ANTIBIOGRAM OF UROPATHOGENIC BACTERIA ISOLATED FROM PATIENTS IN SOME HOSPITALS IN BIRNIN KUDU, JIGAWA STATE, NIGER

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DECLARATION

I declare that the work reported in this dissertation entitled "Antibiogram of Uropathogenic Bacteria Isolated from Patients in some Hospitals in Birnin Kudu, Jigawa State, Nigeria" was carried out by me in the Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, under the supervision of Prof. Y. K. E. Ibrahim and Dr. B. A. Tytler

The information derived from the literature review has been duly acknowledged in the text and a list of the references provided. No part of this dissertation has been presented in any application for another degree or diploma at any University.

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CERTIFICATION

This dissertation entitled "Antibiogram of Uropathogenic Bacteria Isolated from Patients in some Hospitals in Birnin Kudu, Jigawa State, Nigeria" by KACHALLAH Mohammed meets the regulation governing the award of the degree of Masters of Science of Ahmadu Bello University, Zaria, Nigeria and is approved for its contiribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to my parents, Alh. Muhammad Bala and Malama Hamsatu Muhammad Bala.

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ABSTRACT

Urinary tract infection (UTI) is a common infection of human being and if untreated could lead to serious complications. This study was conducted to investigate the antibiotic susceptibility pattern of uropathogens from patients in two hospitals in Birnin kudu, Jigawa State, Nigeria. In this study, the antibiotic resistance profile and the plasmid profile of some multi-antibiotic resistant bacteria isolated from urine samples of patients from Birnin kudu community in North-west, Nigeria were analysed. Rapid diagnostic kit systems were used in identification of the isolated bacteria and agar disc diffusion technique was used for the determination of antibiotic susceptibility profiles of the isolated bacteria. Presence of β- lactamase was determined using standardized β-lactamase identification sticks while acridine orange was used for curing of multidrug resistant isolates. The cultures of some multi- antibiotic resistant isolates irreversibly lost their antibiotic resistance with acridine orange treatment, which suggests that the resistant genes could be harboured in the plasmids. The result showed that 94.3% of the isolates were resistant to Ampicillin, Amoxycillin-clavulanic acid (71.5%), Ceftriaxone (35.4%), Cefuroxime (57.3), Cotrimoxazole (73.1%), Nitrofurantoin (24.6%), Chloranphenicol (36.9), Doxycycline (58.0%), Ciprofloxacin (60.0%) and Gentamicin (61.2%). Out of 36 isolates tested for presence of β- lactamse, 66.1% possessed β- lactamases. Plasmid profile studies revealed the presence of plasmid of size range 5184.8kb – 5673.9bp.

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CHAPTER ONE

1.1 BACKGROUND OF THE STUDY

Urinary Tract Infection (UTI) is an infection that affects part of the urinary tract. When it affects the lower urinary tract, it is known as a simple cystitis (a bladder infection) but when it affects the upper urinary tract, it is known as pyelonephritis (a kidney infection). Lower urinary tract infection is characterized by burning sensation during urination with either frequent urination or urge to urinate or both and is often accompanied with significant pain (Nicolle, 2008). These symptoms may vary from mild to severe (Lane and Takhar, 2011) and in healthy women, can last an average of six days (Colgan and Willliams, 2011). Upper urinary tract infection is characterized by flank pain, fever, or nausea and vomiting in addition to classic symptoms of a lower urinary tract infection (Lane and Takhar, 2011). Rarely, the urine may appear bloody or contain visible pus. In children, the symptoms may be a fever. Infants may feed poorly, vomit, sleep more or show signs of jaundice. In older children, new onset urinary incontinence may occur.

Escherichia coli is the cause of 80-85% of urinary tract infections, with *Staphylococcus saprophyticus* being the cause in 5-10% of the cases (Nicolle, 2008). Other bacterial causes include: *Klebsiella, Proteus, Pseudomonas*, and *Enterobacter*. These are less common and typically related to urinary catheterization (Salvatore, 2011). Urinary tract infection due to *Staphylococcus aureus* occur secondary to blood-borne infections (Lane and Takhar, 2011).

Acute uncomplicated urinary tract infections (UTIs) are a common clinical syndrome that occurs in women with otherwise normal genitourinary tract (Hooton *et al*., 2000) with about 3% of the women in the United States visiting a Physician at least once each year

have uncomplicated urinary tract infection (NCHS, 1979). Sexual intercourse is the cause of 75-95% of bladder infection in young, sexually active women. The risk of infection is related to frequency of sexual intercourse (Nicolle, 2008). Urinary tract infections is very frequent when women first get married, hence the term "honeymoon cystitis" is often used. More women get urinary tract infections than men because women have a urethra that is much shorter and closer to the anus (Dielubanza and Schaeffer, 2011). As a woman's estrogen levels decreases with menopause, the risk of urinary tract infections increases due to the loss of protective vaginal flora. Other risk factors include diabetes (Nicolle, 2008) and having a large prostate (Lane and Takhar, 2011). The use of catheter increases the risk of urinary tract infections. The risk of contracting bacteriuria is 3-6% every day the catheter is used: antibiotics do not stop these infections.

The pathogens causing UTI are consistent across the globe. The pathogenesis of urinary tract infection involves ascending infection with coliform bacteria colonizing the perineum in susceptible women (80–90% *Escherichia coli*, 5–10% *Staphylococcus saprophyticus* with the remainder caused by *Proteus* and other Gram negative rods) (Zalmanovici *et al.,* 2010). While not generally considered a cause of significant mortality, UTI do represent an important cause of morbidity if left untreated. This is more likely to be the case where access to or availability of timely and appropriate medical intervention is limited due to inadequate numbers of health care providers. UTI can be particularly dangerous in pregnant women in whom it has been shown that up to 50% of those with asymptomatic bacteriuria (ABU) go on to develop pyelonephritis. In addition, these women experience higher rates of intrauterine growth restriction and low birth-weight infants. The presence of a UTI has also been shown to increase the risk of preterm

labor, preterm birth, pregnancy-induced hypertension, preeclampsia, amnionitis and anemia (Delzell and Lefevre, 2000).

The antimicrobial misuse in clinical medicine has led, in part, to an increase in microbial resistance; the consequent spread of bacterial resistant strains is a serious public health problem. Urinary tract infection is one of the most common diseases of the community and also of the hospital setting. In 1997, it was reported that 29% of healthy adults outside the hospital environment are colonized by methicillin-resistant *Staphylococcus aureus* (MRSA). By 2008, the figure had increased to 74% (EARSS, 2008). This type of colonization is caused by strains of *Staphylococcus aureus* different from those found in the hospital environment and are often referred to as community-associated MRSA (CA-MRSA). Some studies have shown that CA-MRSA has high potential to become endemic in the community and that this will have a significant impact on the control of MRSA in hospital (Klutymans, 2006; Tang and Stratton, 2010).

Beta-lactamases are enzymes produced by bacteria that provide multi-resistance to β-lactam antibiotics such as penicillins, cephamycins, and carbapenems, although carbapenems are relatively resistant to beta-lactamase. Beta-lactamase provides antibiotic resistance by breaking the antibiotics' structure. These antibiotics (β-lactam antibiotics) all have a common element in their molecular structure: a four-atom ring known as a β-lactam. The lactamase enzyme breaks the β-lactam ring by hydrolysis thereby opening and deactivating the molecule's antibacterial properties. Beta-lactamases produced by Gram-negative organisms are usually secreted, especially when antibiotics are present in the environment (Neu, 1969).

Classification

Group 1

CEPHALOSPORINASE: Group 1 are cephalosporinases not inhibited by clavulanic acid, belonging to the molecular class C

Group 2

Group 2 are penicillinases, cephalosporinases, or both inhibited by clavulanic acid, corresponding to the molecular classes A and D reflecting the original TEM and SHV genes. Additionally, many class A TEM β-lactamases are inhibited by β-lactamase inhibitor protein (BLIP).

Group 3

METALLOENZYME, Molecular Class B

Group 3 are the zinc-based or metallo β-lactamases, corresponding to the molecular class B, which are the only enzymes acting by the metal ion zinc, as discussed above. Metallo B-lactamases are able to hydrolyse penicillins, cephalosporins, and carbapenems.

Group 4

PENICILLINASE, No Molecular Class

Group 4 are penicillinases that are not inhibited by clavulanic acid, and they do not yet have a corresponding molecular class. This group has been omitted from the current scheme, as when it was described originally it included enzymes not classifiable into other groups. It has been concluded that the enzymes that made up this classification in 1995 would be included in one of the other groups once more information becomes available on them and that it was not informative to have a separate group for them (Bush & Jacoby, 2010).

Molecular classification

The molecular classification of β-lactamases is based on the nucleotide and amino acid sequences in these enzymes. To date, four classes are recognised (A-D), correlating with the functional classification. Classes A, C, and D act by a serine-based mechanism, whereas class B or metallo-β-lactamases need zinc for their action.

Resistance in Gram-negative bacteria

Among Gram-negative bacteria, the emergence of resistance to expanded-spectrum cephalosporins has been a major concern. It first appeared in a limited number of bacterial species (*C. cloacae, C. freundii, S. marcescens*, and *P. aeruginosa*) that could mutate to hyperproduce their chromosomal class C β-lactamase. A few years later, resistance appeared in bacterial species not naturally producing AmpC enzymes (*K. pneumoniae, Salmonella* spp., *P. mirabilis*) due to the production of TEM- or SHV-type ESBLs. Characteristically, such resistance has included oxyimino- (for example cefotaxime, ceftriaxone, and ceftazidime, as well as the oxyimino-monobactam

aztreonam), but not 7-alpha-methoxy-cephalosporins (cefoxitin and cefotetan); has been blocked by inhibitors such as clavulanate, sulbactam, or tazobactam, and did not involve carbapenems and temocillin. Chromosomal-mediated AmpC β-lactamases represent a new threat, since they confer resistance to 7-alpha-methoxy-cephalosporins (cephamycins) such as cefoxitin or cefotetan are not affected by commercially available β-lactamase inhibitors, and can, in strains with loss of outer membrane porins, provide resistance to carbapenems (Philippon *et al*., 2002).

1.2 STATEMENT OF THE RESEARCH PROBLEM

Urinary tract infection is one of the common infections of the community and also of the hospital settings, resulting in high rate of morbidity and high economic costs associated with its treatment (Arjunan *et al*., 2010; Rahman *et al*., 2009; Hryniewicsz *et al*., 2001).

Urinary tract infection (UTI) is the second most common infectious presentation in community medical practice. Worldwide, about 150 million people are diagnosed with UTI each year costing the global economy in excess of six (6) billion dollars (Gonzalez and Schaeffer, 1999). In the United States, UTI accounts for 8.3 million out-patient visits and one million hospitalizations annually (CDC, 2004).

UTIs are common in general practice, accounting for 1-3% of all consultations. Almost half of all women report at least one UTI sometime during their lifetime, and after an initial UTI, 20% to 30% of women experience a recurrence (Foxman, 2003).

Some studies carried out have shown that uropathogens such as *Escherichia coli* (46.4-74.2%), *Klebsiella spp* (6-13.45%), *Proteus spp* (4.7-11.9%) and *Enterococcus spp* (5.3-9.54%) represent the main causes of urinary tract infection (Rahman *et al*.,2009; Akram *et al*., 2007; Laupland *et al*., 2007). *Escherichia coli* has been indicated as the most

frequent uropathogens involved in the community–acquired urinary tract infection (Francesco *et al.*,2007; Laupland *et al.*,2007) due to the fact that it belongs to the normal flora of the human intestine and therefore easily colonizing the urinary tract. Some strains of *Escherichia coli* isolated from sexually active patients matched with faecal isolates from their partners, which indicate that the urinary tract infection can be sexually transmitted (Wiles *et al.,* 2008).

The prevalence of UTIs in Benin City was reported as 14.58% (Orhue, 2004). This prevalence level is relatively low compared to the 22% reported for Ibadan (Okesola and Oni, 2009) and much lower compared with figure reported in other parts of the country. For example 35.5% was reported for Jos (Ebie *et al*., 2001) and in Lagos; the figure is 38.6% (Akinyemi *et al.,* 1997). Much higher incidences were reported in some towns, 60% in Lafia (Kolawole *et al*., 2009), 67.2% in Yola, Adamawa State (El-Mahmood *et al.,* 2009) and 77.9% in Enugu (Mbata, 2007).

Antibiotic resistance is a serious public health problem resulting in increased morbidity and mortality. In urinary tract infections, resistance rates against commonly prescribed antibiotics are constantly rising. Nowadays, in many countries more than 20% of uropathogens are resistant to Trimethoprim/ Sulfamethoxazole (TMP/SMX) and cephalosporins. This increasing resistance is also being observed for Flouroquinolones with resistance rates rising up to 10% (Schito *et al.*, 2009; De Backer *et al*., 2008).

Worldwide, Flouroquinolones are being used as the most common antimicrobials for all UTIs, both complicated and uncomplicated. Raul, (2007) explored risk factors for developing Quinolone resistance in uropathogens in the community. UTI is the most common bacterial infection seen in the community, and *E. coli* is responsible for about

70% to 80% of all the uropathogens. Quinolones are one of the most widely used antibiotics in the community for the treatment of UTI, and it is this unfortunate excessive use of the agent that has led to a considerable and worrying increase in the rate of *E. coli* resistant

isolates in many countries. The magnitude of the problem worldwide is becoming very apparent. In as much as the major risk factor associated with the development of these microorganisms is overuse, physicians must practice antibiotic stewardship and avoid the empiric use of quinolones when other antimicrobials may be adequate (Raul, 2007).

Fluoroquinolone resistance is increasing and it is associated with multi-drug resistance. The indiscriminate use of Fluoroquinolones as empirical treatment for the UTIs will facilitate the emergence of resistance to this class of compounds and promote the emergence of multi-drug resistant strains and it should be discouraged as it will undermine the efficacy of Fluoroquinolones to treat more serious infections (James *et al.*, 2006).

The etiology and resistance pattern of community-acquired uropathogens has not been extensively studied in Birnin Kudu community.

It is therefore, important to study susceptibility of some common uropathogens (*Escherichia coli, Klebsiella, Citrobacter, Serratia and Staphylococcus spp*) and the reason for significant variation in antibiotic resistance through molecular characterization of these organisms.

1.3 JUSTIFICATION

Antimicrobial misuse has led to an increased microbial resistance and consequent spread of bacterial resistant strains in both community and hospital settings. Unfortunately there are few publications about the main uropathogens implicated in community-acquired urinary tract infection and their antimicrobial resistance pattern, when compared with nosocomial urinary tract infections.

The cost of microscopy, culture and susceptibility testing is more than the antibiotic treatment itself. These factors have complicated empiric treatment of UTI as data on prevalence of uropathogens and antimicrobial susceptibility are not readily available particularly in developing countries.

Considering the fact that as with many community-acquired infections, resistance rates to antimicrobials commonly used in treatment of UTI is increasing and susceptibility of microorganisms shows significant geographical variations (Gupta *et al*., 1999). Therefore, studies to increase knowledge on etiologic agents of UTIs and their resistance patterns to antibiotics at the local levels are very important as we know there is growing problem of drug resistance which means there is an urgent need for continuous surveillance of antibiotic susceptibility of uropathogens. Appropriate knowledge of local antimicrobial resistance trends is of utmost importance in order to step up evidence based recommendations in empiric antibiotic treatment of UTI.

There are also public health issues as UTI is related to quality of life. Lower urinary tract symptoms which accompany UTI such as urinary urgency, frequency, painful urination, hesitancy, and the sense of incomplete bladder emptying have negative impact on the quality of life (Liao *et al*., 2009). In addition to a reduction in quality of life for women

who are symptomatic, in countries with limited health care resources, unnecessary UTIs might be expected to cause a drain on the already struggling health care apparatus.

1.4 AIM

To determine the antibiogram of uropathogenic bacteria isolated from patients in some hospitals in Birnin kudu, Jigawa State, Nigeria

1.5 SPECIFIC OBJECTIVES

The specific objectives of this work are to:

a. Characterize bacterial uropathogens from patients that are diagnosed with UTI using Rapid identification test kits.
b. Determine the antibiotics susceptibility profiles of the isolated bacterial uropathogens using the agar diffusion technique
c. Explore the presence of β- lactamase in the isolated bacteria
d. Determine if the resistance to antimicrobial agents is plasmid mediated using the Gel electrophoresis method.

1.6 HYPOTHESIS

NULL HYPOTHESIS (H_O)

- Uropathogens isolated from UTI patients are generally susceptible to commonly used antibiotics.

ALTERNATE HYPOTHESIS (H_A)

- Uropathogens isolated from UTI patients in FMC and GHC, Birnin kudu are not susceptible to commonly used antibiotics

1.7 RESEARCH LIMITATION

- Collection of isolates was limited to UTI patients that attended the hospitals from August, 2014 to January, 2015.
- Only isolates that were multi-drug resistant underwent further molecular studies to determine the R-plasmid profiles.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Urinary Tract Infection (UTI)

A urinary tract infection (UTI) is an infection caused by bacteria in parts of the urinary tract. Other organism such as viruses or fungi may be the cause in some cases. In the lower urinary tract, it is known as a simple cystitis. Symptoms from a lower urinary tract infection include painful urination and either frequent micturation or wanting to urinate or both. In the upper urinary tract, it is known as pyelonephritis. Symptoms of a kidney infection also include fever and side and back pain. In old people and young children, the symptoms are not always as clear. The main cause for both types is the bacteria *Escherichia coli.*

Urinary tract infections are the most frequent bacterial infection in women. They occur most frequently between the ages of 16 and 35 years, with 10% of women getting an infection yearly and 60% having an infection at some point in their lives (Nicolle, 2008 and Salvatore, 2011). Recurrences are common, with nearly half of people getting a second infection within a year. Urinary tract infections occur four times more frequently in females than in males (Salvatore, 2011). Pyelonephritis occurs between 20-30 times less frequently (Nicolle, 2008). UTIs are the most common cause of hospital acquired infections accounting for approximately 40%. Rates of asymptomatic bacteria in the urine increase with age from two to seven percent in women of child bearing age to as high as 50% in elderly women in care homes. Rates of asymptomatic bacteria in the urine among men over 75 are between 7-10% (Bhat *et al.,* 2011).

