CHAPTER-I
INTRODUCTION

1.1 Background

Urinary tract infection (UTI) is among the most pedestrian infections described in outpatient setting and hospital patients (Shaifali *et al*., 2012). It is a quotidian, distressing and occasionally, life threatening condition in which the urinary tract is infected with a pathogen causing inflammation (Sibi *et al*., 2011). The clinical manifestations of UTI depend on the portion of the urinary tract involved, the organism(s), the severity of the infection and the patient's ability to mount an immune response to it (Foxman and Brown, 2003).

In clinical microbiology laboratories, UTI accounts a significant part of the workload (Wilson and Gaido, 2004). It is an extremely common condition that occurs in both male and female of all the ages (Griebling, 2005). Most UTIs are caused by the ascent of bacteria from the fecal flora through the urethra to the bladder and kidney, especially in the female due to shorter and wider urethra the microorganisms more swiftly transfer on them (Jones *et al*., 2006).

The urinary tract consists of the kidneys, ureters, bladder and the urethra. All areas above the urethra in a healthy human are sterile (Forbes *et al.*, 2002). However, the entire urinary tract is always at a danger of invasion of bacteria, once any one of its part is infected (Brooks *et al.*, 2004).

The use of hemodialysis which is a form of treatment, for the chronic kidney disease is increasing during past decades comparing the other options (peritoneal dialysis and renal transplantation) which are largely uncommon due to the extremely exorbitant cost, lack of facilities and manpower, and the predominantly urban location of the renal care centers (Arije *et al*., 2000; Bambgoye, 2003; Naicker, 2009). Although hemodialysis treatment modality has led to the increased longevity of patients, it also predisposes them to some infections (Santos *et al*., 1998).

Hemodialysis patients are more susceptible to urinary tract infections and these are common and an important cause of morbidity and mortality in these patients (Lees *et al*., 1985; Rault, 1984; Stamm, 1983; Vij *et al*., 2009). The different studies divulged the high prevalence of UTIs in hemodialysis patients. Kessler *et al*. (1993) reported UTI in hemodialysis patients with frequency of 23%. Likewise, another study revealed the prevalence of urinary tract infection in hemodialysis patients was 37.14% (Swarnalatha *et al*., 2011). Asymptomatic bacteriuria of 28% prevalence is seen in patients undergoing hemodialysis (Bakke and Digranes, 1991; Chaudhry *et al*., 1993).

Escherichia coli (66%) is the most frequent causative agent of UTIs followed by *Enterococci* (8.3%), *Candida* spp. and *Pseudomonas* spp. (7.3% each), *Klebsiella* spp. (5.5%), *Enterobacter* spp. (2.7%), *Proteus* spp. and *Morganella* spp. (<1% each) (Bashir *et al*., 2008).

Moreover, the reported pathogens causing UTIs in hemodialysis are- *Escherichia coli* as the predominant etiologic agent, followed by *Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., *Pseudomonas* spp., *Alcaligenes faecalis, Staphylococcus aureus, Streptococcus faecalis, Pseudomonas spp., Candida albicans* etc. (Jadav *et al*., 1977).

Dialysis patients are at high risk for infections because of the impaired immune defenses, a high severity of illness, and the need for routine puncture of a vascular access site to remove blood for hemodialysis (Horl, 1999). Under normal circumstances, the urine is sterile until it reaches the distal urethra (Forbes *et al*., 2002). Various defense mechanisms of body prevent the infection of urinary tract, and one of the most important defense mechanisms is the flow of urine that washes bacteria out of the body. Likewise, in men, prostate gland produces secretions that prevent bacterial growth and the acidic pH (5.5) and low osmolarity of urine also discourage the bacterial growth (Acharya, 1992). But incase of dialysis patients, where voiding is absent the urinary tract is often overlooked as a source of infection. When diagnosed in a timely manner, these infections are easily curable (Bennett *et al*., 1976; D'Agata *et al*., 2000).

During the past decades, the number of patients with chronic kidney disease (CKD) treated by maintenance hemodialysis is sharply increasing (Ma *et al*., 1999). On the other side, as a result of their frequent receipt of antimicrobials for treatment of urinary tract infections, antimicrobial resistance has been common in patients undergoing dialysis and has added to hazard. The more frequently used antibiotics like Penicillin, Erythromycin, Chloramphenicol, and Ampicillin revealed very low levels of sensitivity (less than 25%) to all organisms in kidney diseased patients as a whole (Jadav *et al*., 1977). Several studies revealed the pattern of rising drugs resistance used in UTIs like-Cotrimoxazole and beta-lactam, fluoroquinolone resistance and MDR among community-acquired urinary isolates (Gupta, 2003; Gupta *et al*, 2001; Kahlmeter, 2000; Talan *et al*, 2000).

The emergence of microbial resistance problem is increasing due to the inappropriate use of antibiotic prophylaxis, self medication with some types of antibiotics and the inadequate dosage of these antibiotics (Adjei *et al*., 2004; Mangiarotti *et al*., 2000). In almost all cases of UTI, empirical antimicrobial treatment initiated before the laboratory results of urine culture are available; thus antibiotic resistance may increase in uropathogens due to frequent use of antibiotics (Tambekar *et al*., 2006). The antimicrobial resistance in patients with UTI is increasing and can vary according to geographical and regional location (Karlowsky *et al*., 2002).

There is a paucity of data pertaining to spectrum of renal diseases in Nepal, particularly over a period of many years (Agrawal *et al*., 2009; Chhetri *et al*., 2008 and Shah *et al*., 2003). Despite the urinary tract infections (UTIs) as the high cause of mortality and morbidity in an increasing population of patients with chronic renal insufficiency undergoing hemodialysis, very limited information is available. UTIs are challenging because of the large number of infections that occur each year. On the other side, the injudicious use of antibiotics and chemotherapeutic agents, their reduced and decreased dosage in renal failure resulting in the emergence of resistant strains which is becoming difficult in the management of UTIs and is becoming a serious public health issue. Particularly in the developing world like ours, where apart

from high level of poverty, ignorance and poor hygienic practices, there is also high prevalence of fake and spurious drugs of questionable quality in circulation. Therefore, in this case the expected result of this research will be a very useful at gaining knowledge about the type of pathogens responsible for UTIs and their susceptibility patterns may help the clinicians to choose the right empirical treatment decreasing the risk of fatal outcomes and improving quality of life of hemodialysis patients and early diagnosis of infections. The study will also contribute to identify risk factors and to formulate strategies focused on particular risk factors and lowering the chances of antimicrobial resistant.

1.2 Objectives

General Objective

To describe the status of UTI in chronic kidney disease patients undergoing hemodialysis.

Specific Objectives

1. To determine the prevalence of UTI in hemodialysis patients attending at National Kidney Centre.
2. To identify the organisms responsible for UTI in hemodialysis patients.
3. To assess the antimicrobial susceptibility patterns of the isolated organisms.

CHAPTER-II
LITERATURE REVIEW

2.1 Overview of UTI

Urinary tract infection (UTI) is a very common infection both in the community and hospital patients, and ranks high amongst the most common reasons that compel a patient to look for medical attention (Gastmeier *et al*., 1998). It is a condition where one or more structures in the urinary tract become infected after bacteria breach its strong natural defenses. In spite of these defenses, UTIs are the most common of all infections and can occur at any time in the life of an individual (Vincent, 2003).

Microbiologically, urinary tract infections exist when pathogenic micro-organisms are detected in the urine, urethra, bladder, kidney or prostate gland (Schaeffer, 1998). In most instances, the usual quantitative definition of UTI is the detection of 10^5 organisms per milliliter from a properly collected midstream "clean-catch" urine sample (Stamm, 2003). In fact urinary tract infection (UTI) is a broad term that encompasses both asymptomatic microbial colonization of the urine and symptomatic infection with microbial invasion and inflammation of urinary tract structures (Cunin, 2000).

The urinary system is primarily concerned with the removal of nitrogenous wastes from the body, which consists of the paired kidneys and ureters and the single bladder and urethra. Based on anatomic location, the urinary tract is divided into- a) lower urinary tract and b) upper urinary tract. The lower urinary tract encompasses the single bladder and urethra and the upper urinary tract encompasses the ureters and kidneys. The noteworthy fact is that the female urethra is relatively short compared to the male urethra and also lies in close proximity to the warm, moist, perirectal region, which is swarming with microorganisms. Due to shorter urethra, bacteria can reach the bladder more easily in female hosts (Forbes *et al*., 2002).

