely from the patient's own gut microbiota (Warren, 1997). Bacteriuria occurs in 3–10% of patients following urinary catheterization (Stensballe, 2007). Catheter-associated bacteriuria is often asymptomatic (Tambyah and Maki, 2000) and there is no good way to distinguish between pathogenic CAUTI and asymptomatic bacteriuria (ASB); even the presence of neutrophils in the urine (pyuria), which are a strong identifier of uncomplicated UTI, is not a good diagnostic indicator of CAUTI (Maki and Tambyah, 2001). Catheter-associated bacteria are largely in a biofilm state and are thus recalcitrant to antibiotic treatment (Maki and Tambyah, 2001). However, if left untreated,

these infections can lead to severe complications such as acute pyelonephritis, bacteremia, urosepsis, and death (Maki and Tambyah, 2001). While the well-adapted uropathogenic E. coli cause the majority of non-catheter-associated UTI in the community, the diversity of species associated with CAUTI is greater. For example, enterococci are rarely associated with community-acquired UTI but play a prominent role in the pathogenesis of CAUTI and are among the predominant pathogens isolated from polymicrobial communities on the surface of indwelling urinary catheters and biliary stents (Dedeic-Ljubovic and Hukic, 2009).

2.6 Laboratory models to study Gram-Positive UTI

Model systems to recapitulate and study infection by Gram-positive uropathogens have been adapted from those used to study UTI caused by Gram-negative bacteria. Transurethral inoculation with 1.5 ml of *S. saprophtyicus* at 1×109 colony forming units (CFU)/ml into the bladders of female albino wistar rats showed that at 7 days post infection, both bladders and kidneys were colonized at similar levels, leukocytes were present in the urine, and bladder inflammation and epithelial damage noted (Gatemann *et al.,* 2007). Subsequent studies in which 50µl of *S. saprophyticus* at 5×107 CFU/ml was transurethrally inoculated into 7–8 week old C3H/HeN female mice showed significantly higher colony forming unit in the kidney compared to the bladder at 6 hours post infection and 2, 7, and 14 days post infection (Kline *et al.,* 2010). Bacteria persistence in the kidneys was observed in C3H/HeN mice but not in C57BL/6 mice, indicating that host factors contribute to the ability of *S. saprophyticus* to cause UTI. Under the same infection conditions, group B Streptococcus showed similar kidney tropism at 1, 7, and 14 days post infection (Kline *et al.,* 2011). Similarly, *E. faecalis* preferentially infects the kidneys of C57Bl/6, outbred Harlan Sprague Dawley, and BALB/c female mice; however, these models

require a 200µl inoculum volume to consistently establish infection (Kau *et al.,* 2011). Since *E. faecalis* is more commonly associated with CAUTI than ascending UTI, foreign body-associated UTI models have been developed in mice and rats to mimic the conditions of patients with in dwelling urinary catheters (Kim *et al.,* 2015). In the murine model, catheter material is inserted transurethrally into the murine bladder prior to bacterial inoculation, where it remains throughout the course of infection. *E. faecalis* establishes a robust infection in the catheter-containing bladder, in the kidneys, and on the catheter material itself where it forms a biofilm that facilitates persistent infection in the face of robust catheter-driven inflammation (Guiton *et al.,* 2013). The CAUTI murine model has recently been used to test the efficacy of novel UTI therapeutics and will continue to be useful in the search for antimicrobial agents aimed at preventing or dispersing Gram-positive biofilms that arise in catheterized individuals (Guiton *et al.,* 2012).

2.7 Epidemiology and animal models for polymicrobial UTI

The presence of multiple recognized uropathogens in midstream urine at titers >100,000 CFU/ml is consistent with a polymicrobial etiology of UTI. Polymicrobial infections occur most often among the elderly, immune compromised, and those with in dwelling catheters, HIV, malignancy, and diabetes. Polymicrobial UTI is less common among young sexually active women. Since the highly polymicrobial microbiota of the gastrointestinal and reproductive tracts are thought to be a major inoculation source leading to UTI, and since truly dual species or polymicrobial UTI do arise, several investigators have sought to examine the consequence of mixed microbial inoculation into the urinary tracts of model organisms. In a rat model, transurethral inoculation of *Staphylococcus saprophyticus or Proteus mirabilis* resulted in ascending pyelonephritis significantly more often when the two organisms were inoculated

together compared to single species infection, suggesting a synergistic virulence between the two species (Hjelm *et al.,* 1989). *P. mirabilis* also synergizes with uropathogenic *Escherichia coli* (UPEC) in the murine urinary tract, such that co-infection gave rise to greater CFU for both P. mirabilis and uropathogenic *Escherichia coli*, compared to either single species infection. The use of complementary, rather than competing, central metabolism pathways in the urinary tract by UPEC and *P. mirabilis* may limit competition and thus promote synergy between these two organisms (Alteri *et al.,* 2015). Co-infection with the urease-positive Gram-negative organisms *P. mirabilis* and *Providencia stuartii* give rise to an increased incidence of urinary stones (urolithiasis) and bacteremia in a murine model of ascending UTI compared to monomicrobial infection (Armbrouster *et al.,* 2014), which may help explain why these organisms commonly co-occur in the urine of individuals with indwelling urinary catheters (Kunin, 2009). Similar studies in mice showed that *Pseudomonas aeruginosa and E. faecalis* co-infection resulted in a more rapid development of pyelonephritis than observed when each species was inoculated alone (Tsuchimori *et al.,* 1994). Moreover, co-infection studies with group B Streptococcus and UPEC in a murine UTI model have demonstrated that the presence of group B Streptococcus can modulate host immunity and alter host susceptibility to persistent high titer infection of the bladder and kidneys by UPEC (Kline *et al.,* 2012). Aged multiparous animals were particularly prone to UPEC infection in the context of group B Streptococcus, demonstrating ~1000-fold higher titer UPEC infection in the presence of GBS compared to age-matched nulliparous controls (Kline *et al.,* 2014). Microscopic and microbial culture examinations, as well as culture-independent DNA sequence analysis of bacterial biofilms found on urinary catheters, show that CAUTI biofilms are often polymicrobial in nature (Barford *et al.,* 2008). By analogy to mixed

species effects in ascending UTI, the nature of the mixed microbial community in CAUTI may also influence the spectrum or severity of sequelae.

Catheterization of urinary tract is one of the most common factors which predisposes the host to complicated UTIs (Bass *et al.,* 2003). Instillation of catheter may lead to damage of mucosal layer, which disrupts the natural barrier and allows bacterial colonization (Kalsi *et al.,* 2003). Organisms can gain entry via extraluminal route by moving across the outer lumen of catheter or by intraluminal route by directly entering the interior of catheter (Dickinson and Bisno, 2007). The organisms most commonly responsible for catheter-associated UTIs are *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Streptococcus faecalis* (Jarvis and Martone, 1992). In case of *E. coli*, the epidemiological, experimental and clinical studies have established the role of multiple virulence factors of *E. coli* like adhesions operative through type-I fimbriae and P fimbriae, O serotypes, K1 capsule, serum resistance, hemolysins, cytotoxic nectrotizing factor (CNF) and siderophores (enterochelin and aerobactin) in relation to uncomplicated and complicated UTIs (Johnson *et al*., 1992).

However, there is paucity of literature in relation to pathogenesis of UTIs caused by Bacteria, Despite advances in antimicrobial therapy, the mortality and morbidity associated Bacteria infections induced UTIs remain significantly high. This unfavorable outcome is due to inability to develop therapeutic strategies to prevent the disease which in turn is due to incomplete understanding about the pathogenesis of the disease.

The potential biological and clinical significance of polymicrobial growth in urine depends on many factors. On one hand, the finding of multiple colony types after urine culture is a valid and justifiable concern to suspect contamination of the urine specimen with periurethral and/or vaginal microbiota. For this reason, most clinical microbiology labs will not evaluate plates with

polymicrobial growth, but rather dismiss them as "contamination" and request another specimen. For instance, in suspected cases of uncomplicated cystitis, *E. faecalis* and group B Streptococcus are often assumed to represent contamination of the urine specimen originating from the periuethral area during collection (Hooton, 2012). In fact, one recent study evaluated titers of these Gram-positives in midstream urine and compared to titers obtained when the same women were subjected to catheterization for urine collection. This study concludes that *Enterococci* (in 10% of cultures) and group B streptococci (in 12% of cultures) were not predictive of bladder bacteriuria at any colony count (Spearman's r=0.322 for enterococci and 0.272 for group B streptococci)." However, only a few patients in this study had Enterococcus or GBS at levels considered significant for a UTI diagnosis (>100,000cfu/ml). Further studies are needed to define:

1) The relationship between Gram-positive bacteria in midstream and catheter-collected urine when patients meet the threshold for UTI (105 CFU/ml)

2) The likelihood of cystitis when these organisms are detected in pure culture versus in the context of multiple other colony types and

3) Whether these relationships are similar across all patient groups. Caution against dismissing Gram-positive pathogens as unimportant was taken. For example, while *S. saprophyticus* is now established as the predominant Gram-positive uropathogen, it was originally considered to be a urinary contaminant (Raz *et al.,* 2005).

There are multiple cited cases of polymicrobial bloodstream infection with identical organisms present in both urine and blood cultures (Siegma-Igra *et al.,* 1993). In these cases, polymicrobial bloodstream infection supports the interpretation of polymicrobial UTI, especially when multiple of the organisms identified in the blood are also identified in urine. Moreover, since cases of

disseminated polymicrobial infection occur most often in compromised persons with underlying risk factors for UTI, the organisms involved are more likely to be members of the 'normal' microbiota that may be overlooked in urine specimens as nonpathogens. In another related case, a woman received a suprapubic catheter following urogynecological surgery and later developed an abscess near the location of bladder catheter insertion. Although hospital microbiology lab returned the result of this urine culture as "contaminated," identification of bacteria from the anaerobically cultured surgically drained pus demonstrated a polymicrobial infection with *E. coli, G. vaginalis, and Peptostreptococcus productus* (Josephson *et al.,* 1988).