Lower urinary tract infection is also known as a bladder infection. The most common symptoms are burning with urination and having to urinate frequently (or wanting to urinate) without vaginal discharge or significant pain. These symptoms can vary from mild to severe (Lane and Takhar, 2011). In healthy women, the symptoms last an average of six days. Some people will have pain above the pubic bone (lower abdomen) or in the lower back. People who have an upper urinary tract infection, or pyelonephritis (a kidney infection) can have flank pain, fever (a high temperature), or nausea and vomiting. Those symptoms are in addition to the normal symptoms of a lower urinary tract infection (Lane and Takhar, 2011). In rare cases the urine looks bloody (Salvatore, 2011) or contains visible pyuria (pus in the urine).

In young children, fever can be the only symptoms of a urinary tract infection (UTI). Many medical associations recommend urine culture for females younger than two year old or uncircumcised males who are younger than a year and have a fever. Infants with UTI sometimes eat poorly, vomit, sleep more, or show signs of jaundice. Older children can have urinary incontinence (Bhat *et al.,* 2011).

Urinary tract infection are frequently not seen in those who are old (Woodford and Goerge, 2011). Sometimes, the only symptoms are incontinence (loss of bladder control), a change in mental status (ability to think), or feeling tired (Lane and Takhar, 2011). The first symptom for some old people is sepsis, an infection of the blood (Salvatore, 2011). Diagnosis can be difficult because many old people are incontinent or have dementia (Woodford and George, 2011).

Sexual intercourse is the cause of 75-90% of bladder infections in young, sexually active women. The risk of infection is related to how often they have sex (Nicolle, 2008). With

UTIs so frequent when women first get married, the term “honeymoon cystitis” is often used. In post-menopausal women, sexual activity does not affect the risk of developing a UTI. Women get more UTIs than men because women have a urethra that is much shorter and closer to the anus (Dielubanza and Schaeffer, 2011). As a woman’s estrogen levels decrease with menopause, the risk of urinary tract infections increases due to the loss of protective vaginal flora (Dielubanza and Schaeffer, 2011).

A urinary catheter is a tube that is put into the bladder to drain the urine. Using a catheter increases the risk for urinary tract infections. The risk of bacteriuria (bacteria in the urine) is 3%-6% every day the catheter is used. Antibiotics do not stop these infections (Dielubanza and Schaeffer, 2011).

Bladder infections are more common in some families. Other risk factors include diabetes, being circumcised, and having a large prostate (Lane and Takhar, 2011). Complicating factors are not completely clear. These factors may include some anatomic problems, functional, or metabolic problems. A complicated UTI is more difficult to treat and usually needs more aggressive evaluation, treatment, and follow-up. In children, UTIs are linked to vesicouretheral reflux and constipation (Bhat *et al.*, 2011).

2.2.1 Classification of UTI

Uncomplicated and Complicated UTI in Pregnancy

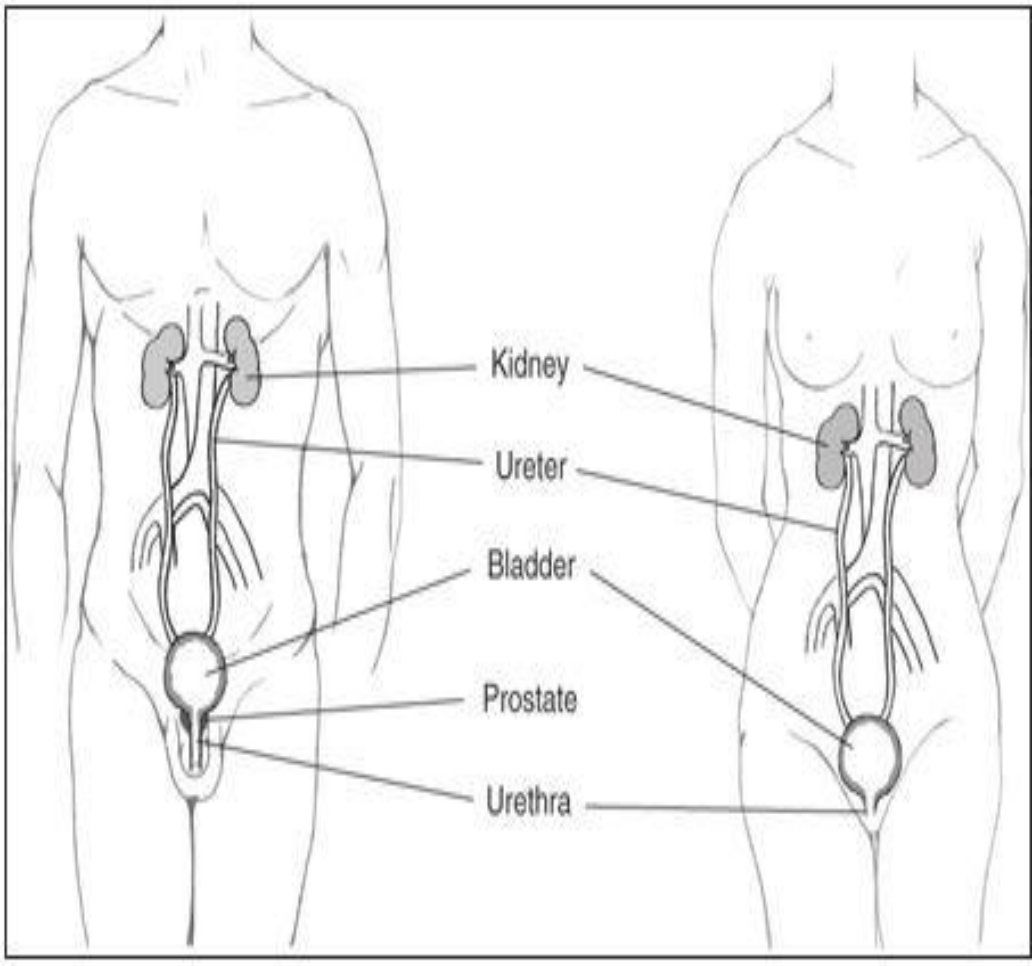
An uncomplicated UTI is one that occurs in a healthy host in the absence of structural or functional abnormalities of the urinary tract. Uncomplicated UTI is encountered most frequently in healthy, young, non-pregnant women (some authorities hold that UTI in all other patient groups is by definition complicated).

A urinary tract infection is said to be complicated if (Colgan and Williams, 2011) it is in the upper tract, the person has diabetes mellitus, the person is pregnant, the person is male, the person has a weakened immune system because of another illness. Complicated UTI is more difficult to treat and usually requires more aggressive evaluation and follow-up. An acute complicated urinary tract infection (UTI) is one of the most common bacterial infections in women (Hooton, 2012). It is estimated that 60% of all women report having a UTI at least once in their lifetime (Foxman, 2002).

Asymptomatic Bacteriuria (ASB)

ASB is defined as persistent bacterial colonization of the urinary tract without urinary symptoms; defined by >100,000 CFU of a single organism. Cystitis has symptoms of increased frequency, urgency, dysuria, hematuria, pyuria, and lack of evidence for systemic illness. Asymptomatic bacteriuria occurs in 4% to 8% of all pregnancies. Significant bacteriuria may exist in asymptomatic patients. Patients with asymptomatic bacteriuria have increased risk of developing pyelonephritis. Untreated asymptomatic bacteriuria leads to the development of symptomatic cystitis in approximately 30 percent of patients and can lead to the development of pyelonephritis in up to 50 percent (Kass, 1970). Asymptomatic bacteriuria is associated with an increase risk of intra-uterine growth retardation and low-birth-weight infants (Harris *et al.,* 1976). The relatively high prevalence of asymptomatic bacteriuria during pregnancy, the significant consequences for women and for the pregnancy, plus the ability to avoid sequelae with treatment justify screening pregnant women for bacteriuria.

The picture below shows the male and female urinary tract system



(AIPM, 2004)

Fig 2.1: The Male and Female Urinary Tract

Upper Urinary Tract Infection

This urinary infection is caused due to the transfer of bacteria from urinary bladder to the kidneys. The kidney infection is also known as Pyelenophritis. This infection shows all the more severe effects than the bladder infection. Hence, one should get themselves

diagnosed as soon as they notice the symptoms of kidney infections because the infection aggravates and damages the kidneys.

Pyelonephritis

Acute pyelonephritis is characterized by fever, flank pain, and tenderness in addition to significant bacteriuria. Other symptoms may include nausea, vomiting, frequency, urgency, and dysuria. Furthermore, women with additional risk factors (immune suppression, diabetes, sickle cell anemia, and neurogenic blader, recurrent or persistent UTIs before pregnancy) are at increased risk for a complicated UTI.

The diagnosis is made when the presence of bacteriuria is accompanied by systemic symptoms or signs such as fever, chills, nausea, and vomiting and flank pain. Pyelonephritis occurs in 2 percent of pregnant women; up to 23 percent of these women have a recurrence during the same pregnancy (Gilstrap *et al.,* 1981).

Lower Urinary Tract Infection

This infection is caused due to the attack of bacterium on the urinary bladder or urethra. The urinary bladder infection is known as cystitis, while urethra infection is known as urethritis.

Acute Cystitis

Acute cystitis is distinguished from asymptomatic bacteriuria by the presence of symptoms such as dysuria, urgency, hematuria, nocturia, suprapubic discomfort and frequency in afebrile patients with no evidence of systemic illness. Up to 30 percent of patients with untreated asymptomatic bacteriuria later develop symptomatic cystistis

(Kass, 1970). A study conducted over a six-year period, found that 1.3 percent of obstetric patients who delivered at a single hospital developed acute cystitis with no symptoms of pyelonephritis (Harris *et al.,* 1976; Gilstrap *et al.,* 1981). Acute cystitis involves only the lower urinary tract; it is characterized by inflammation of the bladder as a result of bacteria or non bacteria causes (example, viral infection). Acute cystitis develops in approximately 1% of pregnant patients, of who 60% have a negative result on initial screening.

2.2.2 Causative Organisms

Escherichia coli is the predominant uropathogen (80 percent), isolated in acute community -acquired uncomplicated UTIs, followed by Staphylococcus saprophyticus with 10 to 15 percent). *Enterococcus, Klebsiella, Enterobacter, and Proteus* species are less common causes (Ronald, 2002). In recurrent uncomplicated UTIs, reinfection occurs when the initially infecting bacteria persist in the fecal flora after elimination from the urinary tract, subsequent recolonizing the bladder (Hooton, 2012). A number of host factors appear to predispose otherwise healthy young women to recurrent UTIs. These include local pH and cervicovaginal antibody changes in the vagina; greater adherence of uropathogenic bacteria to the uroepithelium; and possible pelvic anatomic differences, such as shorter urethra-to-anus distance. Diabetes mellitus, neurologic conditions, chronic institutional residence, and chronic indwelling urinary catheterization are important predisposing factors for complicated UTIs. In affected patients, organisms that are typically less virulent may cause marked illness, although *E. coli* infection remains the most common organism in nearly all patients. *Klebsiella* and groups B *Streptococcus*

infections are relatively more common in patients with diabetes, and *Pseudomonas* infections are relatively more common in patients with chronic catheterization.

(a) Escherichia coli

Escherichia coli is a Gram-negative, facultative anaerobic, rod-shaped bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms (Singleton, 1999). Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination . The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K_2, and preventing colonization of the intestine with pathogenic bacteria. (Hudault *et al*., 2001; Reid *et al.,* 2001).

Escherichia coli and other facultative anaerobes constitute about 0.1% of gut flora (Eckburg *et al*., 2005) and fecal–oral transmission is the major route through which pathogenic strains of the bacterium cause disease. *E coli* can survive outside the body for a limited period of time, which makes them potential indicator organisms to test environmental samples for fecal contamination (Thompson, 2007).

Escherichia coli cells are typically rod-shaped, and are about 2.0 micrometers (μm) long and 0.25–1.0 μm in diameter, with a cell volume of 0.6–0.7 μm^3 (Kubitschek, 1990). It can live on a wide variety of substrates, it uses mixed-acid fermentation in anaerobic conditions, producing lactate, succinate, ethanol, acetate, and carbon dioxide.

Optimal growth of *E. coli* occurs at 37 °C, but some laboratory strains can multiply at temperatures of up to 49 °C (Fotadar *et al*., 2005). Growth can be driven by aerobic or

anaerobic respiration, using a large variety of redox pairs, including the oxidation of pyruvic acid, formic acid, hydrogen, and amino acids, and the reduction of substrates such as oxygen, nitrate, fumarate, dimethyl sulfoxide, and trimethylamine N-oxide (Ingledew and Poole, 1984).

Escherichia coli has the ability to transfer DNA via bacterial conjugation, transduction or transformation, which allows genetic material to spread horizontally through an existing population. This process led to the spread of the gene encoding shiga toxin from *Shigella* to *E. coli* O157:H7, carried by a bacteriophage.

Escherichia coli encompass an enormous population of bacteria that exhibit a very high degree of both genetic and phenotypic diversity. Genome sequencing of a large number of isolates of *E. coli* and related bacteria shows that a taxonomic reclassification would be desirable. However, this has not been done, largely due to its medical importance (Krieg and Holt, 1984) and *E. coli* remains one of the most diverse bacterial species.

A strain is a subgroup within the species that has unique characteristics that distinguish it from other strains. These differences are often detectable only at the molecular level; however, they may result in changes to the physiology or lifecycle of the bacterium. For example, a strain may gain pathogenic capacity, the ability to use a unique carbon source, the ability to take upon a particular ecological niche, or the ability to resist antimicrobial agents. Different strains of *E. coli* are often host-specific, making it possible to determine the source of fecal contamination in environmental samples (Feng *et al*., 2002; Thompson, 2007). For example, knowing which *E. coli* strains are present in a water sample allows researchers to make assumptions about whether the contamination

originated from a human, another mammal, or a bird. Most *E. coli* strains do not cause disease, but virulent strains can cause gastroenteritis, urinary tract infections, and neonatal meningitis. In rare cases, virulent strains are also responsible for hemolytic-uremic syndrome, peritonitis, mastitis, septicemia, and Gram-negative pneumonia.

There is one strain, *E.coli* 0157:H7, that produces a toxin called the Shiga Toxin. This toxin causes premature destruction of the red blood cells which then clog the body's filtering system, the kidneys causing hemolytic-uremic syndrome (HUS). This in turn causes strokes due to small clots of blood which lodge in capillaries in the brain. This causes the body parts controlled by this region of the brain not to work properly. In addition, this strain causes the buildup of fluid leading to edema around the lungs and legs and arms. This increase in fluid buildup especially around the lungs impedes the functioning of the heart, causing an increase in blood pressure.

One of the main causes of urinary tract infections is Uropathogenic *E. coli* (UPEC). It is part of the normal flora in the gut and can be introduced in many ways especially for females; wiping back to front can lead to fecal contamination of the urogenital orifices. Anal intercourse can also introduce this bacterium into the male urethra, and in switching from anal to vaginal intercourse, the male can also introduce UPEC to the female urogenital system.

(b) *Staphylococcus aureus*

Staphylococcus aureus is a Gram-positive coccal bacterium that is a member of the Firmicutes, and is frequently found in the human respiratory tract and on the skin. It is positive for catalase and nitrate reduction. Although *S. aureus* is not always pathogenic, it

is a common cause of skin infections, respiratory disease and food poisoning. Disease-associated strains often promote infections by producing potent protein toxins, and expressing cell-surface proteins that bind and inactivate antibodies.

Staphylococcus aureus is the most common species of *Staphylococcus* to cause *Staph* infections and is a successful pathogen due to combination of nasal carriage and bacterial immuno-evasive strategies (Kluytmans *et al.*, 1997). *S. aureus* can cause a range of illnesses, from minor skin infections, such as pimples, impetigo, boils, cellulitis folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia and sepsis.

Staphylococcus aureus is a facultative anaerobic Gram-positive coccal bacterium also known as "golden staph". *S. aureus* appears as grape-like clusters when viewed through a microscope, and has large, round, golden-yellow colonies, often with hemolysis, when grown on blood agar plates (Ryan and Ray, 2004).

Staphylococcus aureus is catalase-positive. Catalase converts hydrogen peroxide to water and oxygen. Catalase-activity tests are sometimes used to distinguish staphylococci from enterococci and streptococci. Previously, *S. aureus* was differentiated from other staphylococci by the coagulase test. However it is now known that not all *S. aureus* are coagulase-positive (Ryan and Ray, 2004).

Staphylococcus aureus infections can spread through contact with pus from an infected wound, skin-to-skin contact with an infected person by producing hyaluronidase that

destroys tissues, and contact with objects such as towels, clothing, or athletic equipment used by an infected person.

Staphylococcus aureus produces various enzymes such as coagulase which clots plasma and coats the bacterial cell to probably prevent phagocytosis. Hyaluronidase breaks down hyaluronic acid and helps in spreading of *Staphylococcus aureus*. *S.aureus* also produces DNAse which breaks down the DNA, lipase to digest lipids, staphylokinase to dissolve fibrin and aid in spread, and beta-lactamase for drug resistance. Depending on the strain, *S. aureus* is capable of secreting several exotoxins, which can be categorized into three groups. Many of these toxins are associated with specific diseases (Dinges *et al.*, 2000).

Superantigens

This group includes the toxin toxic shock syndrome (TSST-1), enterotoxin type B, which causes TSS associated with tampon use. This is characterized by fever, erythematous rash, hypotension, shock, multiple organ failure, and skin desquamation. Lack of antibody to TSST-1 plays a part in the pathogenesis of toxic shock syndrome. Other strains of *S. aureus* can produce an enterotoxin that is the causative agent of *S. aureus* gastroenteritis. This gastroenteritis is self-limiting, characterized by vomiting and diarrhea one to six hours after ingestion of the toxin with recovery in eight to 24 hours. Symptoms include nausea, vomiting, diarrhea, and major abdominal pain (Becker *et al.*, 2003).