Normally, the urethra has sparse heterogeneous resident microfloras that colonize its epithelium in the distal portion. Some of these organisms are:

Coagulase Negative *Staphylococci* (CoNS) excluding *Staphylococcus saprophyticus* viridians and Non-hemolytic *Streptococci, Lactobacilli, Diphtheroids (Corynebacterium* spp.); Non pathogenic (saprobic) *Neisseria* spp.; anaerobic cocci, *Propionibacterium* spp.; anaerobic gram negative bacilli; commensal *Mycobacterium* spp.; *Mycoplasma* spp. In addition, some enteric bacteria (e.g. *Escherichia coli, Proteus*) which are probably contaminants from the skin, vulva or rectum, may occasionally be found as transient colonizer at the anterior urethra (Forbes *et al*., 2002).

2.2 Classification

Urinary tract infections are categorized either into- a) lower tract infection- located in the bladder (cystitis) and/or urethra (urethritis) or b) upper tract infection- located in the ureters, collecting system, and parenchyma (pyelonephritis) (Heffner and Gorelick, 2008).

Signs and symptoms of cystitis include dysuria, frequency, urgency, malodorous urine, enuresis, hematuria and suprapubic pain. On the other hand, the signs and symptoms of pyelonephritis include fever over 38.5°C, chills along with costovertebral angle or flank pain and tenderness with pyuria and positive urine culture (Dulczak and Kirk, 2005; Ramadan, 2003). Turner *et al*. (2002) reported that if bacteriuria is not treated 30-40 % of patients developed pyelonephritis. Hill *et al*. (2005) found that it can also result in septicemia (17%), transient renal dysfunction (2%) and pulmonary insufficiency (7%).

The bacterial infection can also be symptomatic or asymptomatic. The symptomatic urinary tract infection can be further uncomplicated or complicated. Uncomplicated urinary tract infection is a symptomatic urinary tract infection characterized by frequency, urgency, dysuria or supra pubic pain in a woman with a normal genitourinary tract (Hooton and Stamm, 1997). While complicated urinary tract infection is also a symptomatic urinary infection in a women with functional or structural abnormalities of the genitourinary tract which involve either the bladder or kidneys (Nicolle, 2001).

The asymptomatic urinary tract infection is a persistent, actively multiplying bacteria within the urinary tract without any symptoms of infection (Uncu *et al*., 2002). While up to 90% of the patients with UTIs complain of urinary tract symptoms, one third or more of the patients with these symptoms do not have bacteriuria (Medina-Bambardo *et al*., 2003).

2.3 Pathogens

Several studies in the past have shown that *Escherichia coli* is the most common pathogen of UTI in hospital and community patients and that hospital-acquired UTI, in particular, is characteristically associated with a higher prevalence of *Enterococci* and Coagulase Negative *Staphylococci* (CoNS) (Barrett *et al*., 1999; Gupta *et al*., 1999; Jones *et al*., 1999; Vorland *et al*., 1985; Vromen *et al*., 1999).

E. coli remains by far the primary causative agent of community-acquired UTIs (Kaper, 2004) and Hospital-acquired UTIs (Farrel *et al*., 2003). Other microorganisms followed by *E. coli* were *Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., *Enterococci* spp. and among community acquired UTI also *S. saprophyticus* (Ronald, 2003).

Manikandan *et al*. (2011) reported that *E. coli* (31.5%) was the predominant pathogen causing UTI, followed by *S. aureus* (20.5%), *K. pneumoniae* (15.8%), *P. mirabilis* (7.4%) and *P. aeruginosa* (7.5%).

Kayas *et al*. (2011) in a study of urinary tract infection in children reported the microorganisms according to decreasing frequency were *E. coli* (75.7%), *Klebsiella* spp. (7.2%), *Proteus* spp. (6.3%) and *Enterobacter* spp. (1.8%).

The most frequent causative agents of UTI in diabetic patients accounting *E. coli* for 39.4% of the isolates followed by *Staphylococcus* (18.4%), *Klebsiella* (15.7%), *Enterococcus* (13.1%), *Proteus* (7.8%), *Pseudomonas* and *Candida* (2.6% each) (Sibi *et al*., 2011).

The result of study in UTI by Khaled *et al.* (2006) divulged that the *E. coli* is responsible for the large proportion of infection (53.24%), followed by other strains like *Enterococcus faecalis* (24.04%), *Proteus* spp. (19.53%), *Staphylococcus aureus* (19.20%), *Staphylococcus epidermidis* (7.8%), *Staphylococcus saprophyticus* (13.2), *Klebsiella* spp. (11.96%), *Enterobacter* spp. (5.12%), *Pseudomonas* spp. (3.4%), *Citrobacter* spp. (1.92%) *and Serratia marcescens* (0.8%).

In the study of urinary tract infection in renal transplant recipients, Elkehili *et al*. (2010) reported *E. coli* was one of the predominant organisms in 38.7% of cases, followed by *Klebsiella* spp. (25.8%), *Staphylococcus* spp. (25.8%), and other organisms (9.7%).

Jadav *et al*. (1977) described the *E. coli* (38% in C.R.F. and 33.3% in A.R.F.) as a dominant causative agent in cases of C.R.F. while *Klebsiella* (46.6% in A.R.F. and 28.5% in C.R.F.) was the predominant pathogen in A.R.F. cases. Others microorganisms isolated were *Staphylococcus aureus* (11.9% in C.R.F. and 6.6% in A.R.F.), *Proteus mirabilis* (9.5% in C.R.F. and 6.6% in A.R.F.), *Enterobacter* group (7.1% in C.R.F. and 10.0% in A.R.F.), *Pseudomonas aeruginosa* (7.1% in C.R.F. and 6.6% in A.R.F.), *Streptococcus faecalis* (4.7% in C.R.F.), *Candida albicans* (3.3% in A.R.F.) *and Morganella morganii* (9.5% in C.R.F. and 10% in A.R.F.). While most frequent bacterial species isolated in the hemodialysis patients were *S. aureus* (22.9%), followed by Coagulase Negative *Staphylococci* spp. (CoNS) (19.7%). The occurrence of gram-positive bacteria in the hemodialysis patients was found to be 59.0% in gross infections (Cetin *et al*., 2007).

2.4 Pathogenesis

Urinary tract infections are the consequence of interaction between the bacteria and the host. The increased bacterial virulence appears to be essential to breach strong host resistance while bacteria with minimal virulence characteristic are able to infect the patients who are immunocompromised (Schaeffer, 1998).

The routes of infections can be ascending route, hematogenous route or lymphatic route. The bacteria that cause urinary tract infections typically enter the bladder via the urethra (ascending route). However, infection may also occur via the blood (hematogenous route) or lymph (lymphatic route). It is believed that the bacteria are usually transmitted to the urethra from the bowel, with females at greater risk due to their anatomy. After gaining entry to the bladder, microorganisms especially *E. coli* are able to attach to the bladder wall and form biofilm that resists the body's immune response (Salvatore *et al*., 2011).

2.5 Epidemiology

Urinary tract infections (UTIs) are one of the most prevalent extra-intestinal bacterial infections. Nowadays, it represents one of the most common diseases encountered in medical practice affecting people of all ages from the neonate to the geriatric age group (Kunin, 1994).

Worldwide, about 150 million people are diagnosed with UTI each year (Gupta, 2001). UTI has become the most common hospital-acquired infection, accounting for as many as 35% of nosocomial infections, and it is the second most common cause of bacteremia in hospitalized patients (Akhtar, 2000).

Urinary tract infection (UTI) is one of the most common domiciliary and nosocomial bacterial infections prevalent in both males and females (Dhakal *et al*., 2002).

Women are mostly infected by urinary tract infections (Colgan and Williams, 2011). Mostly between the ages of 16 and 35 years, 10% of women getting an infection yearly and 60% having an infection at some point in their life time (Nicolle, 2008; Salvatore *et al*., 2011).

Recurrent infections are commonly reported, with nearly half of people getting a second infection within a year. Urinary tract infections were seen four times more frequently in female than male (Salvatore *et al*., 2011). Approximately

30% to 40% of patients develop a repeat infection within one year after a first UTI (Foxman, 2002).

Pyelonephritis occurs between 20-30 times less frequently (Nicolle, 2008). Asymptomatic bacteria in the urine increase with age from 2-7% in women of child bearing age to as high as 50% in elderly women in care homes (Dielubanza and Schaeffer, 2011). While rates of asymptomatic bacteria in the urine among men over 75 years are between 7-10% (Woodford and George, 2011).

Urinary tract infections may affect 10% of people during childhood (Salvatore *et al*., 2011). Among children, urinary tract infections are the most common in uncircumcised males less than three months of age, followed by females less than one year. Estimates of frequency among children however, vary widely. In a group of children with a fever, ranging in age between birth and two years, two to 20% were diagnosed with a UTI (Bhat *et al*., 2011).