It has been estimated that up to 20% of women presenting with classic symptoms of UTI have culture-negative urine (Ferry *et al.,* 2007). In the cited example "culture-negative" included samples without a single dominant species (i.e. polymicrobial growth), samples with "secondary pathogens" at titers <104/ml urine, or "doubtful pathogens" at titers <105 CFU/ml urine. Unfortunately, the term sterile is often applied incorrectly to these "culture-negative" contexts. For example, Domann *et al*., (2003) found that 9.2% of urine specimens collected from renal transplant recipients did not produce significant growth under aerobic conditions, but had evidence of intact bacterial rods and/or cocci that were identified as fastidious anaerobes using culture-independent molecular approaches (Domann *et al.,* 2003). Alternatively, what might appear to be a monomicrobial infection when observed by aerobic culture may actually be a polymicrobial infection when characterized by culture-independent techniques. For example, one recent study detailed a case study where a woman had what appeared to be a typical culture-positive UTI with >105 CFU/ml of monomicrobial *E. coli* according to aerobic clinical lab results. The culture-independent approach performed in parallel revealed that urine specimens collected by catheterization and suprapubic aspiration from this patient contained Actinobaculum

and Aerococcus at levels that far exceeded *E. coli* (Wolfe *et al.,* 2012). Other studies that have examined the incidence and significance of Actinobaculum and Aerococcus in urine specimens have shown that up to 90% and 69% of the time, respectively, significant titers of these organisms were found alongside significant titers of typical uropathogens such as *E. coli* (Sierra-Hoffman *et al.,* 2005).

Another study was performed on anaerobic culture for fastidious anaerobes in urine of women who suffer from recurrent chronic episodes of cystitis and compared these results to similar aged groups of young women without UTI (Naboka *et al.,* 2011). The study reports that women with recurrent cystitis were more likely to contain higher titers and a more diverse repertoire of mixed anaerobes in their urine than healthy women without UTI. In fact, this study had two healthy control groups: one with women who reported regular sexual activity and another with women who reported no history of sexual intercourse. Results of the study strongly suggest that sexual activity does not provide a simple explanation for why women with recurrent chronic cystitis have fastidious bacteria in their urine. Taken together, these findings suggest that fastidious organisms and species assumed to represent contamination of urine specimens in urine cultures are routinely overlooked. We argue that additional studies specifically evaluating contested potential uropathogens in specific patient settings such as those who suffer from recurrent UTI, those at risk for complicated UTI, and those with other urologic problems of uncertain etiology (e.g. interstitial cystitis, urinary urgency incontinence, preeclampsia etc.) are still needed (Hooton, 2012).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study design and study population

This study was carried out in Ilorin, the capital of Kwara State, Nigeria from January to March 2019. Samples were collected from HIV positive patients attending routine clinic at Sobi Specialist Hospital and Civil Service Hospital in Ilorin. The research was carried out at the Department of Microbiology, University of Ilorin Teaching Hospital located at Oke Oyi in Ilorin.

A total of 300 HIV positive individuals both male and female receiving HIV care were selected in this study.

3.2 Ethical consideration

Ethical approval was sought and obtained from the Ethics Review Committee of the Ministry of Health, Ilorin with number MOH/KS/EU/777/252. Informed consent was also sought and obtained verbally from the patient before inclusion in the study. The nature and advantage of the research was also explained to each patient.

3.3 Sample collection

Appropriately labeled universal bottles with sex, age and name were given to each participant for the collection of clean catch midstream urine specimen following explanation of the procedure for collection. The samples were immediately transported to the Department of Microbiology, University of Ilorin Teaching Hospital located at Oke Oyi in Ilorin for processing.

3.4 Preparation of culture media

Cystine lactose electrolyte deficient (CLED) agar, Blood agar and Mueller-hinton agar all manufactured by Oxoid were the culture media used for isolation and enumeration of bacteria.

The preparation of each medium was done by strictly adhering to the manufacturer's instructions.

3.4.1 Cystine lactose electrolyte deficient (CLED) agar

Suspended 36.2grams in 1 litre of distilled water was boiled to dissolve the medium completely. It was sterilized by autoclaving at 121°C for 15 minutes. It was thoroughly mixed before pouring.

3.4.2 Blood agar

Suspended 40grams in 1 litre of distilled water was boiled to dissolve the medium completely. It was sterilized by autoclaving at 121°C for 15 minutes. It was thoroughly mixed before pouring.

3.4.3 Mueller-hinton agar

Suspended 38grams to 1 litre of distilled water was boiled to dissolve the medium completely. It was sterilized by autoclaving at 121°C for 15 minutes. It was thoroughly mixed before pouring

3.5 Microbiological analysis

3.5.1 Macroscopic examination

The urine samples were evaluated by its physical appearances (colour, cloudiness, clarity).

3.5.2 Urine culture

Each urine sample was shaken to allow for homogeneity, using a standard sterile wire loop. A loopful of the urine was aseptically and uniformly inoculated unto plates of Blood agar and Cystine lactose electrolyte deficient (CLED) agar. CLED agar is a differential culture medium used in isolating and enumerating bacteria in urine from the suspected cases of Urinary Tract Infection and it supports the growth of all potential urinary pathogens, and a number of contaminants such as diphtheroids, lactobacilli, and micrococci (Tankeshwar, 2015).

The plates were labelled and incubated aerobically at 37°C for 24 hours. The plates were afterwards examined macroscopically and microscopically for bacterial growth (Cheesbrough, 2002).

3.5.3 Centrifuging and microscopic examination of urine

Each urine sample was well mixed and transferred into a conical centrifuge tube, arranged and balanced in a centrifuge machine and spun for 5 minutes at 3200 rpm. After, the supernatant was decanted and a drop of the sediment was placed on a sterile glass slide and covered with a cover slip. This was viewed under x10 and x40 objective lens for the presence of white blood cells, red blood cells, schistosoma haematobium, casts, crystals, epithelial cells.

3.5.4 Identification and significance of urine culture

After incubation of the CLED and blood agar for 24 hours, the plates were carefully examined for morphological characteristics of the colonies of the pure culture growing on the media with reference to their sizes, pattern of their edge, margin, surface texture elevation, consistency and colour (Cheesbrough, 2006).

Asymptomatic bacteriuria (ASB) is said to be positive in urine culture with the presence of a significant quantity of bacteria, of $\geq 10^5$ colony-forming units per milliliter (CFUs/ml) (Banu and Jyothi, 2013). Positive urine culture was further subjected to gram staining and biochemical tests for identification of the bacteria according to Fawole and Oso (2004).

3.6 Antimicrobial susceptibility testing

Using the modified Kirby-Bauer disc diffusion test method, CLSI (2009) a pure culture plate of one of the organisms to be tested was selected. A colony from the plate was emulsified aseptically in a saline solution, thoroughly mixed to ensure that no solid material from the colony is visible in the saline solution. This was repeated until the turbidity of the saline solution

visually matches that of the turbidity of McFarland standard. A 0.5 McFarland standard is prepared by mixing 0.05 mL of 1.175% barium chloride dihydrate ($BaCl_2 \bullet 2H_2O$), with 9.95 mL of 1% (H_2SO_4) sulfuric acid.

A sterile swab stick was dipped into the broth culture of organism and gently squeezed against the inside of the tube in order to remove excess fluid in the swab. Using the swab, the sterile gelled Mueller-Hinton agar plate was streaked to form a bacterial lawn, to obtain uniform growth; the plate was streaked with the swab in one direction, rotate the plate 90° and streaked the plate again in that direction again.

After the plate was left to dry for about 5 minutes, a flame-sterilized forceps was used to place and press the appropriate antimicrobial-impregnated disks on the surface of the agar. The following antibiotic discs (Oxoid, UK) with potencies were used: Augmentin (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), nitrofurantoin (100 µg), imipenem (10 µg), cefoxitin (30 µg), vancomycin (30 µg) and clindamycin (2 µg). the inoculated plates was carefully inverted and incubated for 18 hours at 37° C, After incubation, a metric ruler was used to measure the diameter of the zone of inhibition for each antibiotic used and interpreted by the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2017).

3.6.1 Screening for extended-spectrum beta-lactamase (ESBL) of the isolates

All Gram-negative isolates were subjected to screening tests using ceftazidime (30 µg) and ceftriaxone (30 µg) discs. Those isolates with ceftazidime zone <22 mm and ceftriaxone zone <25 mm were then subjected to confirmatory tests (CLSI, 2017).

3.6.2 Double-disk synergy test

The double-disc synergy test as described by CLSI (2017) was used to confirm ESBL production. Plates were inoculated for routine drug susceptibility using the modified Kirby-

Bauer standardized disc diffusion method (CLSI, 2009). Ceftazidime (30 µg) and ceftriaxone (30 µg) discs were placed on either side of amoxycillin/clavulanic acid (20 + 10 µg) 15 mm apart. ESBL-positive strains showed an expansion of the zone of inhibition of either cephalosporin toward the amoxycillin/clavulanate giving a dumbbell shape (Bradford, 2001). This expansion occurred because the clavulanic acid present in the Augmentin disc inactivated the ESBL produced by the test organism. The ESBL producers were subjected to a second line of antibiotics of nitrofuratoin and imipenem, the results were recorded as either sensitive or resistant according to CLSI (2017).