Exfoliative toxins (EF)

EF toxins are implicated in the disease staphylococcal scalded-skin syndrome (SSSS), which occurs most commonly in infants and young children. It also may occur as

epidemics in hospital nurseries. The protease activity of the exfoliative toxins causes peeling of the skin observed with SSSS (Becker *et al.,* 2003).

Other toxins

Staphylococcal toxins that act on cell membranes include alpha toxin, beta toxin, delta toxin, and several bicomponent toxins. The bicomponent toxin Panton-Valentine leukocidin (PVL) is associated with severe necrotizing pneumonia in children (Gillet *et al.*, 2002). The genes encoding the components of PVL are encoded on a bacteriophage found in community-associated methicillin-resistant *S. aureus* (MRSA) strains.

The treatment of choice for *S. aureus* infection is penicillin; in most countries, however, penicillin resistance is extremely common, and first-line therapy is most commonly a penicillinase-resistant β-lactam antibiotic (Oxacillin or Flucloxacillin). Combination therapy with gentamicin may be used to treat serious infections, such as endocarditis, but its use is controversial because of the high risk of damage to the kidneys.

Antibiotic resistance in *S. aureus* was uncommon when penicillin was first introduced in 1943. Staphylococcal resistance to penicillin is mediated by penicillinase production: an enzyme that cleaves the β-lactam ring of the penicillin molecule, rendering the antibiotic ineffective. Penicillinase-resistant β-lactam antibiotics such as methicillin, nafcillin, oxacillin, cloxacillin, dicloxacillin, and flucloxacillin, are able to resist degradation by staphylococcal penicillinase. Methicillin-resistant *S. aureus* which is one of a number of greatly feared strains of *S. aureus* have become resistant to most β-lactam antibiotics. Resistance is conferred by the *mecA* gene, which codes for an altered penicillin-binding

protein (PBP2a or PBP2') that has a lower affinity for binding β-lactams. This allows for resistance to all β-lactam antibiotics, and obviates their clinical use during MRSA infections. As such, the glycopeptide vancomycin is often deployed against MRSA.

Aminoglycoside antibiotics, such as kanamycin, gentamicin, streptomycin were once effective against staphylococcal infections until strains evolved mechanisms to inhibit the aminoglycosides' action, which occurs via protonated amine and/or hydroxyl interactions with the ribosomal RNA of the bacterial 30S ribosomal subunit (Carter *et al.,* 2000). There are three main mechanisms of aminoglycoside resistance mechanisms which are currently and widely accepted: aminoglycoside modifying enzymes, ribosomal mutations, and active efflux of the drug out of the bacteria.

Aminoglycoside-modifying enzymes inactivate the aminoglycoside by covalently attaching either a phosphate, nucleotide, or acetyl moiety to either the amine or the alcohol key functional group (or both groups) of the antibiotic. This changes the charge or sterically hinders the antibiotic, decreasing its ribosomal binding affinity. In *S. aureus*, the best-characterized aminoglycoside-modifying enzyme is aminoglycoside adenylyltransferase.

Nowadays, *S. aureus* has become resistant to many commonly used antibiotics. In the UK, only 2% of all *S. aureus* isolates are sensitive to penicillin, with a similar picture in the rest of the world. The β-lactamase-resistant penicillins (methicillin, oxacillin, cloxacillin, and flucloxacillin) were developed to treat penicillin-resistant *S. aureus* and are still used as first-line treatment.

Both nosocomial and community acquired MRSA are commonly treated with non-β-lactam antibiotics such as clindamycin and co-trimoxazole. Resistance to these antibiotics has also led to the use of new, broad-spectrum anti-Gram-positive antibiotics, such as linezolid, because of its availability as an oral drug. First-line treatment for serious invasive infections due to MRSA is currently glycopeptide antibiotics (vancomycin and teicoplanin).

Vancomycin-resistant *S. aureus* (VRSA) is a strain of S. *aureus* that has become resistant to the glycopeptides. The first case of *S. aureus* truly resistant to glycopeptide antibiotics was only reported in 2002 (Chang *et al.*, 2003). Three cases of VRSA infection had been reported in the United States as of 2005 (Menichetti, 2005).

(c) Enterobacter spp

Enterobacter spp include *E. cloacae, E. aerogenes, E. agglomerans, E. gergoviae, E. sakazakii, E. cowanii, E. hormaechei, E. taylorae , E. asburiae, E. intermedius, E. amnigenus, E. dissolvens, E. kobei, E. pyrinus and E. nimpressuralis* (Hart, 2006) , *E. cancerogenus* (Abbott, 2007)

Enterobacter spp. is in the family Enterobacteriaceae (Hart, 2006). They are facultatively anaerobic Gram-negative bacilli, 0.6-1 μm in diameter and 1.2-3 μm long, motile by means of flagella and have class 1 fimbriae (Paterson *et al.*, 2005). They produce acid upon glucose fermentation, methyl red negative, oxidase-negative, indole-negative, urease variable and Voges-Proskauer positive, with an optimal growth temperature of 30 °C. It does not belong to the fecal coliforms or thermotolerant coliforms group of

bacteria, unlike *Escherichia coli*, because it is incapable of growth at 44.5°C in the presence of bile salts. The genus *Enterobacter* ferments lactose with gas production during a 48-hour incubation at 35-37°C in the presence of bile salts and detergents.

Enterobacter spp. can cause numerous infections, including cerebral abscess, pneumonia, meningitis, septicemia, and wound, urinary tract (particularly catheter-related UTI), and abdominal cavity/intestinal infections (Farmer *et al.*, 2007). In addition, *Enterobacter* spp. have been noted in intravascular device-related infections and surgical site infections primarily postoperative or related to devices such as biliary stents (Russo and Johnson, 2008).

E. cloacae and *E. aerogenes* are responsible for the majority of *Enterobacter* infections, 65-75% and 15-25 %, respectively (Russo and Johnson, 2008). *Enterobacter* spp. is commonly found in intensive care units and is responsible for 8.6 % of nosocomial infections according to the US Centers for Disease Control and Prevention (Boyce *et al.*, 2004). *Enterobacter* spp are also involved in a considerable proportion of reported bacteremia cases; in one pediatric hospital, *Enterobacter* spp. were noted to be the most common cause of bacteremia, accounting for 14 % of cases (Boyce *et al.,* 2004), while in adults *Enterobacter* spp. are responsible for 1.5-6% of bacteremia cases (Hart, 2006). Infective dose of approximately 1000 cells have been considered infectious, similar to the infectious dose of the pathogenic bacteria *Neisseria meningitidis* and *Escherichia coli* O157 (Iversen and Forsythe, 2003)

Transmission is through direct or indirect contact of mucosal surfaces with infectious agent or in the case of endogenous flora, through transfer to adjacent, susceptible, sterile

body sites (Ryan, 2004). Enterobacteriaceae can also be spread through the fecal-oral route (Bayda *et al.,* 2007).

Enterobacter spp. is commonly found in soil and water; *E. cloacae* and *E. aerogenes* can inhabit the intestines of humans and animals and can also be found in sewage (Hart, 2006).

Most *Enterobacter* spp are susceptible to cefepime (Russo and Johnson, 2008), aminoglycosides, fluoroquinolones, and trimethoprim-sulfamethoxazole. Tigecycline has been shown effective in vitro (Russo and Johnson, 2008).

Enterobacter spp. is resistant to ampicillin; first- and second- generation cephalosporins (Russo and Johnson, 2008) and cephalothin (Farmer *et al*., 2007).

(d) Klebsiella pneumoniae

This is a Gram-negative, nonmotile, encapsulated, lactose-fermenting, facultative anaerobic, rod-shaped bacterium.

Although found in the normal flora of the mouth, skin, and intestines (Ryan and Ray, 2004), it can cause destructive changes to human lungs if aspirated, specifically to the alveoli resulting in bloody sputum. In the clinical setting, it is the most significant member of the *Klebsiella* genus of Enterobacteriaceae. *Klebsiella oxytoca* and *Klebsiella rhinoscleromatis* have also been demonstrated in human clinical specimens. In recent years, klebsiellae have become important pathogens in nosocomial infections.

Members of the *Klebsiella* genus typically express two types of antigens on their cell surfaces. The first, O antigen is a component of the lipopolysaccharide (LPS), of which 9 varieties exist. The second is K antigen, a capsular polysaccharide with more than 80 varieties (Podschum and Ullmann, 1998). Both contribute to pathogenicity and form the basis for serogrouping.

Klebsiella pneumoniae can cause destructive changes to human lungs via inflammation and hemorrhage with cell death (necrosis) that sometimes produces thick, bloody, mucoid sputum. These bacteria gain access typically after a person aspirates colonizing oropharyngeal microbes into the lower respiratory tract.

As a general rule, *Klebsiella* infections are seen mostly in people with a weakened immune system. Most often, illness affects middle-aged and older men with debilitating diseases. This patient population is believed to have impaired respiratory host defenses, including persons with diabetes, alcoholism, malignancy, liver disease, chronic obstructive pulmonary diseases (COPD), glucocorticoid therapy, renal failure, and certain occupational exposures such as paper mill workers. Many of these infections are obtained when a person is in the hospital for some other reason (a nosocomial infection). Feces are the most significant source of patient infection, followed by contact with contaminated instruments.

The most common condition caused by *Klebsiella* bacteria outside the hospital is pneumonia, typically in the form of bronchopneumonia and also bronchitis. These patients have an increased tendency to develop lung abscess, cavitation and pleural

adhesions. It has a high death rate of about 50%, even with antimicrobial therapy. The mortality rate can be nearly 100% for people with alcoholism and bacteremia.

In addition to pneumonia, *Klebsiella* can also cause infections in the urinary tract, lower biliary tract, and surgical wound sites. The range of clinical diseases includes pneumonia, thrombophlebitis, urinary tract infection, cholecystitis, diarrhea, upper respiratory tract infection, wound infection, osteomyelitis, meningitis, and bacteremia and septicemia. For patients with an invasive device in their bodies, contamination of the device becomes a risk; for example, respiratory support equipment and urinary catheters put patients at increased risk. Two unusual infections of note from *Klebsiella* are rhinoscleroma and ozena. Rhinoscleroma is a chronic inflammatory process involving the nasopharynx. Ozena is a chronic atrophic rhinitis that produces necrosis of nasal mucosa and mucopurulent nasal discharge.

Klebsiella ranks second to *E. coli* for urinary tract infections in older people. It is also an opportunistic pathogen for patients with chronic pulmonary disease, enteric pathogenicity, nasal mucosa atrophy, and rhinoscleroma.

Klebsiella organisms are often resistant to multiple antibiotics. Current evidence implicates plasmids as the primary source of the resistance genes (Hudson *et al.,* 2014). *Klebsiella* with the ability to produce extended-spectrum beta-lactamases (ESBL) are resistant to many classes of antibiotics. The most frequent resistances include resistance to aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol, and trimethoprim/sulfamethoxazole (Nathisuwan *et al.,* 2001).

Infection with carbapenem-resistant Enterobacteriaceae (CRE) or carbapenemase-producing Enterobacteriaceae is emerging as an important challenge in health-care settings (Limbago *et al.*, 2011). One of many CREs is carbapenem-resistant *Klebsiella pneumoniae* (CRKP). Over the past 10 years, a progressive increase in CRKP has been seen worldwide; however, this new emerging nosocomial pathogen is probably best known for an outbreak in Israel that began around 2006 within the healthcare system there (Berrie, 2007). In the USA, it was first described in North Carolina in 1996, since then CRKP has been identified in 41 states (Vastag, 2012) and is recovered routinely in certain hospitals in New York and New Jersey. It is now the most common CRE species encountered within the United States.

CRKP is resistant to almost all available antimicrobial agents, and infections with CRKP have caused high rates of morbidity and mortality, in particular among persons with prolonged hospitalization and those critically ill and exposed to invasive devices. The concern is that carbapenem is often used as a drug of last resort when battling resistant bacterial strains. New slight mutations could result in infections for which there is very little, if anything, healthcare professionals can do to treat patients with resistant organisms.

A number of mechanisms cause carbapenem resistance in Enterobacteriaceae. These include hyperproduction of ampC beta-lactamase with an outer membrane porin mutation, CTX-M extended-spectrum beta-lactamase with a porin mutation or drug efflux, and carbapenemase production. The most important mechanism of resistance by CRKP is the production of a carbapenemase enzyme, *blak*pc. The gene that encodes the

*blak*pc enzyme is carried on a mobile piece of genetic material (a transposon; the specific transposon involved is called Tn4401), which increases the risk for dissemination. CRE can be difficult to detect because some strains that harbor *blak*pc have minimal inhibitory concentrations (MICs) that are elevated but still within the susceptible range for carbapenems. Because these strains are susceptible to carbapenems, they are not identified as potential clinical or infection control risks using standard susceptibility testing guidelines. Patients with unrecognized CRKP colonization have been reservoirs for transmission during nosocomial outbreaks.

Place all patients colonized or infected with CRE or carbapenemase-producing Enterobacteriaceae on contact precautions. Acute-care facilities are to establish a protocol, in conjunction with the guidelines of the Clinical and Laboratory Standards Institute to detect nonsusceptibility and carbapenemase production in Enterobacteriaceae, in particular *Klebsiella* spp. and *Escherichia coli*, and immediately alert epidemiology and infection control staff members if identified. All acute-care facilities are to review microbiology records for the preceding 6-12 months to ensure that there have not been previously unrecognized CRE cases. If they do identify previously unrecognized cases, a point prevalence survey (a single round of active surveillance cultures) in units with patients at high risk (e.g., intensive-care units, units where previous cases have been identified, and units where many patients are exposed to broad-spectrum antimicrobials) is needed to identify any additional patients colonized with carbapenem-resistant or carbapenemase-producing *Klebsiella* spp. and *E. coli*. When a case of hospital-associated CRE is identified, facilities should conduct a round of active surveillance testing of

patients with epidemiologic links to the CRE case (e.g., those patients in the same unit or patients having been cared for by the same health-care personnel (Liedo *et al.,* 2009).

***(e) Citrobacter* spp.**

Citrobacter amalonaticus, Citrobacter braakii, Citrobacter farmeri, Citrobacter freundii, Citrobacter koseri, Citrobacter sedlakii, Citrobacter werkmanii, Citrobacter Youngae. *Citrobacter* spp., of the Enterobacteriaceae family, is gram-negative, facultative anaerobic bacteria that appear as rods or coccobacilli at 0.3-1 µm in diameter and 0.6-6 µm long (Abbott, 2007). *Citrobacter* spp. is motile using their peritrichous flagella (Holme and Aucken, 1998). They ferment mannitol with production of gaseous H_2S, and they can also use citrate as sole carbon source (Chen *et al.,* 2002). The genus can be divided in 43 O-serogroups, based on the O antigen of the lipopolysaccharide (LPS) and in 20 chemogroup, based on the sugar composition of the LPS.

Citrobacter are rare opportunistic nosocomial pathogens (Ryan, 2004). *Citrobacter* normally cause urinary tract infections, blood stream infections, intra abdominal sepsis, brain abscesses, and pneumonia and other neonatal infection (Pepperell *et al.*, 2002), such as meningitis, neonatal sepsis, joint infection or general bacteremia (Doran, 1999). CNS infections are more common for infants under 2 months old than for older children or immunocompromised adult patients, but rare cases have been reported. *C. koseri* and *C. ferundii* cause neonatal meningitis that can lead to brain abscesses (Holmes and Aucken, 1998). *Citrobacter* infections can be fatal, with 33-48 % overall death rates, and 30% for neonates (Doran, 1999). Infant survivors may experience significant damage to CNS, including profound retardation, hemiparesis and seizures.

Citrobacter is of worldwide prevalence as it is a component of normal intestinal flora (Ryan, 2004). Neonates (particularly those who are premature) and immunocompromised, elderly or debilitated patients are at increased risk of infection (Chen *et al.*, 2002).

Citrobacter may be spread by direct contact with hospital staff members, mother to child transmission or through ingestion of environmental sources (fecal-oral route) but person-to-person transmission is more prevalent. Parsley contaminated with *Citrobacter* contained in swine manure has caused an outbreak of *Citrobacter* in Canada. The outbreak caused 8 urinary tract infections, and 1 death. *Citrobacter* spp. is susceptible to aminoglycosides, chloramphenicol, imipenim/cilastatin, trimethoprim and trimetoprim/sulfamethazole (Doran, 1995).

2.2.3 Epidemiology

Urinary tract infections are the most frequent bacterial infection in women. They occur most frequently between the ages of 16 and 35 years, with 10% of women getting an infection yearly and 60% having an infection at some point in their lives (Nicolle, 2008; Salvatore, 2011). Recurrences are common, with nearly half of people getting a second infection within a year. Urinary tract infections occur four times more frequently in females than in males (Salvatore, 2011). Pyelonephritis occurs between 20-30 times less frequently (Nicolle, 2008). UTIs are the most common cause of hospital acquired infections accounting for approximately 40%. Rates of asymptomatic bacteria in the urine increase with age from two to seven percent in women of child bearing age to as high as

50% in elderly women in care homes. Rates of asymptomatic bacteria in the urine among men over 75 are between 7-10% (Bhat *et al.,* 2011).

Lower urinary tract infection is also known as a bladder infection. The most common symptoms are burning sensation with urination and frequent urination without vaginal discharge or significant pain. These symptoms can vary from mild to severe (Lane and Takhar, 2011). In healthy women, the symptoms last an average of six days. Some people experience pain above the pubic bone (lower abdomen) or in the lower back. People who have an upper urinary tract infection (a kidney infection) can have flank pain, fever, nausea and vomiting. Those symptoms are in addition to the normal symptoms of a lower urinary tract infection (Lane and Takhar, 2011). In rare cases the urine looks bloody (Salvatore, 2011) or contains visible pyuria (pus in the urine).