2.6 Risk factors of UTI

There are wealth of factors that increase the risk of developing urinary tract infection. Any anatomical or functional abnormalities of the urinary tract that impede urinary flow can increase the host susceptibility to UTI (Tanagho and Mcaninch, 2004).

Anatomic abnormalities include short urethra in females, urinary obstruction, Vesicoureteral reflux (VUR), neurogenic bladder (which is the improper storage of urine in bladder and improper emptying of urine from bladder), and uncircumcison in boys. Uncircumcised boys have a great tendency to harbor organisms in the foreskin due to warm, moist and mucosal environment as a result bacteria migrate up to the urethra and colonize in the bladder (Dulczak *et al.*, 2005 and Heffner and Gorelick, 2008). Another anatomical factor includes posterior urethral valves, or bladder diverticulitis (Heffner and Gorelick, 2008).

Physiological factors include dysfunctional voiding, infrequent voiding, incomplete bladder emptying and constipation. In constipation, stool remains in the rectum for a long period of time, and bacteria tend to colonize in the perineum, as a result increasing the risk for UTI (Dulczak *et al*., 2005).

The risk of UTI can be increased by many factors such as urinary retention, urine stasis, and reflux of urine, unstable bladder, frequent UTIs, constipation, sexual intercourse, chronic illness, and prolonged use of antibiotics. Prolonged use of antibiotics can damage periurethral flora allowing uropathogens to colonize and infect the urinary tract (Tanagho and Mcaninch, 2004).

Various factors make bacteriuria more or less to occur for any individual. These factors are age, gender, race, genetic factors, sexual activity among the teen age girls, and circumcision in boys, nocturnal enuresis and some unhealthy behaviors (Heffner and Gorelick, 2008).

The prevalence of asymptomatic bacteriuria among healthy women increases with advancing age, from about one percent among schoolgirls to >20% among women over 80 years residing in the community (Bengtsson *et al*., 1998; Hooton *et al*., 2000; Nicolle *et al*., 2005). Women are significantly more likely to experience UTI than men. Nearly one in three women will have had at least one episode of UTI requiring antimicrobial therapy by the age of 24 years. Almost half of all women will experience one UTI during their lifetime (Foxman, 2002).

A study in different age and gender groups by Bashir *et al.* (2008) reported that among the uropathogens isolated, *E. coli* was the most frequent in both sexes with 44.45% and 78.1% frequencies in male and female patients, respectively. *Enterococci* caused two folds (9.6%) of UTIs in females than the males (5.55%). *Candida, Pseudomonas* and *Klebsiella* were isolated from 13.89%, 13.89% and 11.11% of the males, respectively.

E. coli caused 80% of cases of acute pyelonephritis in women and 70% of cases in men and was less dominant in older age groups in a study (Czaja *et*

al., 2007). Irrespective of sex, patients with indwelling urinary devices, permanent ureteric stent, spinal cord injuries and on hemodialysis are at increased risk of having asymptomatic bacteriuria (Nicolle *et al*., 2005).

In patients with various diseases, the incidence of urinary tract infection is 20% for diabetes mellitus, 14% for hypertension, 80% for hydronephrosis and nephrolithiasis and greater than 50% for long term indwelling catheters. Twenty five percent of pregnant women with asymptomatic bacteriuria go on to develop acute pyelonephritis (Acharya, 1992).

Twenty five percent to 50% of elderly women and 15%-40% of elderly men in long-term care facilities is bacteriuric (Nicolle, 1997).

Patients undergoing hemodialysis have a prevalence of asymptomatic bacteriuria of 28% (Chaudhry *et al*., 1993). In hemodialysis patients, risk factors include age (p=0.3), sex (p=0.6), diabetes mellitus (p=0.2), immunocompromised state (p=0.2) (D'Agata *et al*., 2000).

The various studies suggest the correlation between UTI and ABO blood group-many blood group antigens, genetically controlled carbohydrate molecules, are found on the surface of uroepithelial cells and may affect bacterial adherence and increase the frequency of urinary tract infection (UTI) in adults (Jantausch *et al*., 1994). Similarly, a study conducted in a Turkey, showed the significant correlation between patients with UTI and ABO-Rh phenotypes. The ABO-Rh phenotype distribution in patients were as follows 36.6% A Rh+, 4.9% A Rh-), 12.2% B Rh+, 2.4% B Rh-), 31.7% O Rh+, 2.4% O Rh-), 4.9% AB Rh+ and no one with AB Rh (Sakallioglu and Sakallioglu, 2007).

2.7 Overview of chronic kidney disease and hemodialysis-

Chronic kidney disease (CKD), is fast emerging as a major public health problem in the twenty-first century, and defined as kidney damage with persistent, gradual and progressive deterioration of kidney function (loss of the capability to excrete wastes, concentrate urine, and conserve electrolytes) (National Kidney Foundation, 2002).

There are various stages of chronic kidney diseases. The new classification systems standardize the various stages of kidney damage. The National Kidney Foundation Disease Outcomes Quality Initiative guidelines define CKD as kidney damage or a glomerular filtration rate of less than 60 mL/min per 1.73 m^2 for at least three months. Three intermediary stages follow, with kidney failure or end-stage renal disease (ESRD), as the final stage, defined by a glomerular filtration rate of less than 15 mL/min per 1.73 m^2. Stage 5 CKD is often called End Stage Renal Disease (ESRD) and is synonymous with the now outdated terms chronic kidney failure (CKF) or chronic renal failure (CRF) (National Kidney Foundation, 2002).

There is no specific treatment clearly shown to slow the worsening of chronic kidney disease. Severe CKD requires renal replacement therapy, which may involve a form of dialysis, and kidney transplant (National Kidney Foundation, 2002). The majority of those with CKD perish because of the lack of funds, as very few can afford regular maintenance dialysis and renal transplantation is often not available (Bambgoye, 2003).

Hemodialysis is the most commonly used modality of Renal Replacement Therapy (RRT) worldwide (Grassmann *et al.*, 2005). It is a form of treatment where accumulated solute and fluid are removed from a patient who has total or near-total loss of kidney function using haemodialysis machines using extracorporeal blood lines and artificial kidney called 'dialyzer' (National Kidney Foundation, 2006).

The other treatment modality like peritoneal dialysis and renal transplantation are also used but they are largely uncommon due to the unaffordable cost,

deficiency of facilities and manpower and the predominantly cities location of the renal care centers (Arije *et al*., 2000; Bambgoye, 2003; Naicker, 2009).

2.8 Chronic kidney disease and infections-

Dialysis patients are more prone to infection than are the general population, both because of the uremia and because of the dialysis itself. This predisposition is multifactorial and is attributed to impairments in lymphocyte and granulocyte function, circulating inhibitors to chemotactic factors, frequent violation of skin, mucosal barriers, baseline hypothermia, iron overload, underlying disorders, low albumin levels, and metabolic acidosis (Cheung and Wong, 2001; Minnaganti *et al*., 2001).

Mainly, there are two major types of white blood cells: neutrophils and lymphocytes. Neutrophils are primarily involved in defending against bacterial infections but bacteria may survive in dialysis patients long enough to produce infections that would not occur in normal individuals. This is because of the inability of neutrophils to ingest and destroy bacteria in the setting of kidney disease. On the other hand, Lymphocytes are primarily involved in protection against infections caused by viruses and fungi. In patients with kidney disease, the activation of lymphocytes to a state where they are most effective in protecting against these infections is impaired, resulting in an increased incidence of viral and fungal infections. Viral infections include simple things like influenza, but also serious conditions such as shingles and hepatitis. Dialysis patients are not only more prone to developing all of these types of viral infections but, when these infections do occur, they tend to be more severe (American Association of Kidney Patients, 2012).

The commonest causes of chronic kidney disease were chronic glomerulonephritis (34.5%), hypertension (32.1%) and diabetes mellitus (17.9%) (Ekrikpo *et al*., 2011). Similarly, the study in Nepal Medical College Teaching Hospital reported the most common cause of CKD were hypertension (54.0%), diabetic nephropathy (18.0%), idiopathic (13.0%) and glomerulonephritis (6.0%) (Chhetri *et al.*, 2008).

Epidemiological studies suggest that there is a higher risk of contracting bacterial infections in CKD patients and that the three most commonly seen infectious complications are urinary tract infections (UTI), pneumonia and sepsis. Patients with CKD treated by dialysis have higher annual mortality rates caused by sepsis compared with the general population, even classified after age, race, and diabetes (Naqvi and Collins, 2006). Overall, the annual percentage of mortality secondary to sepsis is approximately one hundred to three hundred folds higher in dialysis patients (Sarnak and Jaber, 2000). The type of vascular access in use plays an important role in the subsequent development of bloodstream infections. Central venous catheters significantly increase the risk of bacteremia in hemodialysis patients (Powe *et al*., 1999). Those with temporary catheters have been shown to have a 50% higher risk of septicemia than patients with a native fistula. Maximizing the use of arteriovenous fistula as hemodialysis access is likely to lower infection risk (Naqvi and Collins, 2006).