3.6.3 Screening for Methicillin-Resistant *Staphylococcus aureus* (MRSA)

Plates were inoculated with *Staphylococcus aureus* for routine drug susceptibility using the modified Kirby-Bauer standardized disc diffusion method.

Cefoxitin 30-µg disks were applied and the plates were incubated at 37°C for 24 hours. An isolate was considered to be an MRSA strain if the cefoxitin inhibition zone diameter was ≤ 21 mm (Mougeot *et al*., 2001). The MRSA positive strains were subjected to a second line of antibiotics of vancomycin and clindamycin; the results were recorded as either sensitive or resistant according to CLSI (2017).

3.7 Statistical Data Analysis

All data were entered onto the Statistical Package for the Social Sciences (SPSS) version 20 software which was also used for analysis. Descriptive statistics were used for initial data exploration and the level of significance was set at 95%.

CHAPTER FOUR

RESULTS

Table 4.1 Shows the age and gender distribution of the participants.

Out of the 300 participants, 0-20 age groups represents 3.0%, 21-40 age groups represents 50.0%, 41-60 age groups represents 42.0%, 61-80 represents 5.0%. Two hundred and sixty-two (87.3%) females and 38 (12.7%) males were engaged in the study.

Table 4.1: Age and Gender Distribution of the Participants

Age group(years)	Male (%)	Female (%)	Total (%)
0-20	2 (0.7)	7 (2.3)	9 (3.0)
21-40	11 (3.7)	139 (46.4)	150 (50.0)
41-60	20 (6.7)	106 (35.4)	126 (42.0)
61-80	5 (1.7)	10 (3.3)	15 (5.0)
Total	**38 (12.7)**	**262 (87.3)**	**300 (100)**

Table 4.2 shows the significant bacteriuria distribution in respect to age and gender distribution. Out of 300 samples, 67 (22.3%) were positive to bacteria cultures and 233 (77.7%) were negative. The predominant age groups with the highest number of positive bacteriuria were within 21-40 years (10.7%) while the lowest was 0-20 years (0.6%). Six (2.0%) and 61 (20.3%) were the frequencies of bacteriuria for males and females respondents respectively.

Table 4.2: Significant Bacteriuria Distribution against Different Characteristics

Characteristics	Significant bacteriuria			
	Yes (%)	**No (%)**	**Total (%)**	**P-Value**
Age (years)				**0.02**
0-20	2 (0.6)	7 (2.3)	9 (3.0)	
21-40	32 (10.7)	118 (39.3)	150 (50.0)	
41-60	29 (9.7)	97 (32.3)	126 (42.0)	
61-80	4 (1.3)	11 (3.7)	15 (5.0)	
Total	**67 (22.3)**	**233 (77.7)**	**300 (100)**	
Sex				**0.06**
Male	6 (2.0)	32 (10.7)	38 (12.7)	
Female	61 (20.3)	201 (67.0)	262 (87.3)	
Total	**67 (22.3)**	**233 (77.7)**	**300 (100)**	

Table 4.3 shows the significance of the urine culture, Out of the 300 samples, 51 (17%) samples presented mixed growth, 125 (41.7%) samples presented no growth, 57 (19%) samples presented no significant growth and 67 (22.3%) presented significant bacteria growth.

Table 4.3: Significance of the urine culture

Culture Characteristics	Frequency (n=300)	Frequency Percentage (%)
Mixed growth	51	17
No growth	125	41.7
No significant growth	57	19
Significant growth	67	22.3
Positive growth	**67**	**22.3**
Negative growth	**233**	**77.7**

Figure 4.1 shows the frequency of distribution of the isolated bacteria; *Stapylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca, Pseudomonas aeruginosa* and Coagulase Negative *Staphyloccoci* had frequency of occurrence of 28 (41.8%), 17 (25.4%), 12 (17.9%), 5 (7.5%), 3 (4.5%) and 2 (2.9%) respectively.

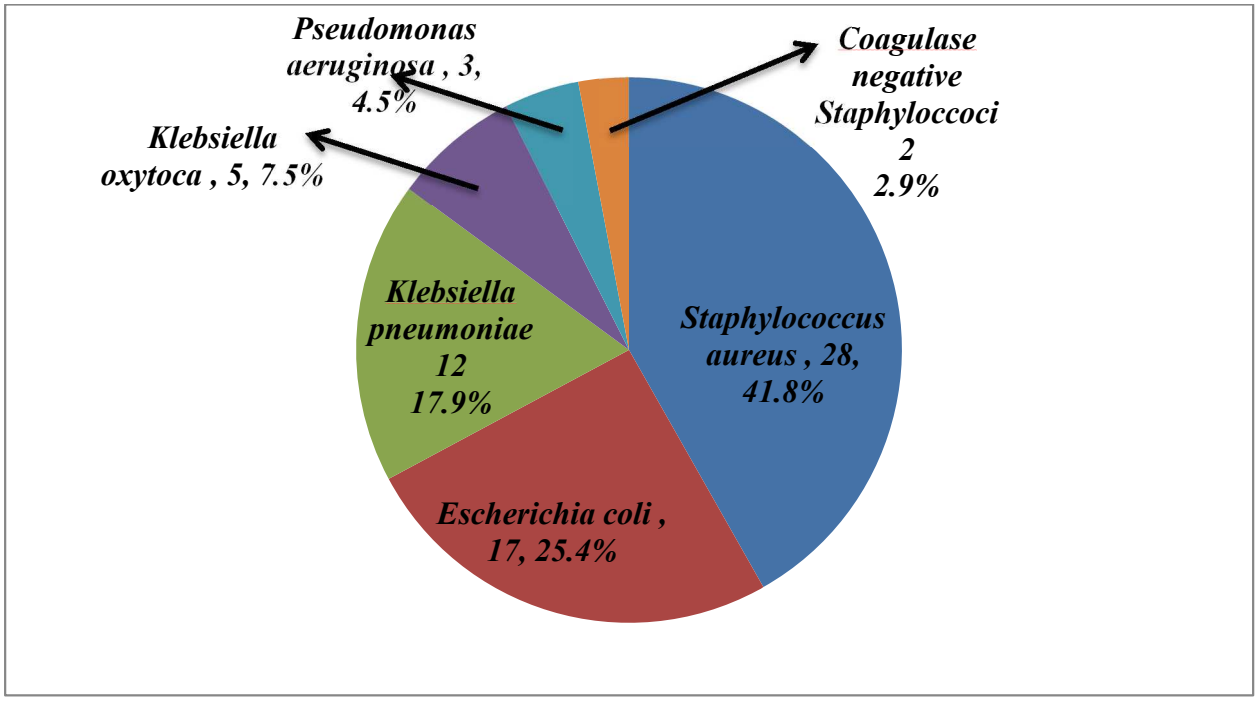

Figure 4.1: Frequency Distribution of Isolated Bacteria (n=67)

Table 4.4 shows the antibiotic susceptibility testing of the isolated bacteria against different standard antibiotics AMC (augmentin), CN (gentamicin), CIP (ciprofloxacin), NA (nalidixic acid), CAZ (ceftazidime), CRO (ceftriaxone), F (nitrofurantoin), IPM (imipenem) and FOX (cefoxitin), the zones of inhibitions were measured and compared with CLSI standard table.

S. aureus were resistant to augmentin (64.3%), ciprofloxacin (64.3%), nalidixic acid (71.4%) and ceftriaxone (53.6%); *E. coli* were resistant to augmentin (70.6%), ciprofloxacin (52.9%), nalidixic acid (64.7%) and ceftriaxone (53.6%); *K. pneumoniae* were resistant to ciprofloxacin (58.3%), nalidixic acid (75.0%) and augmentin (50.0%); *K. oxytoca* were resistant to augmentin (80.0%), nalidixic acid (80.0%) and ciprofloxacin (60%); *Pseudomonas aeruginosa* were resistant to ciprofloxacin (100%), nalidixic acid (100%) and gentamicin (66.7%); Coagulase negative *Staphylococci* were resistant to nalidixic acid (100%), ciprofloxacin (50%) and nitrofurantoin (50%).

Table 4.4: Antibiotics susceptibility testing of the isolated Bacteria

Organisms	No of Isolated Bacteria	Antibiotics Patterns (%)								
		AMC	**CN**	**CIP**	**NA**	**CAZ**	**CRO**	**F**	**IPM**	**FOX**
Staphylococcus	28	S 10(35.7)	15(53.6)	10(35.7)	8(28.6)	NT	13 (46.4)	15(53.6)	NT	20(71.4)
Aureus		R 18(64.3)	13(46.4)	18(64.3)	20(71.4)	NT	15(53.6)	13 (46.4)	NT	8 (28.6)
Escherichia coli	17	S 5(29.4)	13(76.5)	8(47.1)	6(35.3)	14(82.4)	14(82.4)	NT	NT	NT
		R 12(70.6)	4(23.5)	9(52.9)	11(64.7)	3(17.6)	3(17.6)	NT	NT	NT
Klebsiella	12	S 7(58.3)	6(50.0)	5(41.7)	3(25.0)	7(58.3)	7(58.3)	NT	NT	NT
pneumonia		R 5(41.7)	6(50.0)	7(58.3)	9(75.0)	5(41.7)	5(41.7)	NT	NT	NT
Klebsiella	5	S 1(20.0)	2 (40.0)	2(40.0)	1(20.0)	3(60.0)	3(60.0)	NT	NT	NT
oxytoca		R 4(80.0)	3(60.0)	3(60.0)	4(80.0)	2(40.0)	2(40.0)	NT	NT	NT
Pseudomonas	3	S 2(66.7)	1(33.3)	-	-	2(66.7)	3(100)	3(100)	3(100)	NT
Aeruginosa		R 1(33.3)	2(66.7)	3(100)	3(100)	1(33.3)	-	-	-	NT
Coagulase	2	S 2(100)	2 (100)	1(50.0)	-	NT	2(100)	1(50.0)	NT	2(100)
negative		R -	-	1(50.0)	2 (100)	NT	-	1(50.0)	NT	-
Staphylococci										
Mean of Resistance to antibiotics			**40.9%**							

Key: AMC- augmentin, CN- gentamicin, CIP- ciprofloxacin, NA- nalidixic acid, CAZ- ceftazidime, CRO – ceftriaxone, F – nitrofurantoin, IPM – imipenem, FOX –cefoxitin, NT – not tested, S – sensitive and R – resistant showing the zone diameter of inhibition in MM.