In young children, fever can be the only symptoms of a urinary tract infection (UTI). Many medical associations recommended urine culture for females younger than two year old or uncircumcised males who are younger than a year and have a fever. Infants with UTI sometimes eat poorly, vomit, sleep more, or show signs of jaundice. Older children can have urinary incontinence (Bhat *et al.,* 2011).

Urinary tract infection are frequently not seen in those who are old (Woodford and Goerge, 2011). Sometimes, the only symptoms are incontinence (loss of bladder control), a change in mental status (ability to think), or feeling tired (Lane and Takhar, 2011). The first symptom for some old people is sepsis which is an infection of the blood (Salvatore, 2011). Diagnosis can be difficult because many old people are incontinent or have dementia (Woodford and George, 2011).

Sexual intercourse is the cause of 75-90% of bladder infections in young, sexually active women. The risk of infection is related to how often they have sex (Nicolle, 2008). With UTIs so frequent when women first get married, the term “honeymoon cystitis” is often used. In post-menopausal women, sexual activity does not affect the risk of developing a UTI.

Women get more UTIs than men because they have a shorter urethra that is much closer to the anus (Dielubanza and Schaeffer, 2011). As a woman’s estrogen levels decrease with menopause, the risk of urinary tract infections increases due to the loss of protective vaginal flora (Dielubanza and Schaeffer, 2011).

A urinary catheter is a tube that is put into the bladder to drain the urine. Using a catheter increases the risk for urinary tract infections. The risk of bacteriuria is 3-6% every day the catheter is used. Antibiotics do not stop these infections (Dielubanza and Schaeffer, 2011).

Bladder infections are more common in some families. Other risk factors include diabetes, being circumcised, and having a large prostate (Lane and Takhar, 2011). Complicating factors are not completely clear. These factors may include some anatomic problems (relating to physical narrowing), functional, or metabolic problems. A complicated UTI is more difficult to treat and usually needs more aggressive evaluation, treatment, and follow-up. In children, UTIs are linked to vesicoureteral reflux (an abnormal movement of urine from the bladder into ureters or kidneys) and constipation (Bhat *et al.*, 2011).

UTIs are the most common bacterial infections in pregnancy (McNeeley, 1988). About 20% of pregnant women are affected and it is the most common cause of admission in

obstetrical wards (Bacak *et al.*, 2005). Masinde *et al.* reported that symptomatic and asymptomatic bacteriuria have been reported for 17.9% and 13.0% pregnant women, respectively.

Incidence of Asymptomatic bacteriuria during pregnancy has been reported to be 2-10% in the United States (Andrews and Gilstrap, 1992; Sweet, 1977) and 2-5% in the United Kingdom. In Canada, the prevalence rate varies from 4-7%. The prevalence is higher among individuals in lower socioeconomic classes and those with a past history of asymptomatic urinary tract infection (Nicolle, 1994). Other reports have noted that Asymptomatic bacteriuria occurs in 5- 9% of both non-pregnant and pregnant women and that if left untreated in pregnancy, progression to symptomatic UTI including acute cystitis and pyelonephritis occurs in 15 to 45% or 4-fold higher than in non-pregnant women (Barry, 1997). A prevalence rate of 7% in pregnant women has been reported in Ethopia (Gabre-Selassie, 1994).

A study from Bangkok, Thailand (Kovavisarach *et al.*, 2009) reported a prevalence rate for asymptomatic bacteriurea in pregnant women of 10% with an increase risk of asymptomatic bacteriurea in women with a lower educational level. This was consistent with other studies that showed a similar increased risk with either lower educational level or lower socio economic level. Incidence of UTI during pregnancy in India is 8.8% (Mandipa and Rama, 2011).

Masinde published a cross-sectional study from Tanzania in 2009 to determine the prevalence of UTI in both symptomatic and asymptomatic pregnant women. Of the 247 women in the sample, 31.5% were symptomatic and of those women, 18% had bacteriuria while of the 68.5% who were asymptomatic, 13% had bacteriuria.

The incidence of UTI among pregnant women in North Khartoun was 14.0% regardless of the women's age, parity and gestation age. The incidence of UTI among these women is similar to the incidence of UTI among pregnant women in the neighboring countries were 14.6% and 11.6% in Tanzania and Ethopia respectively (Masinde *et al.*, 2009; Assefa *et al.,* 2008). Age, parity and gestational age were not associated with urinary tract infection in neighboring Tanzania (Masinde *et al.,* 2009). However, maternal age, parity and morbid obesity have been previously observed as risk factors for UTI among pregnant women (Arpi and Renneberg, 1984). Likewise in Khartoum, gestational age was not found as risk factor for UTI among pregnant women. Recently, it has been reported that, UTI developed in third trimester. Perhaps the susceptibility of UTI during this period is due to uretral dilatation which started as early as six (6) week and reaching the maximum during 22-24 weeks (Delzell and Lefevre, 2000). Other factors like low socioeconomic status, sexual activity, washing genitals precoitus, and postcoitus, not voiding urine postcoitus and washing genitals from back to front have been observed as risk factors for UTI during pregnancy (Fluit and Schmitz, 2001). Interestingly, high prevalence of urinary tract infection has been reported among Sudanese females with genital mutilation which was widely practiced in Central Sudan. Recently various risk factor of UTI during pregnancy have been reported; perhaps these are varied according to the geographical, social and biological settings (Haider *et al.,* 2010). The earlier documented socio demographic risk factors for urinary tract infection in pregnancy like maternal age and high parity were proven to be associated with urinary tract infection during pregnancy while gestational age was not associated with urinary tract infection in pregnancy. Onyemelukwe *et al.*, (2003) reported that there was no relationship of either

age or parity with bacteriuria in pregnancy. Leigh, (1989) who reported an increasing parity as a risk factor of developing urinary tract infection in pregnancy but no relationship to age in developing urinary tract infection in pregnancy. Onuh *et al*., (2006) reported that there was no relationship between either age or parity and bacteriuria in pregnancy. These differences may be as a result of the different locations in which these studies were being carried out. In Nigeria, Olusanya et al in Sagamu reported a prevalence rate of 23.9% (Olusanya, 1993). Akerele also reported 86.6% in Benin City in 2001 (Akerele, 2001).

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 (WHO, UNAID AND UNICEF, 2011). In people living with HIV/AIDS, almost every part of the genitourinary system is affected with different diseases. In addition, such people are more vulnerable to different bacterial infections including urinary tract infection (UTI) because of high viral load and low CD4 count of the infected individuals (Staiman and Lowe, 2004). Different researchers have shown an increased prevalence rate of UTI in HIV/AIDS patients: prevalence rate of 6.3%–41% was reported from various parts of the world (Michael *et al.*, 2006: Awolude *et al.*, 2010). Antiretroviral therapy (ART), however, improves the health of people infected with HIV/AIDS through decreasing the progression of the infection, restoration of the immunity of the patient, decreasing the viral load, and reducing the opportunistic infections (Schonwald *et al.*, 1999). Studies on the evaluation of the effect of highly active antiretroviral therapy (HAART) show that ART has a significant impact on reduction of the incidence of

bacterial infections including bacteremia, bacterial pneumonia, and urinary tract infections that occur in HIV infected patients (Zolopa *et al.,* 2009). *Staphylococcus aureus* is the predominant bacterial uropathogen among ART user patients (Tumbarello and Tacconelli, 2003; Inyang- Etoh *et al.,* 2009). Escherichia coli (Staiman and Lowe, 2004), Enterococcus species, Pseudomonas aeruginosa, Klebsiella, Acinetobacter, Proteus species, Candida, and Salmonella species are also found among HIV infected patients (Staiman and Lowe, 2004; Awolude *et al.,* 2010).

The incidences of acute urinary tract infections are relatively common in children, with 8 percent of girls and 2 percent of boys having at least one episode by seven years of age (Williams *et al.,* 2006). The most common pathogen is Escherichia coli, accounting for approximately 85 percent of urinary tract infections in children. Renal parenchymal defects are present in 3 to 15 percent of children within one to two years of their first diagnosed urinary tract infection. Clinical signs and symptoms of a urinary tract infection depend on the age of the child, but all febrile children 2 to 24 months of age with no obvious cause of infection should be evaluated for urinary tract infection. Evaluation of older children may depend on the clinical presentation and symptoms that point toward a urinary source (e.g., leukocyte esterase or nitrite present on dipstick testing; pyuria of at least 10 white blood cells per high-power field and bacteriuria on microscopy). Prophylactic antibiotics do not reduce the risk of subsequent urinary tract infections, even in children with mild to moderate vesicoureteral reflux. Constipation should be avoided to help prevent urinary tract infections.

Guidelines regarding the diagnosis, treatment, and follow-up of urinary tract infections (UTIs) in children continue to evolve. Although a somewhat less aggressive approach to

evaluation is now recommended, it is important for primary care physicians to appropriately diagnose and treat UTIs in children. Some underlying etiologies include renal scarring and renal disease which can lead to considerable morbidity later in life.

In a study of infants presenting to pediatric emergency departments, the prevalence of UTI in infants younger than 60 days with a temperature greater than 100.4°F (38°C) was 9 percent (Zorc *et al.,* 2005). The reference standard for the diagnosis of UTI is a single organism cultured from a specimen obtained at the following concentrations: suprapubic aspiration specimen, greater than 1,000 colony-forming units per mL; catheter specimen, greater than 10,000 colony-forming units per mL; or clean-catch, midstream specimen, 100,000 colony-forming units per mL or greater (Hansson *et al.,* 1998: UTI Guideline, 2005). Use of lower colony counts in symptomatic patients has been advocated (Heldrich *et al.,* 2000).

A systematic review found that renal parenchymal defects are identified in 3 to 15 percent of children within one to two years of their first diagnosed UTI (Dick and Feldman, 1996). Long-term complications of UTI associated with renal scarring include hypertension, chronic renal failure, and toxemia in pregnancy. Long-term follow-up data are limited, although one Swedish study found that among patients who had renal scarring from pyelonephritis during childhood, 23 percent developed hypertension and 10 percent developed end-stage renal disease (Jacobson *et al*., 1989). Baseline abnormalities of the urogenital tract have been reported in up to 3.2 percent of healthy, screened infants (Berrocal *et al.,* 2002). Also, obstructive anomalies are found in up to 4 percent and vesicoureteral reflux in 8 to 40 percent of children being evaluated for their first UTI.

2.2.4 Antibiotics Used in Treatment of UTI

(a) Ciprofloxacin

The first members of the quinolone antibacterial class were relatively low-potent drugs such as nalidixic acid, used mainly in the treatment of urinary tract infections owing to their renal excretion and propensity to be concentrated in urine. The discovery of norfloxacin in 1979 and the demonstration that certain structural modifications including the attachment of a fluorine atom to the quinolone ring leads to dramatically enhanced antibacterial potency. Ciprofloxacin is a second-generation fluoroquinolone (Ball, 2000). Chemically, it is 1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid. Its empirical formula is $C_{17}H_{18}FN_3O_3$ and its molecular weight is 331.4 g/mol. It is a faintly yellowish to light yellow crystalline substance (Bolhuis *et al*., 2011). Ciprofloxacin hydrochloride is the monohydrochloride monohydrate salt of ciprofloxacin. It is a faintly yellowish to light yellow crystalline substance with a molecular weight of 385.8 g/mol. Its empirical formula is $C_{17}H_{18}FN_3O_3HCl{\bullet}H_2O$ (Bolhuis *et al.*, 2011).

It is a broad-spectrum antibiotic active against both Gram-positive and Gram-negative bacteria. It functions by inhibiting DNA gyrase, a type II topoisomerase, and topoisomerase IV (Pommier *et al.*, 2010) enzymes necessary to separate bacterial DNA, thereby inhibiting cell division.

Its spectrum of activity includes most strains of bacterial pathogens responsible for respiratory, urinary tract, gastrointestinal, and abdominal infections, including Gram-negative such as *Escherichia coli*, *Haemophilus influenzae*, *Klebsiella pneumoniae*,

Legionella pneumophila, *Moraxella catarrhalis*, *Proteus mirabilis*, and *Pseudomonas aeruginosa*, and Gram-positive such as methicillin-sensitive, but not methicillin-resistant *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, and *Streptococcus pyogenes* bacterial pathogens. Ciprofloxacin and other fluoroquinolones are valued for this broad spectrum of activity, excellent tissue penetration, and for their availability in both oral and intravenous formulations (Brunton *et al.*, 2005).

Ciprofloxacin is used alone or in combination with other antibacterial drugs in the empiric treatment of infections for which the bacterial pathogen has not been identified, including urinary tract infections and abdominal infections (Solomkin *et al.*, 2010). Ciprofloxacin is used to treat a wide variety of infections, including infections of bones and joints, urinary tract infections, endocarditis, gastroenteritis, malignant otitis externa, respiratory tract infections, cellulitis, prostatitis, anthrax and chancroid.

Ciprofloxacin occupies an important role in treatment guidelines issued by major medical societies for the treatment of serious infections, especially those likely to be caused by Gram-negative bacteria, including *Pseudomonas aeruginosa*. For example, ciprofloxacin in combination with metronidazole is one of several first-line antibiotic regimens recommended for the treatment of community-acquired abdominal infections in adults. It also features prominently in treatment guidelines for acute pyelonephritis, complicated or nosocomial urinary tract infection, acute or chronic prostatitis, certain types of endocarditis, certain skin infections and prosthetic joint infections.

Ciprofloxacin is used in complicated urinary tract infections and pyelonephritis due to *Escherichia coli*, but never as first-line agents. Current recommendations by the American Academy of Pediatrics note the systemic use of ciprofloxacin in children should be restricted to infections caused by multidrug-resistant pathogens or when no safe or effective alternatives are available.

As a result of its widespread use to treat minor infections readily treatable with older, less broad spectrum antibiotics, many bacteria have developed resistance to this drug in recent years, leaving it significantly less effective than it would have been otherwise (Jacobs, 2005).

Resistance to ciprofloxacin and other fluoroquinolones may evolve rapidly, even during a course of treatment. Numerous pathogens, including enterococci, *Streptococcus pyogenes* and *Klebsiella pneumoniae* now exhibit resistance. Widespread veterinary usage of the fluoroquinolones, particularly in Europe, has been implicated. Meanwhile, some *Burkholderia cepacia*, *Clostridium innocuum* and *Enterococcus faecium* strains have developed resistance to ciprofloxacin to varying degrees (Linder *et al.*, 2005).

(b) Nitrofurantoin

Nitrofurantoin has been available for the treatment of urinary tract infections (UTIs) since 1953. Its current uses include the treatment of uncomplicated UTIs and prophylaxis against UTIs in people prone to recurrent UTIs. Increasing bacterial antibiotic resistance to other commonly used agents, such as fluoroquinolones and trimethoprim/sulfamethoxazole, has led to increased interest in using nitrofurantoin

(Garau, 2008). The efficacy of nitrofurantoin in treating UTIs combined with a low rate of bacterial resistance to this agent makes it one of the first-line agents for treating uncomplicated UTIs (Gupta *et al.*, 2011).

Nitrofurantoin is not recommended for the treatment of pyelonephritis, prostatitis and intra-abdominal abscess, because of extremely poor tissue penetration and low blood levels.

Nitrofurantoin has been shown to have good activity against *E. coli, Staphylococcus saprophyticus*, Coagulase negative staphylococci, *Enterococcus faecalis*, *Staphylococcus aureus, Streptococcus agalactiae, Citrobacter* specie and *Klebsiella* species. It is used in the treatment of infections caused by these organisms. Many or all strains of the following genera are resistant to nitrofurantoin: *Enterobacter, Klebsiella, Proteus and Pseudomonas* (Gupta *et al,* 2011).

Nitrofuratoin is one of the few drugs commonly used in pregnancy to treat UTIs (Lee *et al.,* 2008). The drug should not be given to women in late pregnancy due to the potential risk of hemolytic anemia in the newborn, as the newborn has not yet developed the enzymatic pathways necessary for glutathione metabolism and the drug may cause oxidative damage to the red blood cells. Newborns of women given this drug late in pregnancy had a higher risk of developing neonatal jaundice (Nordeng *et al.*, 2013).

Organisms are said to be susceptible to nitrofurantoin if their minimum inhibitory concentration is 32μg/ml or less. The peak blood concentration of nitrofurantoin following an oral dose of nitrofurantoin 100 mg is less than 1 μg/ml and may be

undetectable. Its bioavailability is about 90% and the urinary excretion is 40% and tissue penetration is negligible; the drug is well concentrated in the urine: 75% of the dose is rapidly metabolized by the liver, but 25% of the dose is excreted in the urine unchanged, reliably achieving levels of 20μg/ml or more.

At the concentrations achieved in urine (>100 μg/ml), nitrofurantoin is bactericidal. It is bacteriostatic against most susceptible organisms at concentrations less than 32 μg/ml. Nitrofurantoin and the Quinolone antibiotics are mutually antagonistic *in vitro*. It is not known whether this is of clinical significance, but the combination should be avoided (Garau, 2008).

Resistance to nitrofurantoin may be chromosomal or plasmid- encoded and involves inhibition of nitrofuran reductase. Acquired resistance in *E. coli* continues to be rare.

The mechanism of action of nitrofurantoin is unique and complex. The drug works by damaging bacterial DNA, since its reduced form is highly reactive. This is made possible by the rapid reduction of nitrofurantoin inside the bacterial cell by nitrofuran reductase to multiple reactive intermediates that attack ribosomal proteins, DNA, pyruvate metabolism and other macromolecules within the cell. Nitrofurantoin exerts greater effects on bacterial cells than mammalian cells because bacterial cells activate the drug more rapidly. It is not known which of the actions of nitrofurantoin is primarily responsible for its bactericidal activity. The broad mechanism of action for this drug likely is responsible for the low development of resistance to its effects, as the drug affects many different processes important to the bacterial cell.