The higher urinary tract infection susceptibility in the CKD group may be explained partly by a greater incidence of urinary obstructions, which finally leads to infections (Ishani *et al*., 2005). Incase of dialysis patients, the urinary tract is insignificantly neglected may be because of their minimal urine output (D'Agata *et al*., 2000).

2.9 Antibiotics and Antimicrobial resistance

Generally, the most common used antibiotics in the treatment of urinary tract infections include: Cotrimoxazole, Fluroquinolones (e.g.Ciprofloxacin), Nitrofurantoin, Aminoglycosides (e.g.Gentamicin, Amikacin), Cephalosporin and Aminopenicillins (e.g. Ampicillin and Amoxicillin) (Smith, 2004).

For Gram-negative rods, the commonly used drugs are Ampicillin, Piperacillin, Amikacin, Gentamicin, Tobramycin, Cefazolin, Cephalothin, Sulphaperazone, Ciprofloxacin, Imipenem, and Cotrimoxazole etc., and for Gram-positive cocci- Penicillin, Erythromycin, Clindamycin, Tetracycline, Ceftriaxone, Cephalothin, Ciprofloxacin, Imipenem, and Vancomycin are commonly used in practices (Yoon *et al*., 2011).

There has been an onslaught of reports on the inappropriate use of antimicrobial agents and the spread of bacterial resistance among microorganisms causing urinary tract infections in the last three decades (Hryniewicz *et al*., 2001; Kurutepe *et al*., 2005 and Tenever and McGowan, 1996). Hryniewicz *et al*. (2001), Jacoby and Archer (1991), Kurutepe *et al*. (2005) and Mordi and Erah (2006) reported the changing patterns in the etiological agents of urinary tract pathogens and their sensitivities to commonly prescribed antibiotics.

The antibacterial resistance in these antibiotics was found to be very high among the isolates. There are many reasons behind these resistances. Some highlighted are due to prolonged use of antibiotics which can damage periurethral flora, allowing uropathogens to colonize and subsequently to infect the urinary tract, leaving clinicians with very few choices of drugs for the treatment of UTI (Tessema *et al*., 2007). Others may due to the recent development of new antibiotics and the appearance and increase of antibiotic-resistant strains, which are created because of antibiotic abuse and inappropriate choice of antibiotics, have lead to changes in the antibiotic susceptibilities of the pathogens (Erb *et al*., 2007 and Gupta *et al*., 2001).

In addition some bacteria may be inherently resistant or they acquire resistance. This may result from impaired cell wall or cell envelope penetration, enzymatic inactivation, altered binding sites or active extrusion from the cell as a result of efflux mechanisms. Acquired resistance may result from mutation, adaptation or gene transfer **(**Smith, 2004).

Kayas *et al*. (2011) in a study of causative agents and antibiotic susceptibilities in children with urinary tract infection reported the isolated microorganisms according to decreasing frequency were *E. coli* (75.7%), *Klebsiella* spp. (7.2%), *Proteus* spp. (6.3%) and *Enterobacter* spp. (1.8%) and the resistance rates against Trimethoprim- Sulfamethoxazole (Cotrimoxazole) was 71.3%, Ampicillin 82.4%, Amoxicillin-Clavulanate 54.7% and Tetracycline 68.3%, and the least resistance rates were for Ceftriaxone (16%) and Amikacin (8.1%).

Aboderin *et al.* (2009) reported the highest proclivity of *E. coil* isolates in urinary tract infections patients and antimicrobial susceptibility varied in prevalence by agent as follows: Nitrofurantoin (80%), Ofloxacin (24%), Ciprofloxacin (15%), Nalidixic Acid (10%), Cotrimoxazole (5%), and Amoxicillin/Clavulanic Acid (2%). No isolate was susceptible to Amoxicillin, Gentamicin or Tetracycline.

Likewise, in the study conducted by Yoon *et al*. (2011), the most common causative microorganisms for UTI were *E. coli* (81.4%), which showed relatively high susceptibility as compared to Imipenem (100%), Amikacin (97.7%), Aztreonam (97.9%), Cefepime (97.7%) and Ceftriaxone (97.1%), while it showed relatively low susceptibility to Gentamicin (79.0%), Cotrimoxazole (68.7%), Ampicillin/Sulbactam (33.0%) and Ampicillin (28.6%).

In a study, conducted by Jha and Bapat in Nepal in 2005, *E. coli* was the most prevalent organism isolated (49%) and it showed a 100% susceptibility to Nitrofurantoin and considerable resistance to Amoxicillin and Ciprofloxacin. *S. aureus* was 88.8% susceptible to second generation Cephalosporin, 77.7% susceptible to Nitrofurantoin and 75% susceptible to Norfloxacin.

Another study in UTI by Shaifali *et al.* (2012) reported the high susceptibility of antibiotics for the *E. coli* isolates- Nitrofurantoin (86.95%), Amoxicillin (69.56%), Nalidixic acid (65.21%) and Cotrimoxazole (60.86%).

Bashir *et al*. (2008) in a study of UTI in different age groups and gender found *E. coli* as a predominant organism which showed variable antimicrobial resistance to different antibiotics as 92%, 86%, 80%, 62%, 47%, 20% and 4% of the isolates were found to be resistant to Ampicillin, Cotrimoxazole, Ciprofloxacin, Gentamicin, Nitrofurantoin and Amikacin, respectively.

Judicious use of antibiotics and development of novel non antimicrobial-based methods of prevention of UTI are important strategies to help slow the progression of resistance. In the meantime, ongoing surveillance of resistance

trends and enhanced understanding of the determinants of resistance are crucial for optimal management of UTIs (Gupta, 2003).

CHAPTER-III
MATERIALS AND METHODS

The descriptive cross sectional study was performed from November 2011-May 2012 in National Kidney Centre, Banasthali, Kathmandu, which is a referral hospital for hemodialysis patients where we could meet our empirical sample numbers.

3.1 MATERIALS

All the materials required are listed in the Appendix III.

3.2 METHODS

3.2.1 Samples

One hundred and thirty seven hemodialysis patients were randomly selected using a simple random sampling technique.

3.2.2 Data collection

The technique of data collection was interviewed from the patients and data generated from wet laboratory. The tools used were questionnaire which includes some structured questions. Targeted groups were asked to provide sample for our study. Firstly they were explained about our research and then their consent for participation in the study was obtained. The gathered information of patients includes name, age, sex, previous immunosuppressive diseases if present, duration of hemodialysis, signs and symptoms of UTI (dysuria, frequency, urgency, fever, flank pain etc.), health habit (smoking, morning constitutional etc.), antibiotics taken or not etc.

The questionnaire used is presented in Appendix - I.

3.2.3 Specimen collection

The participants were given a sterile, clean, and dry leak proof container and requested for 5-10 ml mid-stream urine sample. Before providing the container, each patient was instructed properly for the collection of sample. For male patients they were first asked to wash the glans thoroughly with warm water and make them collect the mid portion of urine after passing

initial small amount of urine, in a provided wide mouthed sterile container. On the other hand, females were first asked to cleanse the vulva and labia thoroughly with warm water and then they were also asked to collect the mid portion of urine in provided wide mouthed leak proof sterile container. The urine samples were tested immediately after collection and no urine samples were delayed for processing. But incase of any delay the urine samples should be refrigerated or preserved with boric acid. The refrigerated samples should be processed within 24 hours.

3.2.4 Laboratory analysis

a) Macroscopic examination

The specimens obtained in laboratory were observed for its color and appearance and reported accordingly.

b) Microscopic examination

Ten ml of urine sample was taken in a clean sterile centrifuge tube and centrifuged at 3000 rpm for 10 minutes. The supernatant was discarded. The sediment was then examined by wet mount preparation.

Wet mount preparation: Microscopic examination of urinary sediments by wet preparation includes the detection of WBC (pus cells) and RBC. Number of WBC and RBC were estimated as number per HPF i.e. 40X objective of microscope.

3.2.5 Chemical examination

The detection of pH, sugar and albumin in urine were performed by using uristix (dipstick technique). The uristix was dipped into the urine specimen for few seconds and the change in color in test area was noted after 30 seconds. The results were interpreted according to the color change of the test area, comparing with that of the given standard color for detection of pH, sugar and albumin.