Table 4.5 shows the 8 *Staphylococcus aureus* that resist cefoxitin (FOX) which classified them as Methicillin-Resistant *Stapylococcus aureus,* were further tested against a second line of antibiotics (vancomycin and clindamycin). All were susceptible to vancomycin (VA) and only 2 were resistant to clindamycin (DA).

Table 4.5: Second line antibiotics tested against methicillin- Resistant *Staphylococcus aureus* (MRSA)

Number of isolated	Antibiotics pattern (%)	
MRSA	**VA**	**DA**
8	S 8 (100)	S 6 (75.0)
	R -	R 2 (25.0)

Key: VA- vancomycin, DA – clindamycin

Table 4.6 shows the 11 Extended-Spectrum Beta-Lactamase (ESBL) producers that resist ceftazidime (CAZ) and ceftriaxone (CRO) which classified them as Extended-Spectrum Beta-Lactamase (ESBL) producers, were further tested against a second line of antibiotics (nitrofuratoin and imipenem). All were susceptible to imipenem (IPM) and only 2 were resistant to nitrofuratoin (F).

Table 4.6: Second Line Antibiotics tested against Extended -spectrum Beta-lactamase (ESBL)

Number of Isolated ESBL Producers	Antibiotics patterns (%)	
	F	IPM
10	S 8(80.0)	10 (100)
	R 2 (20.0)	-

Key: F – nitrofurantoin, IPM – imipenem

CHAPTER FIVE

DISCUSSION

In this study, the prevalence of UTI among HIV positive patients was 22.3%. The observation of the high prevalence of UTI in this study may require the need for laboratory investigation as a criterion for the commencement of treatment in HIV-positive patients. This finding is in agreement with a study conducted in Jos, Nigeria where 23.5% prevalence was reported by Bigwan and Wakjissa (2013), and higher than the reports in Gondar, Ethiopia where 11.9% was reported (Alemu *et al.,* 2013) and in Jimma, Ethiopia where 10.7% was reported (Debalke *et al.,* 2014), but lower than studies conducted in Ebonyi State, Nigeria (93.8%) (Ifeanyichukwu *et al,* 2013) and Tamil Nadu in India (77.5%) (Xavier *et al.* 2015). This disparity rate might be attributed to differences in sample size (small sample size might over estimate the proportion), geographical variation, and socioeconomic conditions.

There was high prevalence of asymptomatic bacteriuria in our study population, with at least one in five having this condition which also appears to be much higher than those reported in other parts of the country and globally as most studies reports prevalence of 3–15% (De-Pinho *et al.,* 1994; Widmer *et al,* 2010). Only a few studies such as that by Ojoo *et al.* (1996) have recorded such high prevalence as ours. Another study in this environment also reported a high prevalence of 18% among HIV-positive pregnant women (Ezechi *et al.,* 2013).

Compared with HIV-negative population within the same region, it appears that the prevalence of asymptomatic bacteriuria is similar to what was obtained in our study population.

A community-based study on HIV-negative individuals in the same region (Olowe *et al.,* 2013) also found a prevalence of 22.6%, which is comparable to our prevalence of 22.3%.

Similar findings were also reported by Gugino *et al.* (1998) and Widmer *et al.* (2010) as they did not find any difference in the prevalence of asymptomatic bacteriuria between their study population of HIV-positive women and HIV-negative controls.

The usual trend in asymptomatic bacteriuria is for the prevalence to be higher in the female population (Olowe *et al.,* 2013). Our study also reported similar finding. It was found that the prevalence of asymptomatic bacteriuria is higher in women than men in keeping with reported trends which is attributed to the proximity of the urethra to the anus and its short length (Iduoriyekemwen *et al.,* 2012). The study could however not establish a statistically significant association and therefore, cannot make such conclusion in this group of HIV-positive individuals.

In this present study, the highest rate of UTI was recorded between the ages of 21–40 years (50%), which is not in consonance with the findings in Irrua, Nigeria where a relatively higher prevalence was recorded between the ages 20–29 years (53.9%) (Samuel *et al.,* 2012) and in the ages 18–26 in Gondar, Ethiopia (12.7%) (Alemu *et al.,* 2013). The prevalence of asymptomatic bacteriuria in the younger age group was almost twice as high as what was found in the older age group. This might be related to the sexual activity of the younger age group which is a recognized predisposition to bacteriuria (Hermann *et al.,* 2002). It should also be borne in mind, however, that the older age group, especially the elderly, are also at risk of bacteriuria (Hermann *et al.,* 2002; Anathianto, 2011; Olowe *et al.,* 2013).

Urinary tract infections appear to be multifactorial in patients with HIV infections as CD4+ level declines (Omoregie and Eghafona, 2009). Similar findings were reported elsewhere (Akinbami *et al.,* 2013; Adebayo and Salman, 2014; Fenta *et al.,* 2016; Skrzat-klapaczynska *et al.,* 2018). The observation imply that the more immune compromised the patient, the higher the risk of UTI

and possibly more vulnerable to other opportunistic infections. This might probably be due to the impaired immunity at a declining CD4+ count that makes it easier for bacterial pathogens to adhere to the urinary epithelium. Additionally, other reason that has been proposed for this is that in HIV/AIDS, the impaired immunity that occurs makes it easier for bacterial pathogens to adhere to the urinary epithelium (De-Pinho, 1994). Ezechi *et al.,* (2013) in their study on the risk factors for asymptomatic bacteriuria in HIV-positive pregnant women also observed similar findings in their study population of pregnant women. Furthermore, they found high viral load, low hemoglobin, and previous UTI to be associated with asymptomatic bacteriuria. These parameters were however not explored in our study.

The organisms isolated from 67 (22.3%) individual samples yielded significant bacteriuria; the other 233 (77.7%) samples had either no growth, less significant growth or mixed bacterial growth. *Staphylococcus aureus* was found to be the most predominant causative agent of UTI in the present study (41.8%). A similar finding was reported in Ebonyi State, Nigeria (Ifeanyichukwu *et al,* 2013), and Tamil Nadu, India (Xavier *et al.,* 2015). This is not consistent with many others such as report Alemu *et al.,* (2013), Debalke *et al.,* (2014), and Banu and Jyothi (2013). These authors reported *Escherichia coli* (56.1%), (54.3%) and (41.7%) as the commonest urinary tract pathogen respectively. The variation in the type of bacteria isolates might be due to differences in sample size, specimen collection technique, sample processing, and personal and environmental hygiene (Adebayo and Salman, 2014).

Antimicrobial resistance is a major clinical problem in treating infections caused by different bacterial pathogens and has increased over the years. In the present study, *S. aureus* was resistant to augmentin, ciprofloxacin, nalidixic acid and ceftriaxone; *E. coli* was resistant to augmentin, ciprofloxacin, nalidixic acid and ceftriaxone; *K. pneumoniae* was resistant to ciprofloxacin,

nalidixic acid and moderately resistant to augmentin; *K. oxytoca* was resistant to augmentin, ciprofloxacin, nalidixic acid and gentamicin; *P. aeruginosa* was resistant to ciprofloxacin, nalidixic acid and gentamicin while coagulase negative *Staphylococci* was totally resistant to nalidixic acid and moderately resistant to nitrofuratoin and Ciprofloxacin. This was to some extent comparable with the study reported in Gondar, Ethiopia (Alemu *et al.,* 2013), and Jimma, Ethiopia (Debalke *et al.,* 2014).

The 8 *Staphylococcus aureus* that were resistant to cefoxitin which classified them as Methicillin-Resistant *Stapylococcus aureus,* were further tested against a second line of antibiotics vancomycin and clindamycin which are the drug of choice for treating MRSA. The MRSA was 100% susceptible to vancomycin and only 2 out of the 8 were resistant to clindamycin. The development of resistance to β-lactam antimicrobials, occur concurrently with resistance to other antimicrobial agents, these poses a great challenge to the prevention and treatment of *S. aureus* infections (Arias and Murray, 2009). *Staphylococci* have two primary mechanisms for resistance to β-lactam antibiotics: the expression of an enzyme (the PC1 β-lactamase) capable of hydrolyzing the β-lactam ring, thus rendering the antibiotic inactive, and the acquisition of a gene encoding a modified penicillin-binding protein (PBP), known as PBP 2a, found in MRSA and coagulase-negative *staphylococci*, PBP 2a is intrinsically resistant to inhibition by β-lactams (Fuda *et al*., 2005). PBP 2a remains active in the presence of concentrations of β-lactam antibiotics that inhibit most endogenous PBP enzymes, thus substituting for their functions in cell wall synthesis and allowing growth in the presence of the β-lactam inhibitors.