Nitrofurantoin is contraindicated in patients with decreased renal function (Creatinine clearance < 60 ml/min) due to systemic accumulation and subtherapeutic levels reached in the urinary tract. Many of the severe side effects of this drug are more common in the elderly and those with renal impairment, as this causes the drug to be retained in the body and reach higher systemic levels. Thus, the drug is not recommended for the elderly population.

Nitrofurantoin is also contraindicated in babies up to the age of one month, as they have immature enzyme systems in their red blood cells, therefore, nitrofurantoin must not be used because it can lead to haemolytic anaemia. For the same reason, nitrofurantoin should not be given to pregnant women after 38 weeks of pregnancy.

(c) Doxycycline

Doxycycline is an antibiotic useful for the treatment of a number of infections. It belongs to tetracycline antibiotic class. In addition to the general indications for all members of the tetracycline antibiotics group, doxycycline is frequently used to treat Lyme disease, chronic prostatitis, sinusitis, pelvic inflammatory disease (Sweet *et al.*, 1988) acne, rosacea, and rickettsial infections (Walker *et al.*, 2008).

It is used in prophylaxis against malaria. It should not be used alone for initial treatment of malaria, even when the parasite is doxycycline-sensitive, because the antimalarial effect of doxycycline is delayed. This delay is related to its mechanism of action, which is to specifically impair the progeny of the apicoplast genes, resulting in their abnormal cell division (Dahl *et al.*, 2006).

It is used in the treatment and prophylaxis of anthrax caused by *Bacillus anthracis* and Leptospirosis. It is also effective against *Yersinia pestis* and is prescribed for the treatment of Lyme disease and Rocky Mountain spotted fever. In fact, because doxycycline is one of the few medications shown to be effective in treating Rocky Mountain spotted fever, doxycycline is indicated even for use in children for this illness. Otherwise, it is not indicated for use in children under the age of eight years.

When bacteriologic testing indicates appropriate susceptibility to the drug, doxycycline may be used to treat these infections caused by Gram-negative bacteria (FDA, 2008), such as *Escherichia coli* infections, *Enterobacter aerogenes* infections, *Shigella* species infections, *Acinetobacter* species infection, respiratory tract infections caused by *Haemophilus influenzae* and respiratory tract and urinary tract infections caused by *Klebsiella* species.

Some Gram-positive bacteria have developed resistance to doxycycline. Up to 44% of *Streptococcus pyogenes* and up to 74% of *S. faecalis* specimens have developed resistance to the tetracycline group of antibiotics. When bacteriologic testing indicates appropriate susceptibility to the drug, doxycycline may be used to treat these infections caused by Gram-positive bacteria (FDA, 2008) such as upper respiratory infections caused by *Streptococcus pneumoniae*, Skin and soft tissue infections caused by *Staphylococcus aureus*, including methicillin-resistant *Staphylococcus aureus* infections and Anthrax caused by *Bacillus anthracis* infection.

Doxycycline has been used successfully to treat sexually transmitted, respiratory, and ophthalmic infections. Representative pathogenic genera include *Chlamydia*, *Streptococcus, Ureaplasma, Mycoplasma*, and others.

Cautions and side effects are similar to those of other members of the tetracycline antibiotic group. An erythematous rash in sun-exposed parts of the body has been reported to occur in 7.3–21.2% of persons taking doxycycline for malaria prophylaxis. One study examined the tolerability of various malaria prophylactic regimens and found doxycycline did not cause a significantly higher percentage of all skin events when compared with other antimalarials.

Unlike some other members of the tetracycline group, it may be used in those with renal impairment. Doxycycline is contraindicated in the pediatric treatment of acute bacterial rhinosinusitis.

The combination of doxycycline with dairy, antacids, calcium supplements, iron products, and laxatives containing magnesium is not inherently dangerous, but any of these foods and supplements may decrease doxycycline's effectiveness.

Previously, doxycycline was believed to impair the effectiveness of many types of hormonal contraception due to cytochrome P450 induction. Recent research has shown no significant loss of effectiveness in oral contraceptives while using most tetracycline antibiotics including doxycycline, although many physicians still recommend the use of barrier contraception for people taking the drug to prevent unwanted pregnancy (Archer and Archer, 2002).

As with all tetracycline antibiotics, it is contraindicated in pregnancy through infancy and childhood up to eight years of age, due to the potential for disrupting bone and tooth development (Mylonas, 2011). Therefore, doxycycline should not be administered to children under the age of eight except in the treatment of anthrax, or where other medications are contraindicated or ineffective (FDA, 2008).

Doxycycline crosses into breastmilk. The adverse effects on teeth and long bones of children directly administered tetracycline antibiotics is documented, but these effects have not been recorded in infants exposed through breastmilk. Although the dose an infant would receive through breastfeeding would likely be minimal, a theoretical risk exists (Chung *et al*., 2002).

Doxycycline–metal ion complexes are unstable at acid pH; therefore more doxycycline enters the duodenum for absorption compared with the earlier tetracycline compounds. In addition, food has less effect on absorption than on absorption of earlier drugs with doxycycline serum concentrations being reduced by about 20% by test meals compared with 50% for tetracycline (Agwuh and MacGowan, 2006).

(d) Cotrimoxazole

Trimethoprim/Sulfamethoxazole (TMP/SMX) or Co-trimoxazole is an antibiotic used in treatment of a variety of bacterial and protozoal infections. It consists of one part Ttrimethoprim to five parts Sulfamethoxazole. Co-trimoxazole is generally considered bactericidal, although its components are individually bacteriostatic. It is a folate antagonist and functions by inhibiting both folate biosynthesis and metabolism.

Co-trimoxazole was claimed to be more effective than either of its components individually in treating bacterial infections, however, this was later disputed (Brumfit and Hamilton- Miller, 1993). Because it has a higher incidence of adverse effects, including allergic responses, its use has been restricted in many countries to very specific circumstances where its improved efficacy has been demonstrated.

It is effective in a variety of upper and lower respiratory tract infections, renal and urinary tract infections, gastrointestinal tract infections, skin and wound infections, septicaemias, and other infections caused by sensitive organisms.

The synergy between trimethoprim and sulfamethoxazole was first described in the late 1960s. Trimethoprim and sulfamethoxazole have a greater effect when given together than when given separately, because they inhibit successive steps in the folate synthesis pathway. They are given in a one-to-five ratio in their tablet formulations so that when they enter the body their concentration in the blood and tissues is roughly one-to-twenty — the exact ratio required for a peak synergistic effect between the two (Wormser *et al.*, 1982).

Sulfamethoxazole, a sulfonamide, induces its therapeutic effects by interfering with the *de novo* (that is, from within the cell) synthesis of folate inside microbial organisms such as bacteria. It does this by competing with p-aminobenzoic acid (PABA) in the biosynthesis of dihydrofolate (Wormser *et al.*, 1982).

Trimethoprim serves as a competitive inhibitor of dihydrofolate reductase (DHFR), hence inhibiting the *de novo* synthesis of tetrahydrofolate, the biologically active form of folate (Wormser *et al.,* 1982).

The effects of trimethoprim causes a backlog of dihydrofolate (DHF) and this backlog can work against the inhibitory effect the drug has on tetrahydrofolate biosynthesis; this is where the sulfamethoxazole comes in, its role is in depleting the excess DHF by preventing it from being synthesised in the first place (Wormser *et al.,* 1982).

Common side effects of TMP/SMX include nausea, vomiting and diarrhoea. Allergic reactions and Clostridium difficile diarrhea are also possible side effects. Its use near the end of pregnancy is not recommended. Some state it should not be used during breastfeeding while others say it is acceptable.

(e) Ampicillin

Ampicillin is a beta-lactam antibiotic that is part of the aminopenicillin family and is roughly equivalent to amoxicillin in terms of activity. It is active against many Gram-positive and Gram-negative bacteria.

Ampicillin has been used extensively to treat bacterial infections since 1961. Before the introduction of ampicillin, penicillin therapies had only been effective against Gram-positive organisms such as staphylococci and streptococci (Petri *et al.,* 2011). Ampicillin also demonstrated activity against Gram-negative organisms such as *H. influenzae*, coliforms and Proteus spp (Acred *et al.,* 1962).

Ampicillin is active against Gram-negative bacteria including *Streptococcus pneumoniae*, *Streptococcus pyogenes*, some isolates of *Staphylococcus aureus* (but not penicillin-resistant or methicillin-resistant strains), and some *Enterococci*. Activity against Gram-negative bacteria includes *Neisseria meningitidis*, some *Haemophilus influenzae*, and some Enterobacteriaceae. Its spectrum of activity is enhanced by co-administration of sulbactam, a drug that inhibits beta lactamase, an enzyme produced by bacteria to inactivate ampicillin and related antibiotics (Akova, 2008).

It is used for the treatment of infections known to be or highly likely to be caused by bacteria including those implicated in common respiratory infections, sinusitis, bronchitis and pharyngitis, as well as otitis media. In combination with vancomycin, it is effective for the treatment of bacterial meningitis. It is also used for gastrointestinal infections caused by consuming contaminated water or food, such as *Salmonella*, *Shigella*, and *Listeriosis* (Finberg *et al.*, 2012).

Ampicillin is a first-line agent for the treatment of infections caused by *Enterococci*. The bacteria are an important cause of healthcare-associated infections such as endocarditis, meningitis, and catheter-associated urinary tract infections that are typically resistant to other antibiotics.

Ampicillin is relatively non-toxic. Its most common side effects include rash, diarrhea, nausea and vomiting. In very rare cases it causes severe side effects such as angioedema, anaphylaxis and *Clostridium difficile* diarrhea.

Mechanism of Action

Penicillin belongs to the group of beta-lactam antibiotics, it is able to penetrate Gram-positive and some Gram-negative bacteria. It differs from penicillin G, or benzylpenicillin only by the presence of an amino group. That amino group helps the drug penetrate the outer membrane of Gram-negative bacteria.

Ampicillin acts as an irreversible inhibitor of the enzyme transpeptidase, which is needed by bacteria to make their cell walls. It inhibits the third and final stage of bacterial cell wall synthesis in binary fission, which ultimately leads to cell lysis; therefore ampicillin is bactericidal (Petri *et al.*, 2011).

2.2.5 RECOMMENDED PRACTICES FOR UTI PREVENTION

(a) Hygiene

Wearing cotton underwear, avoid drinking sodas, and even to avoid strong laundry detergent in an effort to prevent UTIs. Studies have not been done to evaluate most of these measures. There is no available data obtained on the relationship between urinary tract infection and sodas, carbonated beverages, hygiene and wiping patterns. However, it is intuitive that girls and women should always wipe from front to back after a bowel movement to avoid bringing fecal bacteria towards the vaginal and the urethra. If a woman is predisposed to urinary tract infections, she should carefully watch hygiene. It is helpful to show these women a picture of the vulvar anatomy, explaining the close proximity of the urethra to the anal opening and that an infection occurs when intestinal bacteria enter the urethra. These women should be encouraged to clean with a moist wipe

(such as a baby wipe or other hygienic cleansing wipe) after a bowel movement. It is also helpful to wash the prerineum and perianal area with antibacterial soap prior to intercourse. Patients should also be instructed to avoid any sexual practices that might bring colonic bacteria forward towards the vagina, such as touching the perianal area and then the vaginal area. Voiding after intercourse has been shown to protect against UTI (Foxman and Chi, 1990). There is no evidence that vaginal douching after intercourse decreases UTI incidence and in fact, it may increase the risk of vaginal infections. As such, it is not a recommended practice. It is also advisable that patient with recurrent UTIs should avoid tub baths. This recommendation comes from repeated observations over years of practice that many women who present UTIs gives a history of taking frequent tub baths. It is plausible that the hot water washes away some of the protective mucous coating the urethral and vaginal introitus, making the mucosa drier and more susceptible to bacteria colonization. There were interestingly a few papers in the 70s linking *Pseudomonas* infections, including UTIs, to whirlpools and hot tubs, and this led the Centers for Disease Control (CDC) in the United States to establish standards for chlorination and filtration of these tubs. Patients with UTIs should likely avoid these public tubs as well. Even if the water is correctly chlorinated and filtered, it is extremely hot and drying to the skin.

(b) Diet

Urinary tract infection can be very well prevented by drinking at least 8 glasses of water every day. There is evidence that links overactive bladder but not UTI to regular consumption of carbonated beverages. A large study that examined the prevalence and

incidence of irritative voiding symptoms in men and women over a 12 months period showed a significant association between onset of overactive bladder and weekly consumption of carbonated drinks. There are also several studies linking caffeine to lower urinary tract symptoms, but not infection (Arya, 2006; Lohsiriwat *et al.,* 2011). Although these data don't indicate that dietary factors actually cause UTIs, women who have frequent UTIs often mistake the frequency and urgency caused by a dietary bladder irritant for an infection. This could lead to calls to their health provider requesting therapy and the chance of overtreatment. Thus it would seem prudent for these women who are plagued with frequent UTIs to avoid an excessive amount of carbonated beverages and caffeine. There may well be other dietary bladder irritants, such as citrus and other acidic fruits that can cause urgency. It is helpful for women with frequent UTIs to keep a food diary for a short time and see if they can link certain foods to irritative voiding symptoms.

(c) Natural Remedies

Cranberries and their juice have long been touted for both treatment and prevention of UTI. This was previously thought to be due to acidification of the urine, but more recent research has shown that substances (proanthocyanadins) in the cranberry prevent adhesion of *E. coli* strains to the uroepithelium, including multidrug resistant strains. Studies of cranberry prophylaxis are mixed, but several recent studies of randomized controlled trials comparing prevention of UTIs in users for cranberry products versus placebo or non-placebo controls. They found a risk ratio for cranberry users versus nonusers was 0.62 and statistically significant, leading them to conclude that cranberry

products are associated with protection against UTIs. Further, cranberry products were more effective in certain subgroups including women with recurrent UTIs, children, cranberry juice users (as opposed to tablets) and those who used cranberry products more than twice daily (Wang *et al.,* 2012).

A recent study examined women with recurrent UTIs, randomizing them to either cranberry juice or placebo for 6 months. Those in the cranberry juice did have lower incidence or recurrent UTIs, but it did not reach statistical significance. However, they did have significantly decreased counts of P-fimbriated *E. coli* in their urine during the study periods. These uropathogenic strains with fimbriae capable of attaching to the uroepethelium. Though the cranberry juice didn't significantly reduce the number of recurrent UTIs, the reduction in adherent *E. coli* lends plausibility to a protective effect of cranberry and warrants further large scale studies (Stapleton *et al.,* 2012).

Cranberry juice is safe in pregnancy and there is data from a small study to suggest that it may be efficacious in preventing asymptomatic bacteriuria and symptomatic UTI. However, the juice was poorly tolerated by pregnant women, and there was a high rate of withdrawal (Wing *et al.,* 2008). If used in pregnancy, use of cranberry pill form will likely be more effective as compliance will be higher.

Propolois is a resinous material collected by bees from exudates and buds of plants, then mixed with wax and bee enzymes. It has well documented antibacterial activity. Lavigne *et al.,* (2011) added propolis to proanthocyanidins from the cranberry and studied its effect on human volunteer subjects. They found that once daily ingestion offers some protection against bacterial adhesion, bacterial multiplication and virulence in the urinary tract.

Blueberries and blackberries are widely touted on the internet as effective prevention for UTIs but there are no trials of these found. Bearberry leaves are another folk remedy believed to be helpful in treating mild UTIs, but likewise, no studies of effectiveness have been undertaken. The same holds true for vitamin C. there are no studies of this alone for prevention of UTI. However when vitamin C was added to cranberry extract, D mannose and bromelain, this mixture was effective in reducing recurrent UTIs and improving quality of life in both pre and menopausal women (Efros *et al.,* 2010). More studies are needed on efficacy of these nutraceuticals. In summary, there is emerging evidence that cranberries are effective in the prevention of UTI in women. Both the juice and the capsules seem to be effective, the juice possibly more so, but it should be unsweetened juice to prevent high intake of unnecessary sugars. The capsules may be better tolerated however, particularly in pregnancy. Whichever form is used, it seems that it should be ingested three or more times daily for maximal effectiveness. The optimal dose of cranberry is not known and was studied in only one of the studies included in Wang's meta-analysis (Wing *et al,* 2008). He concluded that the cranberry juice provides the most benefit, and it should be ingested three times daily at a dose of 4 to 6 ounces (Wang *et al.,* 2012). Most over the counter cranberry preparations contain 400 to 500 mg of cranberry extract and are likely also more effective if taken three times/daily. More studies are needed in this area to determine the optimal dose and type of cranberry, cranberries should be used with caution in patients on blood thinners and those with kidney stones.

CHAPTER THREE

3.0 Materials and Methods

3.1 Materials

3.1.1 Equipment

Autoclave (Surgifriend Medicals, England), Incubator (Gallenkamp, UK), Microscope (Wild Heerbrugg, Switzerland), Refrigerator (Haier Thermocool), Hot-Air oven (Baird and Tatlock, England), Gel electrophoresis machine (Max Fill Scie-plas. Model HU10 serial no. 5237), Centrifuge (Eppendorf centrifuge 5417R), Top loading electronic balance (Ohaus, USA, Model PA313), Vortex machine (Touch Plate super Mixer, CAT No. 1291 Lab-line instrument Inc USA).

3.1.2 Reagents and solutions

Solution of crystal violent (May and Baker Ltd, England), Lugol"s iodine (May and Baker Ltd, England), Ethidium bromide (Sigma Chemical Ltd), Hydrogen peroxide (SKG Pharma, Nigeria), Acridine orange (BDH Chemicals Ltd, Poole, England), Oxidase strips (Oxoid Ltd, Basingtoke, UK), Mineral oil (Oxoid Ltd, Basingtoke, UK), Voges-Proskaeuer-1 and Voges-Proskaeuer-2 reagents (VP1&2) (Oxoid Ltd, Basingtoke, UK), Nitrate A and B reagents (Microgen products Ltd, UK), Pyrrolidonyl-α-naphthylamide (PYR) reagent (Microgen products Ltd, UK), Trypton Deaminase (TDA) reagent (Oxoid Ltd, Basingtoke, UK), Kovacs reagent (Oxoid Ltd, Basingtoke, UK), Ninhydrin (Microgen products Ltd, UK), Elution Buffer (10mM Tris-HCl 0.1mM EDTA, pH 8.5) and Agarose gel (Schwarz, England).