3.2.6 Culture of specimen

Semi-quantitative culture technique was used to culture urine specimens and to detect the presence of significant bacteriuria by standard methods (Cheesbrough, 2000). An inoculating loop of standard dimension was used and known volume (0.001ml) of mixed uncentrifuged urine was inoculated on the surface of 5% Blood Agar (BA), MacConkey Agar (MA) and Sabouraud Dextrose Agar (SDA). Urine specimen was thoroughly mixed to ensure uniform suspension of bacteria before inoculating the agar plates. The inoculated MA, BA and SDA plates were aerobically incubated overnight at 37º C.

The criteria to interpret significant bacteriuria given by Kass, Marpal and Sandford as: a) Less than 10^4 organisms/ml- not significant.

b) 10^4-10^5 organisms/ml- doubtful significance (specimen should repeat)
c) More than 10^5 organisms

3.2.7 Identification of isolates

Identification of significant isolate was done by using microbiological techniques as described in the Bergey's manual which involves morphological appearance of the colonies, staining reactions, biochemical properties and serotyping if required in specific cases. Standard protocol provided by Cheesbrough (2000) was followed for identification of bacteria isolated from urine specimens.

Pure culture for identification:

Each of the organisms was isolated in pure form before performing biochemical and other tests. Gram staining of an isolated colony was done from primary culture. For Gram negative organism, a speck of single isolated colony from MA and for Gram positive, the same from BA was transferred into the nutrient broth and incubated at 37ºC for 4 hours. It was then subcultured on dried nutrient agar plate and incubated at 37ºC for 24 hours. Thus, obtained overnight incubated culture of organism on nutrient agar was used to perform Catalase, Oxidase, other biochemical and antibiotic

susceptibility tests. The Gram-staining procedure is mentioned in the Appendix- IV.

Biochemical Test:

Appropriate biochemical tests were performed for the confident identification of the bacterial isolates. For that, the pure colonies on the media plates were inoculated onto different biochemical media.

a) Gram positive organisms were identified primarily on the basis of their response to Gram's staining, Catalase, Oxidase and Coagulase tests.

b) The biochemical tests used for the identification of Gram negative bacterial isolates include Catalase test, Oxidase test, Indole test, Methyl red test, Voges Proskauer test, Citrate utilization test, Triple Sugar Iron (TSI) test, Urease test, Motility test, Sulphide production test and Gas production test.

c) For yeast isolated Germ tube test was performed, which method is mentioned on Appendix-IV.

The composition and preparation of biochemical media and reagents used in the biochemical test are mentioned in the Appendix- IV.

3.2.8 Antibiotic susceptibility test

The antimicrobial susceptibility testing of the isolates towards various antimicrobial disks was done by modified Kirby-Bauer disk diffusion method as recommended by National Committee for Clinical Laboratory Standards (NCCLS) using Mueller Hinton agar (MHA). Antimicrobial agents tested were – Cotrimoxazole (25µg), Nalidixic acid (30µg), Cephalexin (30µg), Cefotaxime (30µg), Cefoxitin (30µg), Ofloxacin (5µg), Nitrofurantoin (300µg), Imipenem (10µg), Norfloxacin (10µg) and Amikacin (30µg). These antibiotics were chosen as they are the antibiotics of choice in the treatment of urinary tract infection and as per literature reviewed.

The Mueller Hinton Agar was prepared and sterilized as instructed by the manufacturer. The pH of the medium 7.2-7.4 and the depth of the medium at 4 mm (about 25 ml per plate) were maintained in petridish. Using a sterile wire loop, a single isolated colony of which the sensitivity pattern is to be determined was inoculated into Mueller Hinton broth tube and was incubated

at 37ºC for 2-4 hrs. After incubation, the turbidity of the suspension was matched with the turbidity standard of McFarland tube number 0.5.Using a sterile swab, a plate of MHA was inoculated with the bacterial suspension using lawn culture technique. The plate was left for about 5 minutes to let the agar surface dry. Using sterile forceps, appropriate antimicrobial discs (6 mm diameter) were placed evenly. After overnight incubation, the plates were examined to ensure confluent growth and the diameter of each zone of inhibition in mm was measured and compared with standardized zone interpretative chart provided by the company.

The preparation and composition of Mueller Hinton Agar medium is mentioned in the Appendix-IV. The detailed about antibiotic discs used and its zone size interpretative chart are mentioned in the Appendix-IV.

3.2.9 Quality control of the test

To obtain reliable microbiological result, it is necessary to maintain quality control. Quality of each test was maintained by using standard procedures. The quality of each agar plates prepared was by incubating one plate of each lot on the incubator. Control strains of *E. coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were used for the standardization of the Kirby-Bauer test and also for correct interpretation of zone of diameter. Quality of sensitivity tests was maintained by maintaining the thickness of MHA at 4 mm and the pH at 7.2-7.4. Similarly antibiotics discs containing the correct amount as indicated were used. Strict aseptic conditions were maintained while carrying out all the procedures.

3.2.10 Data analysis

For data analysis Statistical Package for Social Sciences Version 16 were used. Simple descriptive analysis, Chi-square test was used to determine the risk factors. p-value <0.05 was considered statistically significant. Age, gender, organisms causing UTI, their antibiotic sensitivity and resistance, risk factors for UTI, duration of hemodialysis, and ABO blood group in relation with UTI was included as variables in the analysis.

CHAPTER-IV
RESULTS

This study was conducted among the patients from general hemodialysis ward visiting National Kidney Centre (NKC), Banasthali, Kathmandu, Nepal. A total of 137 MSU samples were collected from the patients and the samples were processed in Microbiology Laboratory of Pathology Department of NKC.

4.1 Clinical information of the patients

Out of one hundred and thirty-seven patients, the male patients were found higher than female patients (64.2% vs. 35.8%). The highest number of patients (18.2%) was found in age group 71-80 years followed by 51-60 years (17.5%). The larger number of female patients (8.0%) was found in the age group 41-50 years. Similarly, higher number of male patients (13.1%) was found in age group 71-80 years.

Table 1: Age and gender wise distribution of patients

Age group	Female	Male	Total
	Number (%)	Number (%)	Number (%)
11-20	0 (0.0)	3 (2.2)	3 (2.2)
21-30	7 (5.1)	11 (8.0)	18 (13.1)
31-40	8 (5.8)	11 (8.0)	19 (13.9)
41-50	11 (8.0)	11 (8.0)	22 (16.1)
51-60	7 (5.1)	17 (12.4)	24 (17.5)
61-70	7 (5.1)	14 (10.2)	21 (15.3)
71-80	7 (5.1)	18 (13.1)	25 (18.2)
81-90	2 (1.5)	3 (2.2)	5 (3.6)
Total	**49 (35.8)**	**88 (64.2)**	**137 (100.0)**

Out of one hundred and thirty-seven total cases, 22.6% had UTI while, 77.4% did not.

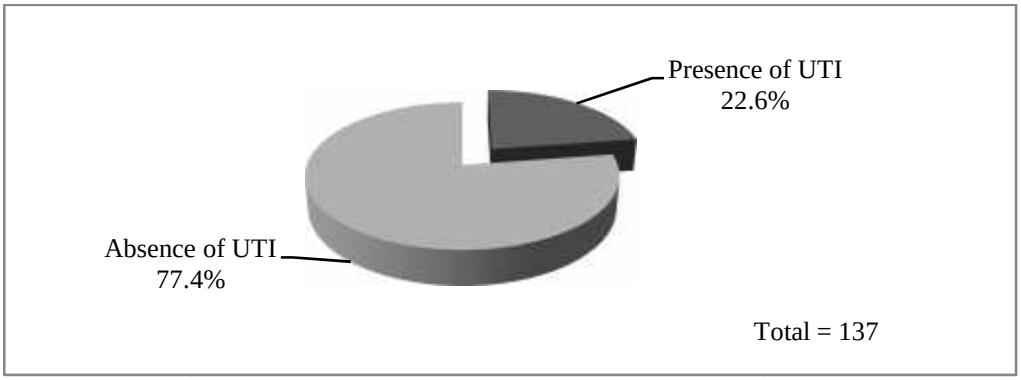

Figure 2: Infection status among patients

The higher proportion of female patients were infected (24.5%) than the male patients (21.6%), however the difference between UTI and gender was statistically insignificant (p>0.05).

Table 2: Gender wise distribution of infection status

Gender	Infected Number (%)	Non infected Number (%)	Total Number (%)	p-value
Male	19 (21.6)	69 (78.4)	88 (100.0)	>0.05
Female	12 (24.5)	37 (75.5)	49 (100.0)	

Among the total of thirty-one culture positive cases of UTI, the symptomatic UTI (54.8%) was found higher in the patients than the asymptomatic UTI (45.2%) and the relationship between UTI with respect to symptoms was statistically insignificant (p>0.05).