Ten Gram negative organisms that resisted ceftazidime and ceftriazone were subjected to double-disk synergy test which confirmed them as Extended-Spectrum Beta-Lactamase (ESBL)

producers was tested against (imipenem and nitrofuratoin) as a second line of antibiotics. All 10 ESBL were susceptible to imipenem and only 2 was resistant to nitrofuratoin. Beta-Lactamase-Producing bacteria (BLPB) can play an important role in polymicrobial infections; they can have a direct pathogenic impact in causing the infection as well as an indirect effect through their ability to produce the enzyme beta-lactamase. These enzyme producing *Enterobacteriaceae* families are associated with a higher morbidity, mortality and fiscal burden (Johann, 2008). Extended spectrum β-lactamases (ESBL) are enzymes produced by a variety of gram negative bacteria which confer an increased resistance to commonly used antibiotics. Extended-spectrum β-lactamases (ESBLs) are usually plasmid-mediated enzymes that confer resistance to a broad range of β-lactams. Initially, resistance to third-generation cephalosporins in gram-negative rods was mainly due to the dissemination of TEM- and SHV-type ESBLs, which are point mutants of the classic TEM, SHV and CTX and enzymes with extended substrate specificity. Treatment of extended spectrum beta-lactamase (ESBL) producing strains of *Enterobacteriaceae* has emerged as a major challenge in hospitalised as well as community based patients (Bhattacharya, 2006).

Uropathogens is of public health challenge; there resistance to antibiotics may be due to the fact that this drug is widely used for prophylaxis against opportunistic infections associated with HIV (Lyamuya *et al.,* 2014). The similarity and differences between reports may be due to the distribution of resistant strains across the country.

Multidrug resistance has serious implications on the health outcome of HIV-infected patients (Rashmi *et al.,* 2013; Murugesh *et al.,* 2014). It is quite alarming to note that almost 40.8% of the isolates in this study were found to be resistant to multiple antibiotics of clinical importance. This was similar to report by Dadi *et al.,* (2019) and higher to the finding of Murugesh *et al.,*(2014) but lower than the observation made by Alemu *et al.,* (2013). The high rate of

resistance to the most commonly prescribed antibiotics observed in this study might be due to easy availability in the community and cost effectiveness which made them subjected to misuse (Eyasu *et al.,* 2014). It could also be due to the use of antibiotics for other nonhuman purposes such as in livestock rearing and animal husbandry activities, which may give force to the growing rate of resistance (Ifeanyichukwu *et al,* 2013; Murugesh *et al.,* 2014). The strength of this prospective study was that it evaluated large urine samples for pathogenic bacteria and highlights the emergence of antimicrobial resistance that may provide precise scientific data for appropriate treatment, prevention, and control of UTI. However, in this study it was not attempted to identify other causative agents (anaerobic bacteria, viruses, and fungus) that would have made a significant contribution to a true prevalence of UTI in HIV-positive patients.

5.1 CONCLUSION AND RECOMMENDATION

In conclusion, the prevalence of UTI in this study was relatively higher than previously reported findings in other places. *S. aureus, E. coli*, and *K. pneumonia* were identified as the major causes of UTI. The isolation of high multidrug-resistant bacteria coupled with methicillin resistant highlights the growing challenge of UTIs that may be impossible to treat. Health professionals should be aware of regional resistance rates to consider the current empirical antimicrobial therapy for UTI generally but HIV patients in particular.

Measures including health education, continuous monitoring of bacteria, and antimicrobial surveillance are crucial among this group of individuals to mitigate the infection and emergence of antimicrobial resistance. Future studies need to focus on exploring a range of causative pathogens and the mechanism of antimicrobial resistance.

REFERENCES

Adebayo, A. and Salman, A. (2014). Prevalence and predictors of asymptomatic bacteriuria in HIV positive pregnant women. *Journal of Medicine and Medical Science Research.* Vol. **3(5)**:48–54.

Ahmed, Z. and Lee, J. (1997). Asymptomatic urinary abnormalities. Hematuria and proteinuria. *Med Clin North Am*. **81**:641-652.

Akinbami, A., Bode-Shojobi, I. and Ajibola, S. (2013). Prevalence of asymptomatic bacteriuria in HIV infected patients in a Tertiary Hospital in Lagos, Nigeria. World *Journal of AIDS.* Vol. **3(2)**:105–110.

Alemu, A., Dagnew, M., Alem, M. and Gizachew, M. (2013). Uropathogenic bacterial isolates and their antimicrobial susceptibility patterns among HIV/AIDS patients attending Gondar University Specialized Hospital, Gondar, Northwest Ethiopia. *Journal of Microbiology Research and Reviews.* Vol.**1**:42–51.

Alteri, C. J., Himpsl, S. D. and Mobley, H. L. (2015). Preferential use of central metabolism in vivo reveals a nutritional basis for polymicrobial infection. *PLoS Pathog.* **11**:1004601.

Ani, O.C. and Mgbechi, E.K. (2008). Prevalence of Urinary Tract Infection (UTI) in sexually active women of Abakaliki, Ebonyi State, Nigeria *Animal Research International.* **5(2)**:876-879.

Ani, O.C., Onwe, C.S. and Nnabugwu, C.C. (2015). Evaluation of female residents for urinary tract infections, Onicha Local Government Area, Ebonyi State, Nigeria. *Nigerian Journal of Parasitology*.**36(1)**:50-54.

Arias, C. A. and B. E. Murray. (2009). Antibiotic-resistant bugs in the 21st century—a clinical super-challenge. *N. Engl. J. Med.* **360:**439-443.

Ariathianto, Y. (2011). Asymptomatic bacteriuria – prevalence in the elderly population. *Aust Fam Physician.* **40**: 805–809

Arinzon, Z., Peisakh, A., Shuval, I., Shabat, S. and Berner, Y. N. (2009). Detection of urinary tract infection (UTI) in long-term care setting: is the multireagent strip an adequate diagnostic tool? *Arch Gerontol Geriatr.* **48**:227–231.

Armbruster, C. E., Smith, S. N., Yep, A. and Mobley, H. L. (2014). Increased incidence of urolithiasis and bacteremia during Proteus mirabilis and Providencia stuartii coinfection due to synergistic induction of urease activity. *J Infect Dis.* **209**:1524–1532.

Banu, A. and Jyothi, R. (2013). Asymptomatic bacteriuria in HIV-positive individuals in a tertiary care hospital. *Journal of HIV and Human Reproduction*. Vol. **1(2)**:54.

Barford, J. M., Anson, K., Hu, Y. and Coates, A. R. (2008). A model of catheter-associated urinary tract infection initiated by bacterial contamination of the catheter tip. *BJU Int.* **102**:67–74.

Bass, PF., Jarvis, J.A. and Mitchell, C.K. (2003). Urinary tract infections. *Prim Care*. **30**:41-61.

Berg, R. C., Underland, V., Odgaard-Jensen, J., Fretheim, A. and Vist, G. E. (2014). Effects of female genital cutting on physical health outcomes: a systematic review and meta-analysis. *BMJ Open.* **4**:006316.

Bhattacharya, S. (2006). ESBL - from petri dish to the patient. *Indian J Med Microbiol.* **24(1)**:20-4.

Bigwan, E. I. and Wakjissa, F. D. (2013). Prevalence of urinary tract infections among HIV patients attending a non-governmental health facility in Jos, Plateau State, Nigeria. *International Journal of Biomedical and Advance Research*. Vol. **4(8)**:528.

Bonadio, M., Meini, M., Gigli, C., Longo, B. and Vigna, A. (1999). Urinary tract infection in diabetic patients. *Urol Int*. **63**:215—9.

Bradford, P.A. (2001). Extended Spectrum β Lactamases in the 21st century: Characterization, Epidemiology and Detection of This Important Resistance Threat. *Clin Microbiol Rev*. **14**:933–51.

Brendler, C.B. (1998). *Evaluation of the urologic patient: history, physical examination and urinalysis*. In: Campbell MF, Walsh PC. Campbell's Urology. 7th ed. Philadelphia: Saunders. 144-156.

Carroll, M.F. and Temte, J.L. (2000). Proteinuria in adults: a diagnostic approach. *Am Fam Physician*. **62**:1333-13340.

Chang, S.L. and Shortliffe, L.D. (2006). Pediatric urinary tract infections. *Pediatr Clin North Am*. **53**:379—400.

Cheesbrough, M. (2002). District laboratory practice in tropical countries part 1.Cambridge University Press. UK. 454.

Cheesbrough, M. (2004). District laboratory practices in tropical countries part 2.Cambridge University Press, UK.

Cheesbrough, M. (2006). *Microbiology tests:* District Laboratory practices in tropical Countries. Volume 2. Cambridge University Press, UK. Pp. 35-70.

Clinical and Laboratory Standards Institute. (2009). *Performance standards for antimicrobial disk susceptibility tests. Approved standard M2-A10*. Clinical and Laboratory Standards Institute; Wayne, PA.

Clinical and Laboratory Standards Institute. (2017). *Performance Standards for Antimicrobial Susceptibility Testing. 27th M100*. Clinical and Laboratory Standards Institute; PA, USA.

Cunningham, A.L., Donaghy, H., Haman, A.N., Kim, M. and Turville, S.G. (2010). Manipulation of dendritic cell function by viruses.*CurrOpinMicrobiol*.**113**: 524- 529.

Dadi, M., Senthilkumar, B.and Berhanu, S. (2019). Prevalence, Antimicrobial Susceptibility Pattern of Bacterial Isolates, and Associated Factors of UrinaryTract Infections among HIV-Positive Patients at Hiwot Fana Specialized University Hospital, Eastern Ethiopia. *Canadian Journal of Infectious Diseases and Medical Microbiology.***19**: 8.