3.1.3 Culture Media

Nutrient agar (Oxoid Ltd, Basingtoke, UK), Nutrient broth (Oxoid Ltd, Basingtoke, UK), Mueller-Hinton agar (Oxoid Ltd, Basingtoke, UK), Mannitol salt agar (Oxoid Ltd, Basingtoke, UK), MacConkey agar (Oxoid Ltd, Basingtoke, UK), Peptone water (Fluka, Spain) and Agarose gel (Schwarz, England)

3.1.4 Antibiotic Discs

Antibiotic sensitivity discs were obtained from Oxoid Ltd, Basingstoke, UK. They are as follow:

Amoxycillin-clavulanic acid (AMC) 30μg, Cotrimoxazole (SXT) 25µg, Nitrofurantoin (F) 300µg, Cefuroxime (CXM) 30µg, Doxycycline (DO) 30µg, Chloramphenicol (C) 30µg, Ceftriaxone (CRO) 30µg, Ciprofloxacin (CIP) 5µg, Gentamicin (CN) 30µg, Ampicillin (AMP) 10µg.

3.1.5 Rapid identification kits

The under listed identification kits were used during the study;

MACROBATH 12E for identification of Enterobactericeae (Oxoid Ltd, Basingtoke, UK) with the following components;

Microwell test strips (MACROBATH 12E, Oxoid Ltd, Basingtoke, UK), Colour chart for reading results (Oxoid Ltd, Basingtoke, UK) and Macrobath identification system software (Oxoid Ltd, Basingtoke, UK)

MACROGEN STAPH for identification of Staphylococcus species (Microgen Bioproducts Ltd, UK) with the following components;

Microwell test strips (Microgen^{TM} STAPH-ID System), Colour chart for reading results (Macrogen products) and Microgen identification system software (Macrogen products)

MACROGEN STREP- ID

Microwell test strips (Microgen^{TM} STREP-ID System), Colour chart for reading results (Microgen products) and Microgen identification system software (Macrogen products

METHODS

3.2.1 Hospitals

Federal Medical Centre

This is a tertiary health institution in Birnin Kudu Local Government Area of Jigawa State. It serves as referral health centre to inhabitants of Jigawa State and part of Bauchi State (Ningi Local Government Area). It has many Specialists in different area of specialization such as Cardiologist, Paediatricians, Gynaecologists and General Practitioners. The Medical Microbiology Department is well equipped with trained Scientist in Medical Microbiology.

Gunduma Heath Centre

This is a General Hospital located in Birnin Kudu Local Government Area and serves mainly the people of Birnin Kudu Area. It has five (5) different wards with several General Practitioners. It also has Medical laboratory with Scientists who specialized in Medical Microbiolgy.

3.2.2 Study population

All out-patients that visited Federal Medical Centre and Gunduma Health Centre complaining of symptoms of UTIs.

3.2.3 Collection of Isolates

A total of 96 isolates were obtained from the Hospitals with Gunduma Health Services having 57 isolates and Federal Medical Centre having 39 isolate.

3.2.4 Ethical clearance

Ethical clearance certificate was obtained from the Research Ethics Committee of the Federal Medical Centre, Birnin Kudu.

3.2.5 Media preparations

Specified quantities of dehydrated media were reconstituted in fresh distilled water according to manufacturers' instruction. 10ml were dispensed in McCartney bottles and sterilized at 121^{o}C for 15 minutes. The sterile media were stored in a refrigerator at 0^{o}C until required.

3.2.6 Preliminary Identification of Isolates

(a) Gram staining technique:

This was carried out as described by Cheesbrough (2006). Smears of the overnight colonies of the isolates were stained with crystal violet and iodine as mordant and thereafter de-colourised with dilute hydrochloric acid. Neutral red was used as a counter stain. Isolates were thereafter classified into two (2) main groups- Gram-positive or Gram-negative bacteria.

(b) Biochemical tests

Biochemical tests were performed on colonies of the bacterial isolates before using the two (2) test kits; The MACROBATH^{TM} 12E and Microgen^{TM} STAPH-ID system. The tests performed were Catalase, Coagulase, and Oxidase, it was carried out as detailed by Cheesbrough (2006).

Catalase test

This is used to differentiate bacteria that produce the enzyme catalase from the non-catalase producing ones. It was carried out as described by Cheesbrough, (2006). Using sterile glass rod, colonies from overnight cultures of the isolates were picked and immersed into a test tube containing 2ml of hydrogen peroxide solution. Immediate production of gas bubbles indicates a positive result.

Coagulase test

This is used in identifying *Staphylococcus aureus* which produces the enzyme coagulase. The slide agglutination test was adopted. A drop of normal saline was placed on a clean glass slide. One colony of the test organism was taken and emulsified in the drop of the saline solution. A drop of plasma was added to the solution and presence of agglutination indicated coagulase positive isolate.

Oxidase test

This test is used in the detection of *Pseudomonas, Neisseria, Vibrio* and *Brusella* species. Colonies of isolates which were Gram- negative rods were smeared on oxidase strips. Development of deep purple colour indicates oxidase positive isolates.

(C) Identification of isolates to species level

Two identification kits were used; one to identify the *Staphylococcus* species and the other to identify members of the Enterobacteriaceae.

(1) The MACROBATH™ 12E

This is a standardized unit system containing dehydrated substrates prepared for fast and easy identification of Enterobacteriaceae and other Gram-negative organisms from samples with high accuracy up to the specie level.

Oxidase test was first performed on all the isolates which were Gram-negative rods. Oxidase negative isolates were selected for the procedures as recommended in the Macrobath 12E Kit.

Suspensions of the isolates from an 18-hour pure culture were prepared in sterile normal saline solution. The dehydrated substrates in each well were reconstituted with saline suspension of the organism to be identified by carefully peeling the back adhesive tape sealing the microwell test strips. Using a sterile micropipette, 4 drops of the bacterial suspension were added to each well while wells 1-3 were covered with mineral oil supplied with the kit. The peeled adhesive tape was replaced back and the specimen identification number written on the end tag with a pen. The inoculated kits were incubated at 37^{o}C for 18 hours. After this inoculation period, Kovacs reagent, VP1 and VP2 and TDA reagents were added to well 8, 10 and 12 respectively, the colour reaction noted and readings documented. These readings were interpreted into numbers which formed the code number. This code was entered into the computer package for the identification of the Gram-negative bacteria isolates.

(2) Microgen™ STAPH-ID system

This is used for identification of the *Staphylococcus, Kocuria, Kytococcus* and *Micrococcus.*

Suspensions of the isolates from an 18-hour pure culture were prepared in the suspending medium supplied in the kit. The dehydrated substrates in each well were reconstituted with the suspension of the organism to be identified by carefully peeling the back adhesive tape sealing the microwell test strips. Using a sterile micropipette, 4 drops of the bacterial suspension were added to each well. Wells 10 and 11 were covered with mineral oil supplied with the kit; the peeled adhesive tape was slowly replaced back and the specimen identification number written on the end tag with a pen. The inoculated kits were incubated at 37^{o}C for 18 hours. Thereafter, readings were taken by removing the adhesive tape and reactions recorded with the aid of the colour chart. A drop of PYR reagent was added to well 12 and a drop of Nitrate A reagent was added to well 9.

These readings were interpreted into numbers which formed the code number. This code was entered into computer package to identify the specie of the Stretococcus, Enterococcus and related species.

3) Microgen^{TM} Strep-ID system

This is used for identification of the *Streptococcus, Enterococcus* and related species.

Suspensions of the isolates from an 18-hour pure culture were prepared in 5ml sterile 0.9% Normal saline, mixed thoroughly and 4 drops of the bacterial suspension were added to each well by carefully peeling the back adhesive tape sealing the microwell test strips. Wells 12 was overlaid with mineral oil supplied with the kit as instructed by the user manual and incubated at 37^{o}C for 18 hours. Reagents were added to the wells as recommended in the test protocol, reactions read and recorded with the aid of the colour chart provided with the strip. All results were interpreted using the Microgen identification software.

3.2.7 Antibiotics Susceptibility Test

The susceptibility of the different species of isolates was determined according to European Committee on Antimicrobial Suscebtibility Testing, EUCAST, (2014). A total of ten (10) antibiotics were used as test antibiotics, namely Ampicillin (10µg), Amoxycillin-clavulanic acid (30µg), Ceftriaxone (30µg), Cefuroxime (30µg), Trimethoprim/Sulphamethoxazole (25µg) Gentamicin (30µg) Ciprofloxacin (5µg), Doxycycline (30µg), Chloramphenicol (30µg), and Nitrofurantoin (300µg).

The standardized cultures was streaked on to the surface of dried Mueller-Hinton agar in such a way as to ensure an even spread with sterile non-toxic cotton swap. The antibiotic discs under test were placed firmly on the surface of agar using sterile forceps. The plates were allowed to stand for an hour to enable the antibiotics to diffuse into the agar. The plates were then incubated at 37^{o}C for 18 hours. After the incubation, the plates were examined for the zones of inhibition, which were measured in millimeter using metric ruler.

The result was interpreted using the interpretation criteria published by EUCAST (2014). The isolates were reported as sensitive (s) and resistant (R) to the various antibiotics depending on the sizes of the zones of inhibition.

3.2.8 Determination of Multiple Antibiotics Resistance Index (MARI)

The multiple antibiotic resistance was determined for each isolates as shown in the below equation. MARI is the number of class of antibiotics to which the isolate is resistant to divided by the total number of class of antibiotics tested **(**Krumpermann, 1983)

$$\text{MARI} = \frac{\text{Number of antibiotics to which isolate is resistant to}}{\text{Total number of antibiotics tested}}$$

3.2.7 Multidrug-resistance (MDR) Classification of Isolates

Classification of resistance patterns into resistance category was established in accordance with the International Expert for Interim Standard Definations for Acquired resistance by Magiorakose *et al,* (2012).

3.2.9 Plasmid curing

This acridine orange method described by Crosa *et al.* (1994) was employed in this determination. The sub-Minimum Inhibitory Concentration (MIC) values of acridine orange of thirty six (36) multi- drug resistant isolates were determined. Sub-MIC of acridine orange was used to cure the isolates of plasmids they might contain. Sterile nutrient broths were inoculated with standardized overnight inocula of the test isolates and solutions of acridine such that the final concentration of acridine in the broth became 625µg/ml. These were then incubated at 37^{o}C for 18 hrs. Thereafter, growths from the tubes were sub cultured and the antibiotic susceptibility of the thirty six (36) isolates were redetermined using same method as described earlier. The antibiotics sensitivity of the acridine-treated isolates was then compared with the values before plasmid curing.

3.2.10 Test for β-lactamase

Overnight cultures of the isolates under the study were prepared and standardized β-lactamase identification sticks were used for this study. Beta-lactamase stick impregnated with a solution of Nitrocefin, phosphate buffer and dimethylsulphoxide. The ends of the stick changes colour from yellow to red as the amide bond in the lactam ring is hydrolysed by the enzyme. The impregnated stick ends were used to touch the growth of the isolates under investigation and placed on the petri dish lid and allowed to stay for 5-

10 minutes before the results were taken. A colour change from yellow to pink/red on the impreganated end of the sticks was taken as positive for the presence of β-lactamase enzyme (Jorgensen *et al*., 1977).

3.2.11 Molecular characterization of some antibiotic resistant isolates

This work was carried out at the Molecular Diagnostic Laboratory, Veterinary Teaching Hospital, Ahmadu Bello University, Zaria, Nigeria.

(a) Isolation of Plasmid DNA of resistant isolates

Suspensions of the overnight Luria- Bertani broth culture of the resistant isolates that are multidrug resistant were centrifuged at 200 rpm for 2 min. The supernatant was discarded and the cell pellets resuspended in 400µl of solution 1(25 mM Tris-HCl, 10mM EDTA and Glucose, RNase, pH 8.0). It was incubated at room temperature for 10 minutes with addition of solution 2 (1% SDS, 0.2M NaOH, pH 12.0) for 10 minutes. Solution 3 (5M Potassium acetate, Glacial acetic acid, pH 5.4) was also added to the mixture, vortexed and incubated for 10 minutes. It was again centrifuged for 10 minutes and the supernatant transferred to a new tube where 800µl of chill absolute ethanol was added to and left for 5 minutes at room temperature. It was again centrifuged and supernatant discarded. It was then washed with 70% ethanol, air dried and pellets eluted using elusion buffer.

(b) Detection of plasmid using Gel Electrophoresis

The agarose gel was prepared by dissolving 1.0g agarose in 100ml of 1× TBE by heating it in a microwave oven. This was cooled, few drops of ethidium bromide was added and mixed completely. Combs were inserted into the gel trays and agarose was poured and allowed to solidify. The combs were removed from the tank which created wells. More solution of TBE was poured into the tank until the gel completely immersed in the TBE

solution. The extracted plasmid DNA samples mixed with loading dye (5µl) were loaded into the wells and gel electrophoresis tank connected to the power source and allowed to run at 70 volts for 1h. A standard DNA ladder (1kbp) was loaded in the first well which served as control (Nworu *et al*., 2010).

C) Determination of Molecular weight of plasmids

The resulting gel was observed in gel documentation system and the plasmid sizes of the isolates were determined using the Biorad Image Lab software

CHAPTER FOUR

4.0 RESULTS

4.1 Identification and Distribution of Uropathogens

Uropathogens isolated from the urine samples with significant bacteriuria presented in Table 4.1 showed that *E. coli* constituted the predominant organisms (67.7%). Other uropathogens isolated were *S. aureus, Klebsiella spp* and *Streptococcus pyogene*.

However, using the rapid test kit of Microbath 12E and Microgen STAPH^{TM} ID system, a significant proportion of the isolates did not correlate with the previous identifications (Table 4.2 and 4.3). Only 64.6% of the isolates identified by the rapid kit test matched with the identification made in the Medical Microbiology Laboratory of the Hospitals. Percentage correlation varied among the two hospitals, with some having correlation values. The percentage correlation also varied from one uropathogen species to another. For example, with *E. coli*, the correlations in the two hospitals were similar, about 64.6%. However, for *Staphylococcus aureus*, it ranged from 40% in FMC to 50% in GHC and for *Klebsiella spp*, it was 100% in FMC as against 50% in GHC.

Some of the *E. coli* previously classified by the conventional method was by the rapid test kit, identified variously as *Citrobacter*, *Enterobacter*, *Serratia*, *Providentia*, *Salmonella spp* and *Hafnia specie*. Similarly, some of the *Klebsiella species* were also later found to be *Yersinia enterocolitica, Serratia liquefaciens*.

Some of the presumptively classified *Staphylococcus aureus* isolates were found to belong to different *Staphylococcus species* such as *S. xylosus, S. haemolyticus, S. cohnii, S. intermedius and S. hominis.*

Table 4.1. Distribution of Uropathogens in FMC and GHC, Birnin Kudu, Jigawa State using Conventional Method.

Isolates	FMC	GHC
E. coli	26	39
Klebsiella spp	6	10
S. aureus	5	8
Streptococcus pyogene	2	0
Total	39	57

Table 4.2: Distribution of Uropathogens in Federal Medical Centre and Gunduma Health Centre, Birnin Kudu using Rapid Identification Kit

Organism	Frequency F. M. C. (n=39)	G. H. C. (n=57)	Total n (%)
Enterobacteriaceae			
E. coli	17	25	42 (43.75)
K. pneumoniae	2	0	2 (2.08)
K. oxytoca	4	5	9 (9.38)
C. diversus	2	1	3 (3.13)
C. freundii	0	4	4 (4.17)
S. marcescens	2	3	5 (5.21)
S. liquefaciens	0	3	3 (3.13)
P. rettgeri	1	0	1 (1.04)
Y. enterocolitica	0	2	2 (2.08)
Enterobacter gergoviae	0	2	2 (2.08)
S. arizonae	1	3	4 (4.17)
H. alvei	1	0	1 (1.04)
Non-Enterobactericeae			
A. iwoffii	2	1	3 (3.13)
***Staphylococcus* spp**			
S. aureus	2	4	6 (6.25)
S. hominis	1	0	1(1.04)
S. xylosus	2	0	2(2.08)
S. intermedius	0	1	1(1.04)
S. hemolyticus	0	2	2(2.08)
S. cohnii	0	1	1(1.04)
***Streptococcus* spp**			
Streptococcus pyogenes	2	0	2 (2.08)
Total	39	57	96 (100)

Table 4.3: Comparative distribution of Uropathogens in FMC and GHC, Birnin Kudu, Jigawa State based on the analytical procedure.

Organism	Distribution of Isolates			
	FMC		GHC	
	Conventional	Rapid	Conventional	Rapid
E. coli	26	17	39	25
Klebsiella spp	6	6	10	5
Staph. aureus	5	2	8	4
Strep. pyogenes	2	2	-	-
	39	27	57	34

Key:
E. coli= Escherichia coli, Staph= Staphylococcus, Strep= Streptococcus, spp= Species

4.2 Antibiotic Resistance Profile of Isolates

Antibiotic resistance profiles of the bacterial isolates showed that most were susceptible to the inhibitory effect of Nitrofurantoin, Chloramphenicol and Ceftriaxone. They were resistant to Ampicillin and moderately susceptible to Ciprofloxacin, Gentamicin, Doxycycline and Cefuroxime. *E.coli and Klebsiella spp* are moderately sensitive to Amoxycillin-Clavulanic acid and Doxycycline. *Citrobacter* spp were more resistant to the inhibitory effects of the test antibiotics which recorded 100.0% resistance against Ampicillin, Amoxycillin-Clavulanic acid, Doxycycline, Ciprofloxacin, and Gentamicin and on the other hand recorded 71.4% susceptibility against Ceftriaxone, Chloramphenicol, Cefuroxime and 42.9% susceptibility against Nitrofurantoin.