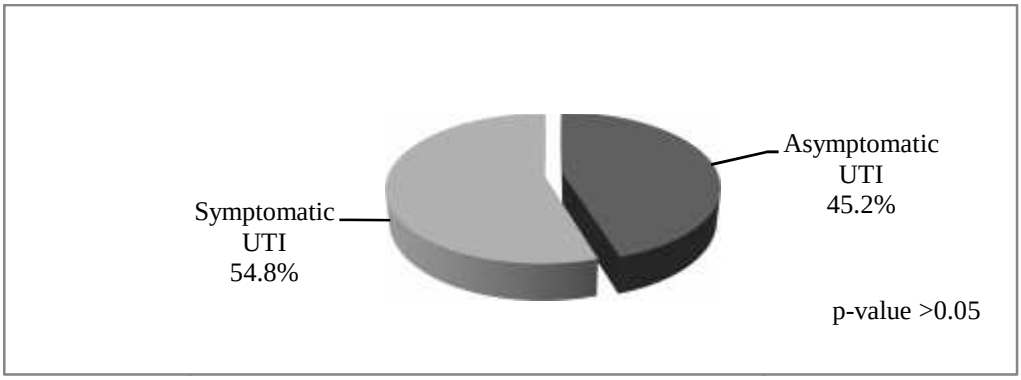

Figure 3: UTI with respect to symptoms

In total of thirty one UTI positive cases, the highest growth rate (22.6%) was obtained from the patient's age group 71-80 years and the least growth rate (6.5%) was obtained from the patient's age group 81-90 years.

Table 3: UTI among age group

Age group	UTI infected Number (%)	UTI non infected Number (%)	Total Number (%)
11-20	0 (0.0)	3 (2.8)	3 (2.2)
21-30	4 (12.9)	14 (13.2)	18 (13.1)
31-40	4 (12.9)	15 (14.2)	19 (13.9)
41-50	5 (16.1)	17 (16.0)	22 (16.1)
51-60	6 (19.3)	18 (17.0)	24 (17.5)
61-70	3 (9.7)	18 (17.0)	21 (15.3)
71-80	7 (22.6)	18 (17.0)	25 (18.2)
81-90	2 (6.5)	3 (2.8)	5 (3.6)
Total	**31 (100.0)**	**106 (100.0)**	**137 (100.0)**

The commonest cause of Chronic Kidney disease in the patients was found to be the hypertension (30.7%). The UTI cases were found higher in the patients with diabetes mellitus (DM) along with hypertension (HTN) (32.2% of total UTI infected samples).

Table 4: UTI with risk factors (in relation with DM and HTN)

Risk factors	UTI infected Number (%)	UTI non infected Number (%)	Total (%)
DM	7 (22.6)	15 (14.2)	22 (16.0)
HTN	6 (19.4)	36 (34.0)	42 (30.7)
DM, HTN	10 (32.2)	26 (24.5)	36 (26.3)
Miscellaneous	8 (25.8)	29 (27.3)	37 (27.0)
Total	**31 (100.0)**	**106 (100.0)**	**137 (100.0)**

[DM: Diabetes mellitus, HTN: Hypertension]

The duration of hemodialysis in patients ranged from <1 month to >25.1 months. The highest UTI 32.2%, (10/31) was found in the duration between 10.1-15.0 months. The lowest UTI 3.2%, (1/31) was found in duration between 15.1-20.0 months.

Table 5: UTI and hemodialysis duration

Hemodialysis duration in month	UTI infected Number (%)	UTI non infected Number (%)	Total Number (%)
<1	5 (16.1)	12 (11.3)	17 (12.4)
1.0-5.0	4 (12.9)	37 (34.9)	41 (29.9)
5.1-10.0	6 (19.4)	20 (18.8)	26 (19.0)
10.1-15.0	10 (32.2)	14 (13.2)	24 (17.5)
15.1-20.0	1 (3.2)	6 (5.7)	7 (5.1)
20.1-25.0	3 (9.7)	6 (5.7)	9 (6.6)
>25.1	2 (6.5)	11 (10.4)	13 (9.5)

The UTI was found higher in blood group A Rh positive patients (45.2%) followed by blood group B Rh positive patients (19.4%). The UTI infection was lower in O Rh negative (3.2%) blood group patients.

Table 6: UTI and ABO blood group

Blood group	UTI infected Number (%)	UTI non infected Number (%)	Total
A Rh positive	14 (45.2)	33 (31.1)	47 (34.3)
B Rh positive	6(19.4)	31 (29.2)	37 (27.0)
O Rh positive	5 (16.1)	31 (29.2)	36 (26.3)
AB Rh positive	5 (16.1)	9 (8.5)	14 (10.2)
O Rh negative	1 (3.2)	0 (0.0)	1 (0.7)
Others	0 (0.0)	2 (1.9)	2 (1.5)
Total	**31 (100.0)**	**106 (100.0)**	**137 (100.0)**

4.2 Bacterial isolates

E. coli (32.3%) was found to be the most predominant organism among bacterial isolates followed by CoNS (22.6%). *E. coli* infected more male patients (36.8%) than female patients (25.0%). The other organisms include *K. pneumoniae* (12.9%), *S. aureus* (9.7%), *P. mirabilis* (6.5%) and *M. morganii* (6.5%). One fungus *Candida albicans* was isolated from female patient.

Table 7: Bacterial isolates in gender

Bacteria isolated	Female Number (%)	Male Number (%)	Total Number (%)
Gram negative			
E. coli	3(25.0)	7 (36.8)	10 (32.3)
P. mirabilis	2 (16.7)	0 (0.0)	2 (6.5)
K. pneumoniae	2 (16.7)	2(10.5)	4 (12.9)
M. morganii	2(16.7)	0(0.0)	2 (6.5)
Gram positive			
S. aureus	1(8.3)	2(10.5)	3 (9.7)
CoNS	1 (8.3)	6 (31.6)	7 (22.6)
Streptococcus spp.	0(0 .0)	2(10.5)	2 (6.5)
Fungi			
Candida albicans	1 (8.3)	0(0.0)	1 (3.2)

4.3 Antibiotic susceptibility pattern of isolated organisms

Out of total Gram negative isolates Amikacin 100.0% (18/18), Imipenem 100.0% (18/18) and Nitrofurantoin 72.2% (13/18) were effective drugs while, Nalidixic acid was the most ineffective drug 94.4% (17/18). The organisms were resistant to different lower generation Cephalosporins (>50%).

Table 8: Antibiotic susceptibility pattern of Gram negative organisms

Antibiotics	Sensitive Number (%)	Intermediate Number (%)	Resistant Number (%)	Total Number (%)
Amikacin	18 (100)	0 (0.0)	0 (0.0)	18 (100.0)
Cephalexin	0 (0.0)	3 (16.7)	15 (83.3)	18 (100.0)
Cefotaxime	7 (38.9)	1 (5.6)	10 (55.6)	18 (100.0)
Cotrimoxazole	3 (16.7)	4 (22.2)	11 (61.1)	18 (100.0)
Cefoxitin	1 (5.6)	2 (11.1)	15 (83.3)	18 (100.0)
Imipenem	18 (100)	0 (0.0)	0 (0.0)	18 (100.0)
Nalidixic acid	1 (5.6)	0 (0.0)	17 (94.4)	18 (100.0)
Nitrofurantoin	13 (72.2)	3 (16.7)	2 (11.1)	18 (100.0)
Ofloxacin	5 (27.8)	5 (27.8)	8 (44.4)	18 (100.0)
Norfloxacin	4 (22.2)	3 (16.7)	11 (61.1)	18 (100.0)

Out of twelve Gram positive organisms isolates, Imipenem 91.7% (11/12), Cefotaxime 58.3% (7/12), Nitrofurantoin 58.3% (7/12) and Ofloxacin 41.7% (5/12) were effective drugs while, Cephalexin and Cefoxitin (75% resistant each) were the least effective drugs.