Debalke, S., Cheneke, W., Tassew, H. and Awol, M. (2014). Urinary tract infection among antiretroviral therapy users and non-users in Jimma University Specialized Hospital, Jimma, Ethiopia. *International Journal of Microbiology.* **968716:**6.

Dedeić-Ljubović, A and Hukić, M. (2009). Catheter-related urinary tract infection in patients suffering from spinal cord injuries. *Bosn J Basic Med Sci*. **9**:2–9.

Demilie, T., Beyene, G., Melaku, S. and Tsegaye, W. (2014). Diagnostic accuracy of rapid urine dipstick test to predict urinary tract infection among pregnant women in Felege Hiwot Referral Hospital, Bahir Dar, North West Ethiopia. *BMC Res Notes.* **7**:481.

Deokar, S. and Badhankar, M.G. (2009). Studies on emergence of drug resistance in HIV associated bacterial urinary tract infections. *Am J Inf Dis.* **5**:183- 187.

De-Pinho, A. M., Lopes, G.S., Ramos-Filho, C.F., Santos, O.R., De-Oliveira, M.P., Halpern, M., Gouvea, C.A. and Schechter, M. (1994). Urinary tract infection in men with AIDS. *Genitourin Med*. **70**: 30–34.

Dickinson, G.M. and Bisno, A.L. (2007). Infections associated with indwelling devices: infections related to extravascular device. *Antimicrob Agents Chemother*. **33**:602-7.

Domann, E., Hong, G., Imirzalioglu, C., Turschner, S., Kühle, J., Watzel, C., Hain T., Hossain, H. and Chakraborty, T. (2003). Culture-independent identification of pathogenic bacteria and polymicrobial infections in the genitourinary tract of renal transplant recipients. *J Clin Microbiol.* **41**:5500–5510.

Edwards, M. S and Baker, C. J. (2005). Group B streptococcal infections in elderly adults. *Clin Infect Dis.* **41**:839–847.

Ekwealor, P.A., Ugwu, M.C., Ezeobi, I., Amalukwe ,G., Ugwu,B.C., Okezie, U., Stanley, C. and Esimone, C. (2016). Antimicrobial Evaluation of BacterialIsolates from Urine Specimen of Patients with Complaints of Urinary Tract Infections in Awka, Nigeria.*International Journal of Microbiology*.

Eyasu, M., Kassahun, K., Yilikal, A., Yewondwossen, T. and Alemayehu, K. (2014). *Food Medicine and Healthcare Administration and Control Authority of Ethiopia*, Addis Ababa, Ethiopa, 3rd edition.

Ezechi, O.C., Gab-Okafor, C.V., Oladele, D.O., Kalejaiye, O.O., Oke, B.O., Ekama, S.O., Audu, R.A., Okoye, R.N. and Ujah, I.A.O. (2013). Prevalence and risk factors of asymptomatic bacteriuria among pregnant Nigerians infected with HIV. *J Matern-Fetal Neonat Med*. **26(4)**:402–406.

Fassett, R.G., Horgan, B.A.and Mathew, T.H. (1982). Detection of glomerular bleeding by phase-contrast microscopy. *Lancet*. **1**:1432-1434.

Fawole, M. O., and Oso, B. A. (2004). Characterization of Bacteria.In *LaboratoryManual of Microbiology*. Spectrum Books Limited, Nigeria. 22-33.

Fenta, G., Legese, M. and Weldearegay, G. (2016). Bacteriuria and their antibiotic susceptibility patterns among people living with HIV attending tikuran bessa specialized and zewditu memorial hospital ART clinics, addis ababa, Ethiopia. *Journal of Bacteriology & Parasitology*. Vol. **7(5):**1–7.

Ferry, S. A., Holm, S. E., Stenlund, H., Lundholm, R. and Monsen, T. J. (2007). Clinical and bacteriological outcome of different doses and duration of pivmecillinam compared with placebo therapy of uncomplicated lower urinary tract infection in women: the LUTIW project. *Scand J Prim Health Care.* **25**:49–57.

Fogazzi, G.B. and Garigali, G. (2001). The clinical art and science of urine microscopy. *Curr Opin Nephrol Hypertens*. **12**:625-632.

Foxman, B. (2003). Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. *Dis Mon*. **49(2)**:53–70.

Fuda, C. C., Fisher, J.F. and Mobashery,S. (2005). Beta-lactam resistance in *Staphylococcus aureus*: the adaptive resistance of a plastic genome. *Cell. Mol. Life Sci.* **62:**2617-2633.

Gallagher, E.J., Schwartz, E. and Weinstein, R.S. (1990). Performance characteristics of urine dipsticks stored in open containers. *Am J Emerg Med.* **8**:121-123.

Gatermann, S., John, J.and Marre, R. (2007). Staphylococcus saprophyticus urease: characterization and contribution to uropathogenicity in unobstructed urinary tract infection of rats. *Infect Immun.* **57**:110–116.

Gugino, L., Russo, T., Wactawski-Wende, J., Goodnough, S..L, Tristram, D.A. and Mylotte, J. (1998). Asymptomatic bacteriuria in human immunodeficiency (HIV)-infected women. *Prim Care Update Ob Gyns.* **5(4)**:146.

Guiton, P. S., Cusumano, C. K., Kline, K. A., Dodson, K. W., Han, Z., Janetka, J. W., Henderson, J. P., Caparon, M. G. and Hultgren, S. J. (2012). Combinatorial small-molecule therapy prevents uropathogenic *Escherichia coli* catheter-associated urinary tract infections in mice. *Antimic rob Agents Chemother.* **56**:4738–4745.

Guiton, P. S., Hannan, T. J., Ford, B., Caparon, M. G. and Hultgren, S. J. (2013). *Enterococcus faecalis* overcomes foreign body-mediated inflammation to establish urinary tract infections. *Infect Immun.* **81**:329–339.

Hanno, P.M., Wein, A.J. and Malkowicz, S.B. (2001). *Clinical manual of urology*. 3d ed. New York: McGraw-Hill.

Hermann, V., Palma, P., Geo, M.S. and Lima, R.S. (2002). Urinary tract infections: pathogenesis and related conditions. *Int Urogynecol J.* **13**:210–213.

Hidron, A.I., Kempker, R., Moanna, A., and Rimland, D. (2010).Methicillin-resistant *Staphylococcus aureus* in HIV-infected patients. *Infect Drug Resist*. **3**:73-86.

Hjelm, E., Lundell-Etherden, I. and Mårdh, P. A. (1989). Ascending urinary tract infections in rats induced by *Staphylococcus saprophyticus and Proteus mirabilis. Acta Pathol Microbiol Immunol Scand [B]*. **95**:347–350.

Hooton, T. M. (2012). Clinical practice. Uncomplicated urinary tract infection. *N Engl J Med.* **366**:1028–1037.

House, A.A. and Cattran, D.C. (2002). Nephrology: 2. Evaluation of asymptomatic hematuria and proteinuria in adult primary care. *CMAJ*. **166**:348-353.

Iduoriyekemwen, N.J., Sadoh, W.E.and Sadoh, A.E (2012). Asymptomatic bacteriuria in HIV positive Nigerian children. *J Med Biomed Res.* **11(1)**:88–94.

Ifeanyichukwu, I., Emmanuel, N. and Chika, E. (2013). Frequency and antibiogram of uropathogens isolated from urine samples of HIV infected patients on antiretroviral therapy. *American Journal of Bioscience.* Vol. **1(3)**:50–53.

Iweriobor, B.C., Obi, C.L. and Akenyemi, O. (2012).Uropathogens isolated from HIV infected patients from Limpopo Province, South Africa. *Afr J Biotech*. **11**: 10598- 10604.

Jain, P., Parada, J.P., David, A. and Smith, L.G. (2005). Overuse of the indwelling urinary tract catheter in hospitalized medical patients. *Arch Intern Med.* **155**:1425–1429.

Jarvis, W.R. and Martone, W.J. (1992). Predominant pathogens in hospital infections. *J Antimicrob Chemother.* **29**:19—24.

Johann, D.D., Pitout Kevin. and Laupland, B. (2008). Extended Spectrum β-Lactamase Producing Enterobacteriaceae: An emerging public-health concern. *Lancet Infect Dis.* **8**:159-66.

Johnson, J.R., Berggren, T. and Manivel, J.C. (1992). Histopathologic- microbiologic correlates of invasiveness in a mouse model of ascending unobstructed urinary tract infection. *J Infect Dis*. **165**:299-305.

Josephson, S., Thomason, J., Sturino, K., Zabransky, R.and Williams, J. (1988). Gardnerella vaginalis in the urinary tract: incidence and significance in a hospital population. *Obstet Gynecol*. **71**:245–250.

Kalsi, J., Arya, M., Wilson, P. and Mundy, A. (2003). Hospital-acquired urinary tract infection. *Int J Clin Pract*. **57**:388-91.

Katz, A.R. (2003). Urinary tract infections and acupuncture.American Medical *Journal of Public Health*.**9**:702-723.

Kau, A. L., Martin, S .M., Lyon, W., Hayes, E., Caparon, M. G. and Hultgren, S. J. (2011). *Enterococcus faecalis* tropism for the kidneys in the urinary tract of C57BL/6J mice. *Infect Immun.* **73**:2461–2468.

Kavouras, S.A. (2002). Assessing hydration status. *Curr Opin Clin Nutr Metab Care*. **5**:519-524.

Kemajou, T.S., Ajugwo, A.O., Oshoma, C.E. and Enabulele, O.I. (2016).Antibiotic Resistance of Bacterial Isolates from HIV Positive Patients with Urinary Tract Infection (UTI) in Portharcourt, *Nigeria. J AIDS Clin Res*. **7**:594.