The multiple antibiotic resistance index (MARI) for the isolated *E. coli* showed that a high percentage of the *E. coli* exhibit multiple antibiotic resistance. Of the 42 isolates, 73.81% had MARI of ≥ 0.3 and 86% *Klebsiella spp* and 100% *Citrobacter spp* isolates had MARI of ≥ 0.3 and ≥ 0.4 respectively. Also, 87.5% of *Serratia spp* and 60% of Gram negative uropathogen isolates had MARI of ≥ 0.3 as shown in Table 4.6 and 4.7.

As shown in Table 4.7, fifty- seven resistance patterns distributed among the ninety-six isolates that exhibited resistance to at least one of the test antibiotics were observed. Only few isolates showed similar patterns of resistance. Three isolates were resistant to the ten test antibiotics; three was resistance to the combination of Amoxycillin-clavulanic acid, Cefuroxime, Ceftriaxone and Ampicillin and seven isolates exhibited resistance to only Ampicillin. Out of ninety six isolates, sixty-one were categorized as multi- drug resistance (MDR) bacteria.

Table 4.4: Percentage Resistance of Isolates from Birnin Kudu in FMC and GHC

Antibiotics	% Resistance of Isolates					
	E. coli	***Klebsiella spp***	***Citrobacter spp***	***Serratia spp***	***Staph spp***	***Average***
	n=42	**n=11**	**n=7**	**n=8**	**n=14**	
Ampicillin	92.9	100.0	100.0	100.0	78.6	94.3
Amoxy-clav	62.0	45.5	100.0	99.9	50.0	71.5
Ceftriaxone	35.7	18.2	71.4	37.5	14.3	35.4
Cefuroxime	42.9	45.5	71.4	62.5	64.3	57.3
Gentamicin	26.2	63.6	100.0	37.5	78.6	61.2
Doxycycline	57.1	54.5	100.0	50.0	28.6	58.0
Ciprofloxacin	52.4	72.7	100.0	75.0	0.0	60.0
Cotrimoxazole	71.4	81.8	85.7	62.5	64.3	73.1
Chloramphenicol	35.7	45.5	71.4	25.0	7.1	36.9
Nitrofurantoin	9.5	45.5	42.9	25.0	0.0	24.6

Key: Amoxy-clav= Amoxycillin-clavulanic acid, Staph= Staphylococcus, spp= species

Table 4.5: Multiple Antibiotic Resistance Indices (MARI) of Enterobacteriacea Isolates

MARI	Percentage Frequency *E. coli* (n=42)	*Kleb.Spp* (n=11)	*Citro. Spp* (n=7)	*Serratia spp* (n=8)	Total (n=68)
0.0	7.1	0.0	0.0	0.0	4.4
0.1	16.7	0.0	0.0	12.5	11.8
0.2	16.7	0.0	0.0	0.0	10.3
0.3	9.5	27.3	0.0	37.5	14.7
0.4	11.9	0.0	14.3	12.5	10.3
0.5	9.5	27.3	0.0	0.0	10.3
0.6	16.7	27.3	0.0	25.0	17.6
0.7	7.1	2.3	71.4	12.5	14.7
0.8	4.8	2.3	14.3	0.0	5.9
0.9	0.0	0.0	0.0	0.0	0.0
1.0	0.0	0.0	0.0	0.0	0.0

Key:Kleb=*Klebsiella*,
Citro= *Citrobacter*,
spp= species

Table 4.6: Multiple Antibiotic Resistance Indices (MARI) of Gram positive

MARI	% Frequency		
	Staph.Spp **(n=13)**	***Strep.pyogene*** **(n=2)**	**Total (n=15)**
0.0	0.0	0.0	0.0
0.1	7.7	50.0	13.3
0.2	30.8	0.0	26.7
0.3	30.8	50.0	33.3
0.4	7.7	0.0	6.7
0.5	23.1	0.0	20.0
0.6	0.0	0.0	0.0
0.7	0.0	0.0	0.0
0.8	0.0	0.0	0.0
0.9	0.0	0.0	0.0
1.0	0.0	0.0	0.0

Key:
Staph= *Staphylococcus*, Strep= *Streptococcus*, Spp= Species, n= Number of isolates in each bacteria groups

Table 4.7: Resistance Profiles and Categories of Uropathogens Isolate from FMC and GHC, Birnin Kudu

S/No.	Resistance Profile	Resistance Category	No. of Isolate
1	AMC-SXT-F-CXM-DO-C-CRO-CIP-CN-AMP	MDR	3
2	AMC-SXT-CXM-DO-C-CRO-CIP-CN-AMP	MDR	8
3	AMC-SXT-F-CXM-DO-C-CIP-CN-AMP	MDR	2
4	AMC-SXT-F-CXM-DO-C-CRO-CIP-AMP	MDR	1
5	AMC-SXT-F-CXM-C-CRO-CIP-CN-AMP	MDR	1
6	AMC-SXT-F-DO-CRO-CIP-CN-AMP	MDR	1
7	AMC-SXT-CXM-DO-CRO-CIP-CN-AMP	MDR	3
8	AMC-SXT-CXM-DO-C-CRO-CN-AMP	MDR	1
9	AMC-SXT-F-CXM-DO-CRO-CIP-AMP	MDR	1
10	AMC-SXT-F-CXM-DO-C-CRO-AMP	MDR	1
11	AMC-SXT-CXM-C-CRO-CIP-CN-AMP	MDR	1
12	AMC-SXT-CXM-DO-C-CRO-CIP-AMP	MDR	1
13	SXT-F-CXM-DO-C-CIP-CN-AMP	MDR	3
14	AMC-SXT-DO-C-CIP-CN-AMP	MDR	1
15	AMC-SXT-CXM-DO-C-CIP-AMP	MDR	2
16	AMC-SXT-F-DO-C-CIP-AMP	MDR	1
17	AMC-SXT-CXM-DO-CIP-CN-AMP	MDR	1
18	AMC-SXT-CXM-CRO-CIP-AMP	MDR	1
19	AMC-SXT-F-C-CN-AMP	MDR	1
20	AMC-SXT-CXM-DO-CIP-AMP	MDR	1
21	SXT-CXM-DO-CIP-CN-AMP	MDR	1
22	AMC-CXM-DO-CRO-CIP-AMP	MDR	1
23	AMC-SXT-DO-C-CIP-AMP	MDR	2
24	AMC-SXT-CXM-DO-C-AMP	MDR	1
25	AMC-DO-CIP-CN-AMP	MDR	1
26	SXT-DO-CRO-CIP-AMP	MDR	1
27	AMC-SXT-DO-CIP-AMP	MDR	1
28	AMC-SXT-DO-C-AMP	MDR	2
29	AMC-SXT-CXM-DO-AMP	MDR	1
30	AMC-SXT-CXM-CIP-AMP	MDR	1
31	AMC-SXT-DO-AMP	MDR	2
32	AMC-SXT-CIP-AMP	MDR	1
33	AMC-CXM-CRO-AMP	NMDR	4
34	AMC-F-CXM-AMP	MDR	2
35	SXT-C-CIP-AMP	MDR	1
36	SXT-DO-C-AMP	MDR	1

37	AMC-SXT-C-AMP	MDR	1
38	CXM-DO-CRO-CIP	MDR	1
39	DO-CIP-AMP	MDR	1
40	SXT-DO-AMP	MDR	3
41	AMC-SXT-AMP	NMDR	2
42	SXT-CRO-AMP	MDR	1
43	AMC-CRO-AMP	NMDR	1
44	SXT-CXM-AMP	NMDR	1
45	AMC-CXM-AMP	NMDR	1
46	SXT-DO-CIP	MDR	1
47	CXM-CRO-CN	MDR	1
48	SXT-AMP	NMDR	2
49	AMC-SXT	NMDR	1
50	CIP-AMP	NMDR	1
51	CXM-CRO	NMDR	1
52	CXM-AMP	NMDR	2
53	AMC-AMP	NMDR	1
54	AMP	NMDR	7
55	CXM	NMDR	1
56	SXT	NMDR	1
57	CIP	NMDR	1
Total		90	

Key: AMC= Amoxicillin-clavulanic acid, SXT= Cotrimoxazole, F= Nitrofurantoin, CXM= Cefuroxime, DO= Doxycycline, C= Chloramphenicol, CRO= Ceftriaxone, CIP= Ciprofloaxcin, CN= Gentamicin, AMP= Ampicillin, MDR= Multidrug resistant, NMDR= Not Multidrug resistant

4.3: Determination of Nature of Resistance

Of the 36 isolates that underwent plasmid curing using acridine orange, 66.7% became susceptible to one or more antibiotics of same concentration to which they were previously resistant. For instance, 70% of the isolates that were resistant to Nitrofurantoin became susceptible and more than 30% of the isolates that were resistant to Amoxicillin – Clavulanic acid, Cefuroxime, and Gentamicin also became susceptible. In contrast, resistance profile to chloramphenicol didn't change in spite of plasmid curing treatment.

Gel Electrophoresis of plasmid DNA showed that the ten (10) isolates examined carried plasmid DNA of average sizes of 5.4kbp.

Table 4.8: Sensitivity Profile of Some Isolates with reduction in resistant Profile after Plasmid Curing

% reduction	*E. coli* (n=15)	*Klebsiella* spp (n=7)	*Citrobacter* spp (n=4)	*Serratia* spp (n=2)	*Staph* spp (n=4)
1- 20	2	1	0	2	0
21- 40	3	2	3	0	0
41- 60	2	1	0	0	1
61- 80	1	0	0	0	0
81- 100	0	1	0	0	0

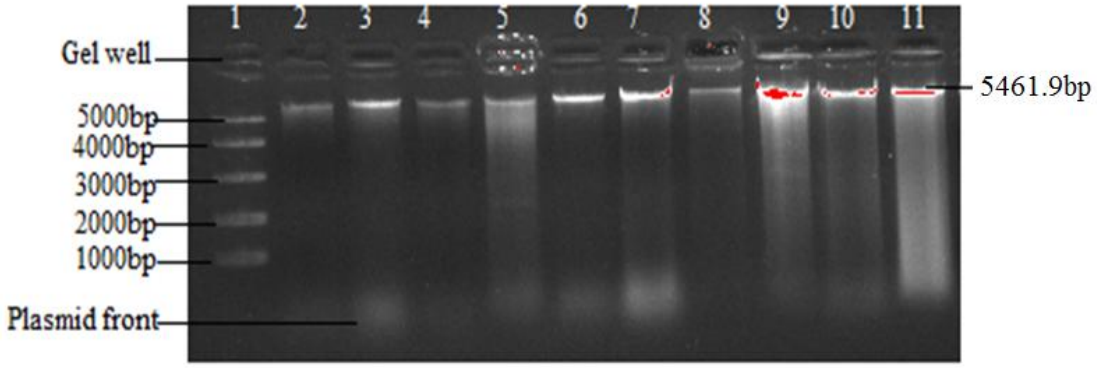


Plate 1: **Gel Electrophoresis showing plasmid band of some uropathogens**

Lane 1= 1Kb DNALadder, Lane 2= *E. coli*, Lane 3= *E. coli*, Lane 4= *E. coli*, Lane 5= *K.oxytoca*, Lane 6= K. *oxytoca*, Lane 7= *S. liquefaciens*, Lane 8= *S. arizonae*, Lane 9= *E. coli*, Lane 10= *K. oxytoca*, Lane 11= *K. oxytoca*

Table 4.9: Plasmid sizes of some Antibiotic Resistant Bacteria Isolated from Urine of Patients in Birnin Kudu

Isolate No.	No. of Plasmid	Plasmid sizes
BKG 15	1	5184.8bp
BKG 17	1	5239.1bp
BKG 21	1	5347.8bp
BKG 24	1	5347.8bp
BKG 29	1	5456.5bp
BKG 27	1	5565.2bp
BKG 36	1	5673.9bp
BKF 14	1	5565.2bp
BKF 16	1	5565.2bp
BKF 25	1	5673.9bp

Table 4.10: Proportion of MDR Isolates that are Beta-lactamase Producer

Isolate	Percentage
E. coli (n=15)	60.0
Klebsiella spp (n=7)	42.9
Citrobacter spp (n=4)	75.0
Staphylococcus spp (n=4)	100.0

Key:

MDR= Multidrug resistant, Spp=Species,

CHAPTER FIVE

5.0 DISCUSSION

World Health Organization (WHO, 2003) defined health as not just freedom from disability or the absence of morbidity but as "complete physical, mental and social well-being". Symptoms of lower urinary tract infection include bothersome sensations such as urinary urgency, frequency, painful urination, hesitancy, and the sense of incomplete bladder emptying. A study by Liao *et al*., (2009) had examined the effect of such symptoms on the quality of life and reported that it generally has a negative effect on such life quality.

Although urinary tract infection is not a significant cause of mortality, it still represents an important cause of morbidity since UTI have the potential for serious and life-threatening consequences if appropriate treatment is not given. Likely complications include pyelonephritis which can lead to renal scaring and sepsis (Litwin and Saigal, 2007).

However, because of the serious and life-threatening consequences of this disease together with the increasing prevalence of antimicrobial resistance (Nerurkar *et al.*, 2012; Sood and Gupta, 2012) this study was carried out to determine the incidence of bacteriuria in Birnin Kudu community and the attendant risks in terms of its management.

The result of this study in two (2) health facilities using the conventional method of identification showed that *E. coli*, *Klebsiella* spp, *S. aureus*, and *Streptococcus pyogenes* are majorly responsible for UTI in Birnin Kudu community. A large proportion of the

isolates obtained from the laboratories did not correlate with the organisms identified using rapid diagnostic kit. The discrepancies observed in the use of these two methods could be as a result of inclusion of other test reactions in the rapid identification kit systems that enabled them to identify these organisms better than the conventional methods. Most Medical Laboratory results of identification are based on the few biochemical tests such as indole, whereas the one used in this study, is based on the outcome of about twelve (12) different biochemical tests which are well standardized. It is a semi-automatic system, with a test software computer guided to interpret the organisms identified during the study for a higher index of specificity and accuracy in organism identification.

The prevalence of Gram negative organism as uropathogens has been remarkably consistent world-wide with Gram-negative organisms accounting for most infections (Gupta *et al.*, 1999; Moges and Genetu, 2002). The high incidence of *E. coli* as uropathogens may be due to the fact that:

E. coli is part of the normal flora in the gut and can be introduced in many ways especially for females;

I. Wiping from back to front has been suggested as a possible means of fecal contamination of the urogenital orifices.
II. Anal intercourse can also introduce this bacterium into the male urethra, and in switching from anal to vaginal intercourse, the male can also introduce uropathogenic *E. coli* (UPEC) to the female urogenital system.

The incidence level of *Klebsiella* spp and *Citrobacter* spp in this study is in agreement with previous studies (Moges and Genetu, 2002). Other less common Gram-negative organisms isolated in this study such as *Salmonella* spp, *Acinetobacter* spp, *Yersinia* spp, *Enterobacter* spp, *Providentia* spp, and *Hafnia* spps have also been reported to be more common among HIV infected patients (Staiman and Lowe, 2004; Awolude *et al.,* 2010; Michael *et al*., 2006). The high proportion of Gram-negative organism among the UTI isolates in this study agrees with the findings of Ebie *et al.,* (2001); Drew *et al*. (2005) and Aiyegoro *et al*. (2007).

Epidemiological surveillance of antimicrobial resistance is indispensable for empirical treatment of infections, implementing control measures, and preventing the spread of antimicrobial-resistant microorganisms (Stelling *et al*., 2005). Result of the antibiotic sensitivity showed that many of the isolated bacteria in this study are resistant to the commonly prescribed antibiotics in Birnin Kudu. The high rates of resistance found in this study may imply inappropriate use of many of these antimicrobial drugs in man and animal which promotes development of resistant strains. The high percentage of resistance to the Cotrimoxazole and Amoxycillin-clavulanic acid may not be unconnected with the indiscriminate use of the drugs in the treatment of various ailments such as cough. An alternative to the first-line drug against UTI before culture and sensitivity is Nitrofurantoin. It is cheap and readily available in developing countries. It has also been shown to be very safe in pregnancy (Delzell and Lefevre, 2000). The consistent and high-level susceptibility of *E. coli* to Nitrofurantoin may be influenced by Nitrofurantoin's narrow spectrum of activity, limited indication (treatment of acute cystitis) and narrow tissue distribution (James *et al.,* 2002). The relative high potency of Ceftriaxone and

hence low resistance against the isolated uropathogens may be attributed to the restricted use of the drug. Ceftriaxone is a third generation Cephalosporin that is resistant to effects of β-lactamase enzyme.

Resistance to Fluoroquinolone was generally low about two decades ago (Christiaen *et al*., 1998) that only 1% of the pathogens were resistant to fluoroquinolones. But in contrast to the result obtained in this study in which about two-third were resistant. This might be due to the several brands of Ciprofloxacin with questionable qualities circulating in the country. In recent years, use of fluorquinolones has increased in many countries and emergence of resistance amongst bacterial isolates to fluoroquinolones has been observed (Pickering, 2004). The increased level of resistance, among others, may not be connected to inappropriate prescribing of antibiotics and poor infection control strategies (Tolun *et al.,* 2004). The situation in Birnin Kudu, in terms of antimicrobial drug use, is not different from that of many part of this country, where people usually procure antimicrobial drugs without prescription or without performing the necessary antibiotic susceptibility test. The widespread and more often the misuse of antimicrobial drugs have led to a general rise in the emergence of resistant bacteria, particularly to Ciprofloxacin.

The multiple antibiotic resistance index (MARI), a measure of exposure of organism to antibiotics show that most of the isolated organisms exhibited resistance to many antibiotics belonging to different classes of antibiotics. It indicates that a very large proportion of the bacterial isolates have been exposed to several antibiotics. A similar study by Christopher *et al*., (2013) reported uropathogen isolates as being resistant to Ampicillin, Amoxicillin/clavulanic acid, Ciprofloxacin and Cotrimoxazole.