Table 9: Antibiotic susceptibility pattern of Gram positive organisms

Antibiotics	Sensitive Number (%)	Intermediate Number (%)	Resistant Number (%)	Total Number (%)
Amikacin	3(25.0)	3 (25.0)	6(50.0)	12(100.0)
Cephalexin	1 (8.3)	2 (16.7)	9 (75.0)	12 (100.0)
Cefotaxime	7(58.3)	1 (8.3)	4 (33.3)	12 (100.0)
Cotrimoxazole	4 (33.3)	3 (25.0)	5 (41.7)	12 (100.0)
Cefoxitin	0 (0.0)	3 (25.0)	9 (75.0)	12 (100.0)
Imipenem	11(91.7)	1 (8.3)	0 (0.0)	12 (100.0)
Nitrofurantoin	7(58.3)	5 (41.7)	0 (0.0)	12 (100.0)
Ofloxacin	5(41.7)	0 (0.0)	7(58.3)	12 (100.0)
Norfloxacin	3(25.0)	1 (8.3)	8(66.7)	12 (100.0)

For *E. coli* isolates, Amikacin and Imipenem (each 100.0% susceptibility) and Nitrofurantoin (90.0% susceptibility) were effective drugs, while first generation cephalosporin (i.e. Cephalexin) and second generation cephalosporin (i.e. Cefoxitin) and Nalidixic acid were least effective.

Table 10: Antibiotic susceptibility pattern of *E. coli* (N=10)

Antibiotics	Sensitive Number (%)	Intermediate Number (%)	Resistant Number (%)	Total Number (%)
Amikacin	10 (100.0)	0 (0.0)	0(0.0)	10 (100.0)
Cephalexin	0 (0.0)	0 (0.0)	10 (100.0)	10 (100.0)
Cefotaxime	2 (20.0)	0(0.0)	8 (80.0)	10 (100.0)
Cotrimoxazole	1 (10.0)	1 (10.0)	8 (80.0)	10 (100.0)
Cefoxitin	1 (10.0)	0 (0.0)	9 (90.0)	10 (100.0)
Imipenem	10 (100.0)	0 (0.0)	0 (0.0)	10 (100.0)
Nalidixic acid	1 (10.0)	0 (0.0)	9 (90.0)	10 (100.0)
Nitrofurantoin	9 (90.0)	1 (10.0)	0 (0.0)	10 (100.0)
Ofloxacin	3 (30.0)	2 (20.0)	5 (50.0)	10 (100.0)
Norfloxacin	3 (30.0)	2 (20.0)	5 (50.0)	10 (100.0)

For CoNS, the drugs Imipenem (100.0%), Cefotaxime (42.9%) and Nitrofurantoin (42.9%) were effective. No isolates were sensitive to old generation Cephalosporins- Cephalexin and Cefoxitin. The drugs Amikacin, Cotrimoxazole, Norfloxacin and Ofloxacin showed effectiveness less than 30%.

Table 11: Antibiotic susceptibility pattern of Coagulase Negative *Staphylococci* (CoNS) (N=7)

Antibiotics	Sensitive Number (%)	Intermediate Number (%)	Resistant Number (%)	Total Number (%)
Amikacin	2 (28.6)	1 (14.3)	4 (57.1)	7 (100.0)
Cephalexin	0 (0.0)	1 (14.3)	6 (85.7)	7 (100.0)
Cefotaxime	3 (42.9)	1 (14.3)	3 (42.9)	7 (100.0)
Cotrimoxazole	2 (28.6)	1 (14.3)	4 (57.1)	7 (100.0)
Cefoxitin	0 (0.0)	3 (42.9)	4 (57.1)	7 (100.0)
Imipenem	7 (100.0)	0 (0.0)	0 (0.0)	7 (100.0)
Nitrofurantoin	3 (42.9)	4(57.1)	0 (0.0)	7 (100.0)
Norfloxacin	2 (28.6)	1 (14.3)	4 (57.1)	7 (100.0)
Ofloxacin	2 (28.6)	0 (0.0)	5 (71.4)	7 (100.0)

Incase of *K. pneumoniae* isolates, Amikacin and Imipenem (100.0% each) were most effective drugs. On the other side, Cefoxitin, Nalidixic acid and Norfloxacin were least effective drugs (100.0% resistant each).

Table 12: Antibiotic susceptibility pattern of *K. pneumoniae* (N=4)

Antibiotics	Sensitive Number (%)	Intermediate Number (%)	Resistant Number (%)	Total Number (%)
Amikacin	4 (100.0)	0 (0.0)	0 (0.0)	4 (100.0)
Cephalexin	0 (0.0)	1 (25.0)	3 (75.0)	4 (100.0)
Cefotaxime	2 (50.0)	1 (25.0)	1 (25.0)	4 (100.0)
Cotrimoxazole	2 (50.0)	1 (25.0)	1 (25.0)	4 (100.0)
Cefoxitin	0 (00.0)	0 (0.0)	4 (100.0)	4 (100.0)
Imipenem	4 (100.0)	0 (0.0)	0 (0.0)	4 (100.0)
Nalidixic acid	0 (0.0)	0 (0.0)	4 (100.0)	4 (100.0)
Nitrofurantoin	2 (50.0)	1 (25.0)	1 (25.0)	4 (100.0)
Ofloxacin	2 (50.0)	0 (0.0)	2 (50.0)	4 (100.0)
Norfloxacin	0 (0.0)	0 (0.0)	4 (100.0)	4 (100.0)

Among three species of *S. aureus* isolates, antimicrobial susceptibility varied as follows: the drugs Cefotaxime, Nitrofurantoin, Ofloxacin and Imipenem (66.7% susceptibility each), Amikacin and Cephalexin (33.3% susceptibility each) and no isolate was susceptible to Cefoxitin and Norfloxacin.

Table 13: Antibiotic susceptibility pattern of *S. aureus* (N=3)

Antibiotics	Sensitive Number (%)	Intermediate Number (%)	Resistant Number (%)	Total Number (%)
Amikacin	1 (33.3)	1 (33.3)	1 (33.3)	3 (100.0)
Cephalexin	1 (33.3)	1 (33.3)	1 (33.3)	3 (100.0)
Cefotaxime	2 (66.7)	1 (33.3)	0 (0.0)	3 (100.0)
Cotrimoxazole	0 (0.0)	2 (66.7)	1 (33.3)	3 (100.0)
Cefoxitin	0 (0.0)	0 (0.0)	3 (100.0)	3 (100.0)
Imipenem	2 (66.7)	1 (33.3)	0 (0.0)	3 (100.0)
Nitrofurantoin	2 (66.7)	1 (33.3)	0 (0.0)	3 (100.0)
Ofloxacin	2 (66.7)	0 (0.0)	1 (33.3)	3 (100.0)
Norfloxacin	0 (0.0)	0 (0.0)	3 (100.0)	3 (100.0)

Antibiotic susceptibility pattern of *P. mirabilis* (N=2)

All the two isolates of *P. mirabilis* were sensitive to Amikacin and Imipenem (100% each) and resistant to Nalidixic acid (100%). Only one isolate was sensitive to Cefotaxime and Nitrofurantoin. No isolate was sensitive to Cephalexin, Cotrimoxazole, Cefoxitin, Ofloxacin and Norfloxacin.

Antibiotic susceptibility pattern of *M. morganii* (N=2)

All the two isolates of *M. morganii* were sensitive to the drugs Amikacin, Cefotaxime and Imipenem (100.0% each) and resistant to Nalidixic acid (100.0%). Only one isolate was sensitive to Nitrofurantoin and Norfloxacin. No isolate was sensitive to Cephalexin, Cotrimoxazole, Cefoxitin and Ofloxacin.

Antibiotic susceptibility pattern of *Streptococcus* spp. (N=2)

All the isolates of *Streptococcus* spp. (6.5%) were sensitive to Cefotaxime, Cotrimoxazole, Nitrofurantoin and Imipenem (100.0% each) while; no isolates were sensitive to Cephalexin and Cefoxitin. Only one isolate was sensitive to Ofloxacin and Norfloxacin and resistant to Amikacin.

CHAPTER-V
DISCUSSION

The proclivity of organisms causing urinary tract infection is always exorbitant in hemodialysis patients. But the very notorious disease is not given much emphasis and often neglected. UTI being an unreportable disease in many circumstances is important cause of morbidity and mortality in hemodialysis patients. The early diagnosis of UTI is necessary to preclude subsequent complications of UTI such as sepsis, nephrectomy and death in these patients. A very limited study has been conducted regarding UTI in hemodialysis patients.

Total of one hundred and thirty seven, urine samples from hemodialysis patients were studied. The overall prevalence rate of UTI in hemodialysis was about one-fourth. This implies the hemodialysis patients are at higher risk to UTI. The finding is bolstered by the finding of Kessler *et al.* (1993) which reported the UTI in hemodialysis patients with frequency of 23.0%. The finding of Swarnalatha *et al*. (2011) is however higher than ours (37.14%). Thus, UTI is a seemingly common but having greater potency to revamp into high severity of illness because of the impaired immune defenses in hemodialysis patients.