Khan, M.A., Shaw, G. and Paris, A.M. (2002). Is microscopic haematuria a urological emergency. *BJU Int*. **90**:355-357.

Kiel, D.P. and Moskowitz, M.A. (1987). The urinalysis: a critical appraisal. *Med Clin North Am*. **71**:607-624.

Kim, H. Y., Choe, H. S., Lee, D. S., Yoo, J. M. and Lee, S. J. (2015). A novel rat model of catheter-associated urinary tract infection. *Int Urol Nephrol.* **47**:1259–1263.

Kline, K. A., Ingersoll, M. A., Nielsen, H. V., Sakinc, T., Henriques-Normark, B., Gatermann, S., Caparon, M. G. and Hultgren, S. J. (2010). Characterization of a novel murine model of *Staphylococcus saprophyticus* urinary tract infection reveals roles for Ssp and SdrI in virulence. *Infect Immun.* **78**:1943–1951.

Kline, K. A., Schwartz, D. J., Gilbert, N. M., Hultgren, S. J. and Lewis, A. L. (2012). Immune modulation by group B Streptococcus influences host susceptibility to urinary tract infection by uropathogenic *Escherichia coli. Infect Immun.* **80**:4186–4194.

Kline, K. A., Schwartz, D. J., Gilbert, N. M. and Lewis, A. L. (2014). Impact of host age and parity on susceptibility to severe urinary tract infection in a murine model. *PLoS One.* **9**:977-998.

Kline, K. A., Schwartz, D. J., Lewis, W. G., Hultgren, S. J. and Lewis, A. L. (2011). Immune activation and suppression by group B streptococcus in a murine model of urinary tract infection. *Infect Immun.* **79**:3588–3595.

Kunin, C. M. (2009). Blockage of urinary catheters: role of microorganisms and constituents of the urine on formation of encrustations. *J Clin Epidemiol*. **42**:835–842.

Lifshitz, E. and Kramer, L. (2000). Outpatient urine culture: Does collection technique matter?. *Arch Intern Med*. **160**:2537-2540.

Litwin, M.S., Saigal, C.S., Yano, E.M., Avila, C., Geschwind, S.A. and Hanley, J.M. (2005). Urologic Diseases in America Project: analytical methods and principal findings. *J Urol*.**173**:933—7.

Lyamuya, E., Moyo, S., Komba, E., M. and Haule, M. (2014). "Prevalence, antimicrobial resistance and associated risk factors for bacteriuria in diabetic women in Dar Es Salaam, Tanzania," *African Journal of Microbiology Research*. Vol. **5(6)**:683–689.

Maki, D. G and Tambyah, P. A. (2001). Engineering out the risk for infection with urinary catheters. *Emerg Infect Dis.* **7**:342–347.

Mehnert-Kay, S. A. (2005). Diagnosis and management of uncomplicated urinary tract infections. *Am Fam Physician.* **72**:451–456.

Miguelles, S. and Connors, M. (2010). Long term Non-progressive disease among untreated HIV- infected individuals: Clinical implications of understanding immune control of HIV. *Journal of the American Medical Assoc*. **304**:194- 201.

Mittal, R., Chhibber, S., Sharma, S. and Harjai, K. (2004). Macrophage inflammatory protein-2, neutrophil recruitment and bacterial persistence in an experimental mouse model of urinary tract infection. *Microbes Infect*. **6**:1326-32.

Mougeot, C., Guillaumat-Tailliet, J. and Libert, J.M. (2001). *Staphylococcus aureus*: new detection of intrinsic resistance using the diffusion method. *Path Biol*. **49**:199-204.

Murugesh, K., Deepa, S., Ravindranath, C. and Venkatesha, D. (2014). Multidrug resistant uropathogens in HIV: are they a threat to community?. *International Journal of Scientific Study*. Vol. **2(3)**:38–42.

Naboka, I. L, Kogan, M. I., Vasileva, L. I., Gudima, I. A., Miroshnichenki, E. A. and Ibishev, K. S. (2011). Bacterial mixed infection in women with chronic recurrent cystitis. *Zh Mikrobiol Epidemiol Immunobiol.* **1**:8–12.

Ojoo, J., Paul, J., Batchelor, B., Amir, M., Kimari, J., Mwachari, C., Bwayo, J., Plummer, F., Gachihi, G., Waiyaki, P. and Gilks, C. (1996). Bacteriuria in a cohort of predominantly HIV-1 seropositive female commercial sex workers in Nairobi, Kenya. *J Infect.* **33**:33–37.

Olowe, O., Ojo-Johnson, B., Makanjuola, O., Olowe, R. and Mabayoje, V. (2015). Detection of bacteriuria among human immunodeficiency virus seropositive individuals in Osogbo, south-western Nigeria. *European Journal of Microbiology and Immunology*. Vol. **5(1)**:126–130.

Olowe, O.A., Makanjuola, O.B., Olabiyi, K.O., Akinwusi, P.O., Alebiosu, C.O. and Isawumi, M.A. (2013). Asymptomatic bacteriuria among elderly and middle-aged rural community-dwellers in South-Western Nigeria. *Infect. Drug Res.* **6**:55–58.

Omoregie, R. and Eghafona, N. (2009) Urinary tract infection among asymptomatic HIV patients in Benin City, Nigeria. *British Journal of Biomedical Science*. Vol. **66(4)**:190–193.

Ovalle, A. and Levancini, M. (2001).Urinary tract infections in pregnancy. *CurrOpin Urol*. **11(1)**:55–9.

Rabinovitch, A. (2001). *Urinalysis and collection, transportation, and preservation of urine specimens: approved guideline*. 2d ed. Wayne, Pa.: National Committee for Clinical Laboratory Standards. NCCLS document GP16-A2.

Rashmi, K., Ravikumar, K., Nimitha, J. and Bhagyashree, H. (2013). Asymptomatic bacteriuria in HIV/AIDS patients: occurrence and risk associated with low CD4 counts. *Journal of Evolution of Medical and Dental Sciences*. Vol. **2(19)**:3358–3366.

Raz, R., Colodner, R. and Kunin, C. M. (2005). Who are you--Staphylococcus saprophyticus? *Clin Infect Dis.* **40**:896–898.

Roberts, A.P., Robinson, R.E. and Beard, R.W. (1999). Some factors affecting bacterial counts in Urinary tract infections. *B. Medical Journal*. **(1)**:400-3.

Samuel, S., Salami, T., Adewuyi, G., Babatope, E. and Ekozien, M. (2012). Prevalence of urinary tract infections among a cohort of HIV positive patients accessing care in a rural health centre in Nigeria. *Journal of Microbiology and Biotechnology Research Scholars.* Vol. **2(4)**: 507–510.

Schroeder, A. R., Chang, P. W., Shen, M. W., Biondi, E. A. and Greenhow, T. L. (2015). Diagnostic accuracy of the urinalysis for urinary tract infection in infants<3 months of age. *Pediatrics.* **135**:965–971.

Siegel, A.J., Hennekens, C.H., Solomon, H.S. and Van Boeckel, B. (1979). Exercise related hematuria. Findings in a group of marathon runners. *JAMA*. **241**:391-392.

Siegman-Igra, Y., Kulka, T., Schwartz, D. and Konforti, N. (1993). The significance of polymicrobial growth in urine: contamination or true infection. *Scand J Infect Dis*. **25**:85–91.

Sierra-Hoffman, M., Watkins, K., Jinadatha, C., Fader, R. and Carpenter, J. L. (2005). Clinical significance of Aerococcus urinae: a retrospective review. *Diagn Microbiol Infect Dis.* **53**:289–292.

Skrzat-Klapaczynska, A., Matlosz, B. and Bednarskal, A. (2018). Factors associated with urinary tract infections among HIV-1 infected patients. *PLoS One.*Vol. **13(1)**:e0190564.

Springberg, P.D., Garrett, L.E., Thompson, A.L., Collins, N.F., Lordon, R.E. and Robinson R.R. (1982). Fixed and Reproducible Orthostatic Proteinuria: Results of a 20-Year Follow-up Study. *Ann Intern Med*. **97**:516–519.

Stensballe, J., Tvede, M., Looms, D., Lippert, F. K., Dahl, B., Tonnesen, E. and Rasmussen, L. S. (2007). Infection risk with nitrofurazone-impregnated urinary catheters in trauma patients: a randomized trial. *Ann Intern Med.* **147**:285–293.

Sutton, J.M. (1990). Evaluation of hematuria in adults. *JAMA*. **263**:2475-2480.

Tambyah, P. A and Maki, D. G. (2000). Catheter-associated urinary tract infection is rarely symptomatic: a prospective study of 1,497 catheterized patients. Arch Intern Med. **160**:678–682.

Tankeshwar, Acharya (2015). CLED Agar: composition, uses and typical colony characteristics. Bacteriology, Biochemical tests in Microbiology, laboratory diagnosis of Bacterial Disease. Retrieved from https://microbeonline.com/cled-agar-composition-uses-typical-colony-characteristics/.

Timothy, O.O., Olusesan, F.J., Adesola, B.O., Temitayo, A.A., David, F.O. and Ige, O.O. (2014). Antibiotic resistance pattern of bacterial isolates from cases of urinary tract infections among hospitalized and out-patients at a tertiary health facility in South Western Nigeria. *Ann Trop Med Public Health.*

Tsuchimori, N., Hayashi, R., Shino, A., Yamazaki, T. and Okonogi, K. (1994). Enterococcus faecalis aggravates pyelonephritis caused by Pseudomonas aeruginosa in experimental ascending mixed urinary tract infection in mice. *Infect Immun.* **62**:4534–4541.