Development of resistance to most of the antibiotics used in the management of UTI in this community should be a cause for concern to health workers.

In this country, there is indiscriminate use of drugs especially antibiotics by the general populace and this medicine belongs to the prescription group of drugs and are readily accessible from patent medicine vendors, open drug market and street drug hawkers (Paul *et al*., 1997). All these sources of drugs encourage fake and substandard drugs and even where the source of the drugs is genuine, the storage conditions became a major source of concern to health care workers. Other sources of concern include improper dosage regimens and extremely short duration of therapy which are very common among patients.

Multiple antibiotic resistance indexes showed that 73.5% and 60% of the Gram negative and Gram positive uropathogens respectively have displayed MARI ≥ 0.3 and is taken as significant, revealing that they were resistant to more than three classes of antimicrobial agents tested. This observation suggests that the isolates in this study may probably have originated from an environment where antibiotics are often misused (Paul *et al*., 1997). Broadspectrum antibiotics are sometimes reported to be given in place of narrow–spectrum antibiotics as a substitute for culture and sensitivity testing, with the consequent risk of selection of antibiotic-resistant mutants (Krumperman, 1983). The high MARI from the isolated uropathogen indicates a serious public health risk. Out of ninety six isolates, 63.5% of them categorized as multi- drug resistance (MDR) bacteria. Occurance of these MDR isolates as an aetiological agent of UTI is a cause of concern because most of the commonly used antibiotics may not be useful in the management of UTI and treatment of UTI would take longer duration in the study area.

Result of the plasmid curing experiment showed that plasmid DNA contributes to the antibiotics resistance observed among the isolates. More than two-third (66.7%) of the multi-drug resistant isolates lost their ability, partly, to resist the inhibitory effect of antibiotics they were previously resistant to. Therefore, plasmid curing experiment and Plasmid sizes (5184-5673.9bp) obtained in this study suggested that the antibiotics resistance was mostly plasmid mediated as seen in the sizes of plasmid though they are mobilizable plasmids that do not have the ability to encode the functions necessary to promote cell to cell DNA transfer. Mobilizable resistance plasmid tend to be relatively small, often less than 10 kb in size, encoding only a handful of genes including the resistance gene(s) and all the plasmid sizes obtained during this study are less than 10 kb. Mobilizable plasmid when helped by a conjugative plasmid co-resident in the cell may be transferred from one cell to another cell (Wilkins, 1995). On other hand, some isolates showed no variation in their susceptibility profile after plasmid curing experiment this means that the resistance may be chromosomally encoded. The presence of plasmid of same sizes indicates that resistance is likely from the same source. Plasmids are major mechanism for the spread of antibiotic resistance genes in bacterial populations (Fang *et al.,* 2008). Multiple resistance genes are harbored on R-plasmids some of which are conjugative (Pitout *et al.,* 2009). *E. coli* have been reported to transfer antibiotic resistance genes to enteric pathogenic and normal flora bacteria. (Platt *et al.,* 1986). The result of this work also showed that more than two-third of the Multiple Antibiotic Resistant (MAR) isolates were β- lactamase producer. This means that most strains of the isolated bacterial species (*E. coli, Klebsiella* spp*, Citrobacter* spp and *Stapylococcus* spp)

were β-lactamase producer which imply that substantial number of the MAR isolates developed resistance to the drugs as a result of β-lactamases production.

CHAPTER SIX

6.0 Summary, Conclusion and Recommendations

6.1 Summary

Using a standardized rapid test kits, 96 bacterial isolates obtained from four different families were identified. These include Enterobacteriaceae, Staphylococcus, Streptococcus and Acinetobacter in decreasing order of frequency.

Most isolates were susceptible to Nitrofurantoin (75.4%), Ceftriaxone (64.6) and Chloramphenicol (63.1%). Majority of the isolates showed resistance to Ampicillin (94.3%), Cotrimoxazole (73.1%) and Amoxycillin-clavulanic acid (71.5%).

Multiple antibiotic resistance indexes showed that 73.5% and 60% of the Gram negative and Gram positive uropathogens respectively were multi-drug resistant. Most of the antibiotic resistant isolates were β- lactamse producers and are plasmid encoded.

6.2 Conclusion

There is high incidence of antibiotic resistance among uropathogens in the study area.

E. coli is the predominant uropathogen in the study area. Commonly available antibiotics may not be useful in the management of UTI in the study area.

The result gave information about antibiotics resistance of uropathogens at Birnin Kudu, Nigeria which may be due to geographic variation or indiscriminate use of

antibiotics. Plasmid profile revealed that the antibiotic resistance in this geographical area is plasmid borne

6.3 Recommendations

1. Periodic monitoring of uropathogens should be done so as to establish local and national antimicrobial resistance monitoring systems in Nigeria to provide information for the development of UTI treatment guidelines.
2. Antibiotics such as Ceftriaxone, Nitrofurantoin and Chloramphenicol showed good activity against uropathogens in this study and can be used in the empirical treatment of UTI.
3. Further study is recommended with other antibiotics such as Sparfloxacin and Gartifloxacin to test their suitability as it is obvious that most of the antibiotics used in Birnin Kudu are no more effective against the causative agents.
4. There is a necessity for constant antimicrobial sensitivity surveillance and susceptibility testing to be conducted prior to antibiotics use.

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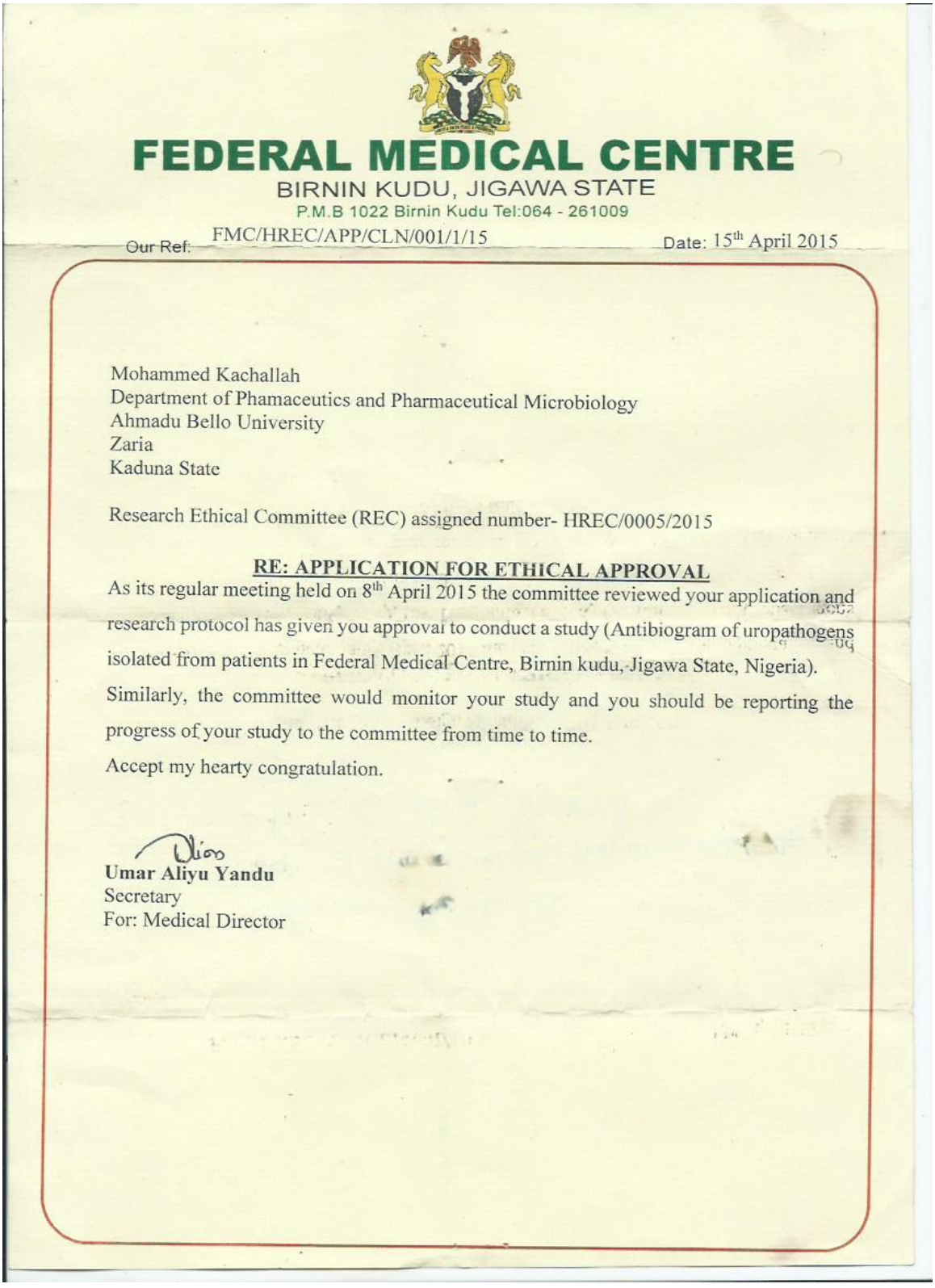
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APPENDICES

APPENDIX 1: Ethical clearance certificate to conduct research study in Federal Medical Centre, Birnin Kudu



FEDERAL MEDICAL CENTRE
BIRNIN KUDU, JIGAWA STATE
P.M.B 1022 Birnin Kudu Tel:064 - 261009

Our Ref: FMC/HREC/APP/CLN/001/1/15 Date: 15th April 2015

Mohammed Kachallah
Department of Phamaceutics and Pharmaceutical Microbiology
Ahmadu Bello University
Zaria
Kaduna State

Research Ethical Committee (REC) assigned number- HREC/0005/2015

RE: APPLICATION FOR ETHICAL APPROVAL

As its regular meeting held on 8th April 2015 the committee reviewed your application and research protocol has given you approval to conduct a study (Antibiogram of uropathogens isolated from patients in Federal Medical Centre, Birnin kudu, Jigawa State, Nigeria).

Similarly, the committee would monitor your study and you should be reporting the progress of your study to the committee from time to time.

Accept my hearty congratulation.

Umar Aliyu Yandu
Secretary
For: Medical Director

APPENDIX II: Zone Diameter Interpretive Standared Chart Using European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2014).

Antibiotics	Disc potency (Mcg)	Diameter of zones of inhibition (mm) R	S
Ampicillin	10	< 14	≥ 14
Amoxy-clav	30	< 19	≥ 19
Cefuroxime	30	< 18	≥ 18
Ceftriaxone	30	< 20	≥ 23
Ciprofloxacin	5	< 19	≥ 22
Gentamicin	30	< 14	≥ 17
Doxycycline	30	< 10	≥ 14
Cotrimoxazole	25	< 13	≥ 16
Nitrofurantoin	300	< 14	≥ 17
Chloramphenicol	30	< 17	≥ 17

APPENDIX III: Interpretation of Antibiotic Susceptibility Tests of the different Bacterial Isolates.

S/No.	Isolates	AMC	SXT	F	CXM	DO	C	CRO	CIP	CN	AMP
1	BKF1	S	R	S	R	R	S	R	R	R	R
2	BKF2	R	R	S	S	S	S	S	S	S	R
3	BKF3	R	R	S	R	R	R	R	R	R	R
4	BKF4	S	S	S	S	R	S	S	R	S	R
5	BKF5	S	R	S	S	R	S	S	S	S	R
6	BKF6	R	S	S	S	R	S	S	R	R	R
7	BKF7	S	R	S	S	R	S	S	R	S	R
8	BKF8	R	R	R	S	S	R	S	S	R	R
9	BKF9	R	R	S	S	R	S	S	S	S	R
10	BKF10	R	R	R	R	R	R	R	R	R	R
11	BKF11	R	R	S	S	S	S	S	R	S	R
12	BKF12	R	R	R	S	R	S	R	R	R	R
13	BKF13	R	S	R	S	R	S	S	R	R	R
14	BKF14	R	R	S	R	R	S	R	R	R	R
15	BKF15	S	S	S	S	S	S	S	R	S	R
16	BKF16	S	R	R	R	R	S	S	R	R	R
17	BKF17	S	S	S	R	R	S	R	R	S	S
18	BKF18	R	R	S	R	R	R	R	R	S	R
19	BKF19	R	S	S	R	R	S	R	R	S	R

20	BKF20	R	R	R	R	S	R	R	R	R	
21	BKF21	S	S	S	R	S	S	R	S	R	S
22	BKF22	R	R	S	R	R	S	R	R	R	R
23	BKF23	S	S	S	R	S	S	R	S	S	S
24	BKF24	S	R	S	S	S	S	S	S	S	R
25	BKF25	S	R	R	R	R	R	S	R	R	R
26	BKF26	S	R	S	S	R	S	S	S	S	R
27	BKF27	S	S	S	S	S	S	S	S	S	S
28	BKF28	S	R	S	S	R	S	S	S	S	R
29	BKF29	S	S	S	R	S	S	S	S	S	S
30	BKF30	R	R	S	R	R	R	S	R	S	R
31	BKF31	S	S	S	S	S	S	S	R	S	S
32	BKF32	R	R	S	S	R	R	S	R	S	R
33	BKF33	R	R	S	R	R	S	S	S	S	R
34	BKF34	S	S	S	S	S	S	S	S	S	S
35	BKF35	R	S	S	R	S	S	R	S	S	R
36	BKF36	S	R	S	S	S	S	S	S	S	R
37	BKF37	S	S	S	R	S	S	S	S	S	R
38	BKF38	R	S	S	R	S	S	R	S	S	R
39	BKF39	S	R	S	S	S	S	S	S	S	S
40	BKG1	R	R	S	S	S	S	S	S	S	R
41	BKG2	S	S	S	S	S	S	S	S	S	R

42	BKG3	R	R	S	R	R	R	R	R	R	R
43	BKG4	R	S	S	R	S	S	R	S	S	R
44	BKG5	S	R	S	S	S	S	S	S	S	R
45	BKG6	R	R	S	R	R	R	R	R	R	R
46	BKG7	R	S	S	S	S	S	S	S	S	R
47	BKG8	R	R	S	R	S	S	R	R	S	R
48	BKG9	R	S	S	R	S	S	R	S	S	R
49	BKG10	R	S	R	R	S	S	S	S	S	R
50	BKG11	R	R	S	R	R	R	R	R	R	R
51	BKG12	R	R	S	R	R	S	R	R	S	R
52	BKG13	R	R	S	S	R	R	S	R	R	R
53	BKG14	R	R	S	S	R	S	S	R	S	R
54	BKG15	R	R	R	R	R	R	R	R	R	R
55	BKG16	S	S	S	S	S	S	S	S	S	R
56	BKG17	R	R	S	R	R	R	S	R	S	R
57	BKG18	R	R	S	S	R	R	S	S	S	R
58	BKG19	R	R	S	S	R	S	S	S	S	R
59	BKG20	S	R	S	S	S	S	R	S	S	R
60	BKG21	R	R	R	S	R	R	S	R	S	R
61	BKG22	R	R	S	R	R	R	R	S	R	R
61	BKG23	R	R	R	R	R	R	S	R	R	R
63	BKG24	R	R	R	R	R	R	S	R	R	R
64	BKG25	R	S	R	R	S	S	S	S	S	R

65	BKG26	S	S	S	S	S	S	S	S	S	S
66	BKG27	R	R	S	R	R	S	S	R	R	R
67	BKG28	R	R	S	R	R	R	R	R	R	R
68	BKG29	R	R	S	R	R	S	S	R	S	R
69	BKG30	R	R	R	R	R	R	R	R	S	R
70	BKG31	R	R	S	S	R	R	S	S	S	R
71	BKG32	R	R	S	R	R	R	R	R	R	R
72	BKG33	S	R	S	S	S	R	S	R	S	R
73	BKG34	R	R	R	R	R	R	R	R	R	R
74	BKG35	R	R	R	R	R	S	R	R	S	R
75	BKG36	R	R	S	R	R	R	S	R	S	R
76	BKG37	S	S	S	S	S	S	S	S	S	R
78	BKG38	R	R	R	R	R	R	R	R	R	R
79	BKG39	R	R	S	R	R	S	R	R	R	R
80	BKG40	S	R	S	S	R	R	S	S	S	R
81	BKG41	R	R	R	R	R	R	R	S	S	R
82	BKG42	S	S	S	S	S	S	S	S	S	R
83	BKG43	R	R	S	R	S	R	R	R	R	R
84	BKG44	R	R	S	S	S	R	S	S	S	R
85	BKG45	S	S	S	S	S	S	S	S	S	R
86	BKG46	S	S	S	S	S	S	S	S	S	R
87	BKG47	S	S	S	S	S	S	S	S	S	R
88	BKG48	R	R	S	R	R	R	R	R	R	R

89	BKG49	R	S	S	S	S	S	R	S	S	R
90	BKG50	S	S	S	R	S	S	S	S	S	R
91	BKG51	S	R	S	R	S	S	S	S	S	R
92	BKG52	R	S	S	R	S	S	S	S	S	R
93	BKG53	S	R	S	S	R	S	S	S	S	S
94	BKG54	R	R	S	R	R	R	R	S	S	R
95	BKG55	R	R	S	R	R	S	R	R	S	R
96	BKG56	R	R	R	R	S	R	S	R	R	R

APPENDIX IV: Comparison of Resistance of some Antibiotics to 36 Multi Antibiotic Resistant Isolates from urine of Patients in Birnin Kudu, before and after Plasmid Curing.

Antibiotic	Frequency		% Reduction in Resistance
	Before curing	After curing	
Amoxy-Clav Acid	33	20	39.00
Cotrimoxazole	35	30	14.00
Nitrofurantoin	10	3	70.00
Cefuroxime	31	21	32.25
Doxycycline	31	25	19.35
Chloramphenicol	23	23	0.00
Ceftriaxone	22	19	13.63
Ciprofloxacin	33	29	12.12
Gentamicin	23	15	34.78
Ampicillin	35	34	2.85

APPENDIX V: GRAPH FOR DETERMINATION OF PLASMID SIZE