Von Bonsdorff, M., Koskenvuo, K., Salmi, H.A. and Pasternack, A. (1981). Prevalence and causes of proteinuria in 20-year old Finnish men. *Scand J Urol Nephrol*. **15**:285-290.

Wagenlehnerg, F. M and Naber, K. G. (2012). Current challenges in the treatment of complicated urinary tract infections and prostatitis. *Clin Microbiol Infect.* **12(3)**:67–80.

Warren, J.W. (1997). Catheter-associated urinary tract infections. *Infect Dis Clin North Am.* **11**:609–622.

Widmer, T. A., Theron, G. and Grove, D. (2010). Prevalence and risks of asymptomatic bacteriuria among HIV-positive pregnant women. *South Afr J Epidemiol Infect.* **25(1)**: 28–32.

Wolfe, A. J., Toh, E., Shibata, N., Rong, R., Kenton, K., Fitzgerald, M., Mueller, E. R., Schreckenberger, P., Dong, Q., Nelson, D. E. and Brubaker, L. (2012). Evidence of uncultivated bacteria in the adult female bladder. *J Clin Microbiol.* **50**:1376–1383.

Xavier, T. F., Auxilia, A. and Kannan, M. (2015). Isolation and characterization of UTI pathogens from HIV positive patients of Karur District, Tamil Nadu, India. *International Journal of Current Microbiology and Applied Sciences.* Vol. **4(1)**:558–563.

APPENDIX

Appendix 1: Morphological, biochemical and microscopic characteristics of the bacteria isolates

Characteristics	**Isolated species**					
	S. aureus	*E. coli*	*Kleb. pneumoniae*	*Kleb.* oxytoca	*Pseudomonas aeruginosa*	CONS
Color	golden yellow	pink	greyish	whitish	blue-green	yellowish
Elevation	raised	convex	dume-shape	raised	convex	raised
Colony shape	round	circular	circular	circular	circular	round
Surface	smooth	smooth	mucoid	mucoid	smooth	smooth
Structure	opaque	opaque	opaque	opaque	translucent	opaque
Gram reaction	+ve	-ve	-ve	-ve	-ve	+ve
Cell – Shape	cocci	Rod	rod	rod	rod	cocci
Motility	non	motile	non	non	motile	non
Spores forming	no	No	no	no	no	no
Indole	-ve	+ve	-ve	+ve	+ve	-ve
Citrate	+ve	-ve	+ve	+ve	+ve	+ve
Coagulase	+ve	-ve	-ve	-ve	-ve	-ve
Catalase	+ve	+ve	+ve	+ve	+ve	+ve
Oxidase	-ve	-ve	-ve	-ve	+ve	-ve

Key: CONS= Coagulase Negative Stapylococcoci

Appendix 2: Macroscopy and Microscopy Characteristics of the Positive Samples

S/N	Sex	Age	Macroscopy/ Appearance	Microscopy/HPF							
				WBC	RBC	Schistosoma haematobium	cast	Epiht cell	Crystals	Bacteria cells	Yeast Cells
1	F	60	Deep amber & turbid	0-2	-	-	-	-	-	+++	-
2	F	25	Amber & turbid	num	-	-	++	-	-	-	
3	F	38	Amber & turbid	4-6	0-2	-	-	+++	-	-	-
4	F	50	clear	0-2	-	-	-	-	-	-	-
5	F	40	Amber & turbid	num	0-2	-	-	+	-	+++	-
6	F	18	Amber & clear	-	-	-	-	-	-	-	-
7	F	38	Amber & cleaar	4-6	-	-	-	+	-	-	-
8	F	29	Amber &clear	0-2	-	-	-	-	-	-	-
9	F	51	Amber &clear	0-2	2-4	-	-	-	-	-	-
10	F	45	Amber & clear	2-4	0-1	-	-	-	-	-	-
11	F	55	Amber & clesr	0-2	-	-	-	-	-	-	-
12	F	14	Deep amber & turbid	10-12	-	-	-	+	-	-	-
13	F	45	Deep amber & turbid	10-12	-	-	-	-	-	-	-
14	F	36	Amber & slightly turbid	0-2	-	-	-	-	-	-	-
15	F	60	Amber & turbid	0-2	-	-	-	-	-	+++	-
16	M	26	Amber &clear	-	-	-	-	-	-	-	-
17	F	45	Amber & clear	-	-	-	-	-	-	-	-
18	F	40	Slightly turbid	2-4	-	-	+++	+++	-	-	-
19	F	31	Amber & clear	0-2	-	-	-	-	-	-	-
20	F	37	Slightly turbid	0-2	0-2	-	-	+++	-	-	-
21	F	32	Amber & clear	-	-	-	-	+	+	-	-
22	F	56	Amber & turbid	20-22	-	-	++	-	-	-	-
23	F	39	Amber & turbid	0-2	-	-	-	++	-	-	-
24	F	25	Amber & clear	-	-	-	-	-	+	-	-
25	F	46	Deep amber	6-8	0-2	-	+	+	-	-	-

			& turbid								
26	F	28	Amber & cloudy	10-12	-	-	+++	++++	-	-	-
27	F	40	Deep amber & turbid	num	-	-	+	+	-	-	-
28	F	35	Amber &clear	-	-	-	-	-	-	-	-
29	F	29	Amber & clear	0-2	-	-	-	++	-	-	-
30	F	38	Amber & turbid	4-6	0-2	-	-	+++	-	-	-
31	F	46	Amber & slightly turbid	2-4	-	-	-	+++	-	-	-
32	F	52	Amber & clear	0-2	-	-	-	+	-	-	-
33	F	53	Slightly turbid	6-8	-	-	+	++	-	-	-
34	F	38	Amber & slightly turbid	6-8	0-2	-	+	+	-	-	-
35	F	50	Amber& clear	-	-	-	-	-	-	-	-
36	F	50	Amber & clear	0-2	-	-	-	-	-	-	-
37	F	50	Amber & clear	0-2	-	-	-	-	-	-	-
38	F	60	Amber & clear	0-2	0-2	-	-	-	-	-	-
39	F	28	Amber& clear	4-6	-	-	-	-	++	-	-
40	F	60	Amber & cloudy	2-4	0-2	-	-	-	+++	-	-
41	F	45	Amber & cloudy	0-2	-	-	-	+++	+	-	-
42	F	33	cloudy	0-2	0-2	-	-	+	-	-	-
43	F	45	Amber & slightly turbid	8-10	-	-	+	+	-	-	-
44	F	38	Slightly cloudy	0-2	-	-	-	++++	-	-	-
45	F	58	Amber & cloudy	2-4	0-1	-	-	+++	-	-	-
46	F	48	Deep amber & cloudy	22-24	-	-	-	++++	-	-	-
47	F	60	Amber & clear	4-8	0-2	-	-	+	-	-	-
48	F	60	Cloudy, turbid & bloody	num	num	-	-	-	-	-	-
49	M	43	Amber & cloudy	-	-	-	-	-	-	-	-
50	F	30	Deep amber & cloudy	0-2	-	-	-	++	-	-	-
51	F	35	Amber &	18-20	0-2	-	-	+	-	-	-

			turbid								
52	F	39	Amber & clear	0-1	-	-	-	+	-	-	-
53	M	43	Deep amber & cloudy	6-8	0-2	-	-	+	-	-	-
54	F	66	Amber & clear	0-2	-	-	-	++	-	-	-
55	F	39	Amber & clear	2-4	-	-	++	++	-	-	-
56	M	61	Amber & slightly cloudy	0-2	-	-	-	-	++	-	-
57	F	65	turbid	8-10	-	-	-	+	-	-	-
58	F	22	Amber & cloudy	4-6	0-2	-	-	+++	-	-	-
59	M	67	Amber & slightly turbid	4-6	-	-	-	+	-	-	-
60	F	33	Amber & cloudy	0-2	-	-	-	+	-	-	-
61	F	37	cloudy	0-2	-	-	-	+++	-	-	-
62	F	36	Amber & slightly turbid	10-12	0-2	-	-	++	-	-	-
63	M	41	Deep amber & cloudy	2-4	-	-	-	++	-	-	-
64	F	32	amber & clear	0-2	-	-	-	-	-	-	-
65	F	35	Deep amber & turbid	0-2	-	-	+++	+	-	-	-
66	F	43	Slightly turbid	0-2	-	-	+++	-	-	-	-
67	F	43	Deep amber & turbid	-	-	-	++	+++	-	-	-

Key: WBC – white blood cells, RBC – red blood cells, Epith cells – Epithelial cells, HPF – high power field, num – numerous, + present, - absent.

MINISTRY OF HEALTH

P.M.B. 1386, FATE ROAD, ILORIN, KWARA STATE. 031-220349

Our Ref: MOH/KS/EU/777/252 *Your Ref:* _____ *Date:* 16th November, 2018.

Badmus, Ajoke Rahmat

Department of Microbiology,
College of Pure and Applied Sciences,
Kwara State University,
Malete, Kwara State.

APPROVAL TO CARRY OUT A RESEARCH TITLED: **"Resistance Patterns of Bacterial Isolates From Urine Samples of HIV Positive Patients in Ilorin Metropolis."**

Sequel to your request and the interest of the State Ministry of Health in Health related research activities to improve the health of the citizen. I am directed to forward to you the approval of the Ministry of Health to carry out the dissertation as itemized in your protocol. This approval dates from 16th November, 2018 to 16th November, 2019.

2. You are mandated to acknowledge the State Ministry of Health by your presentations/publications and deposition of the final copy of the research findings/publications.

3. Best wishes in your research project.

Mrs Sadiq T.R
For: Honourable Commissioner

CMD/Officer in charge.

.....

.....

Above for your information and necessary action, please