Among the total UTI cases, 54.8% were symptomatic and 45.2% were asymptomatic. Therefore, without a proper understanding of the characteristics of UTI or symptoms, early detection of this disease is elusive which can mislead in treatment. A study in haemodialysis patients by Chaudhry *et al.* (1993) showed 28% asymptomatic bacteriuria, which was lower prevalence rate than our result obtained. The symptomatic and asymptomatic forms of UTI in different circumstances make the disease even more serious which can affect patients in both avenues either manifesting symptoms or without manifesting symptoms. Thus, a severe renal injury can occur before the UTI is diagnosed. For this reason, an early and accurate diagnosis through careful examination and tests can help in preventing severe renal injuries through adequate treatment and careful follow-up.

The highest prevalence of UTI was found in female (24.5%) as compared to male (21.6%), however the prevalence of UTI was independent of the gender (i.e. $p>0.05$). This result is embraced with result obtained by Bashir *et al*. (2008), Elkehili *et al*. (2010), Jha and Bapat (2005) etc. which also showed high prevalence of UTI in female. The reason for higher growth rate in female is attributed to their anatomical structure (short urethra and proximity to anal orifice) which leads to easy access for coliform bacilli, sexual activity, history of UTI, anatomical factors affecting bladder emptying (cystoceles, urinary incontinence, residual urine) etc. Therefore, the routine check ups in female patients can help in reducing UTI.

In the age group 71-80 years the highest prevalence of UTI was found. The similar findings were obtained in a study by Arul Prakasam *et al*. (2012) in India, in which an increased prevalence of UTI was recorded among the extreme age group of male 51-60 years (54.28%). With increasing age the prevalence of UTI increases in both men and women. The increasing episodes of UTI in men after middle age may be mostly attributable to obstructive uropathy such as an enlarged prostrate (Lipsky, 1989). Steven (1989) reported that about 10% women have urinary tract infections at the age of seventy. This may be due to a variety of anatomical and functional changes which arise with aging, such as hormonal changes (e.g. decreased estrogen which is essential to maintain the normal acidity of vaginal fluid), reduced uromucoid secretions, decreased renal ability and increased bacterial adherence to uroepithelial cells (O'Donnell and Hofmann, 2002). Others may be due to decreasing immunity with increasing age, prominent risk factors in higher ages, metabolic factors such as diabetes, living in a long-term care facility, functionally abnormal genitourinary tract or a predisposing medical condition etc. Thus, the older aged patients should have regular screening of UTI so that UTI can be checked in CKD patients during hemodialysis.

The commonest causes of chronic kidney disease in patients was hypertension (30.7%) followed by diabetes mellitus along with hypertension (26.3%), diabetes mellitus (16.0%) and others (27%). This finding is consonant to the finding by Ekrikpo *et al*. (2011) and Seok *et al*. (2010). The similar study

conducted in Nepal Medical College Teaching Hospital by Chhetri *et al*. (2008) divulged the hypertension as a major cause of CKD. The growth rate was found higher in patients with diabetes mellitus and hypertension (32.2%). UTI recurs easily if it is accompanied with anatomical anomalies of the urinary system. The hypertension and diabetes are among the risk factors that make the body more susceptible to developing kidney infections. In diabetics due to the unfavorable metabolic changes such as elevated blood sugar levels, which suppress the immune system (Sklar *et al.,* 1987). For this reason, the patients should be encouraged to embrace healthy lifestyles, their families, and caregivers should be educated on infectious risks through raised general public awareness which help to reduce all these risk factors and finally UTI can be checked.

The UTI cases were found higher in duration between 10 to15 months of dialysis (32.2%). This may be due to the long term care facility, exposure to different risk groups during care facility etc. Therefore, the patients having longer routines of dialysis should avoid exposure to the different risk groups. And more meticulous patient management by medical professionals and increased attention to infections and their prevention may reduce UTI in the CKD population undergoing hemodialysis.

In our studies the highest UTI was found among 'A Rh+' blood group. However, the prevalence of UTI and the blood group of the patients is not correlated ($p>0.05$). The result is championed by the finding of Sakallioglu and Sakallioglu (2007). Many blood group antigens, genetically controlled carbohydrate molecules, are found on the surface of uroepithelial cells and may affect bacterial adherence and increase the frequency of urinary tract infection (UTI) in adults (Jantausch *et al*., 1994). Therefore, A Rh+' blood group patients should be aware of UTI which help them to prevent from UTI.

Gram negative bacteria were more frequently isolated than Gram positive microorganisms. In addition a fungus was also found. This result is in agreement with other reports (Jadav *et al*., 1977). This is because of inability of neutrophils and lymphocytes in protecting against these infections, resulting

in an increased incidence of bacterial, viral and fungal infections. Altogether eight different bacterial isolates were found in this study. *E. coli* is the major bacterial culprit in urinary tract infections. As expected, among the isolates *E. coli* was the predominant urinary pathogen accounting for 32.3% (10/31) followed by Coagulase Negative *Staphylococcus* (CoNS) (22.6%), *Klebsiella* spp. (12.9%), *S. aureus* (9.7%). Also *M. morganii* (6.5%), *Proteus mirabilis* and *Streptococcus* spp. (6.5% each), *Candida albicans* (3.2%) were found. In a similar study done by Jadav *et al*., (1977) the organisms isolated were *E. coli* (38%), *Klebsiella* spp. (28.5%), *P. mirabilis* (9.5%), *M. morganii* (9.5%), *S. aureus* (4.7%), *and S. faecalis* (2.3%).

The preponderance of *E. coli* seen in this study is in harmony with many other studies by different researchers- Elkehili *et al.* (2010), Jadav *et al.* (1977), Jha and Bapat (2005), Kaper **(**2004), Manikandan *et al.* (2011), Sibi *et al*.(2011), Yoon *et al*.(2011) etc. This may due to the fact that *E. coli*, a common inhabitant of the gastrointestinal tract of humans and animals, gains easily access to the urinary tract. Incase of the debilitated or immunosuppressed host or when the gastrointestinal barriers are violated, even nonpathogenic-commensal strains of *E. coli* can cause infection (Kaper *et al.*, 2004).

In our studies the CoNS (22.6%) is being second and *S. aureus* (9.7%) being fourth predominant organism causing UTI. The reasons may be due to the fact that *Staphylococcus* is a normal inhabitant of the skin but, given the decreased resistance to infection by *Staphylococcus* that often occurs in patients with kidney disease, these bacteria are much more likely to invade the body. Once these bacteria have access to the blood stream, they frequently spread to bones, joints and the heart, causing potentially lethal destruction of these tissues. If the infection spreads from the catheter or graft into the blood stream, it may travel to other parts of the body producing additional problems in those areas.

K. pneumonie was isolated as the third commonest pathogen in frequency causing UTI. *Klebsiella* species are ubiquitous in both the environment and on mammalian mucosal surfaces. The detection rate for these bacteria in normal

human stool samples ranges from 5 to 38% although they are identified in 77% of stool samples of hospitalized patients (Thom, 1970). The urinary tract is the most common site of *Klebsiella* infection however it also causes pneumonia in compromised hosts (Carpenter, 1990). *Klebsiella* accounts for up to 17-29% of all nosocomial UTI and even shows higher incidence among specific groups of patients (Lye *et al*., 1992; Podschun and Ullmann, 1998).

P. mirabilis, even though not a common cause of UTI in the normal urinary tract, but is found frequently in individuals with structurally abnormal urinary tracts or with devices.

A mounting body of evidence indicates that UTI is responsible for a large proportion of antibiotic consumption in and out of the hospital. No wonder that the infection has a large socio-economic impact and contributes to the emergence of antibiotic resistance in the hospital and the community (Magee *et al.* 1999 and Mobley, 2000).The dialysis patients are often in the forefront of the epidemic of resistance (Berns and Tokars, 2002).

It is noteworthy fact that for gram-negative organisms, the more frequently used antimicrobials like Nalidixic acid, Cephalexin, Cefoxitin, Cotrimoxazole, Norfloxacin, Ofloxacin etc. revealed lowest levels of susceptibility (<30%). In contrast, Amikacin, Nitrofurantoin and Imipenem demonstrated the best sensitivity and most consistent activity (>70%). Incase of gram-positive organisms the antimicrobial agents tested for isolates, showed following sensitivity in decreasing order Imipenem (91.7%), Cefotaxime (58.3%), Nitrofurantoin (58.3%), Ofloxacin (41.7%), Cotrimoxazole (33.3%), Norfloxacin (25%), Amikacin (25%), Cephalexin (8.3%). *E .coli* were high sensitive to the antibiotics-Amikacin (100%), Imipenem (100%), Nitrofurantoin (90%). In contrast, the commonly used antibiotics like old generation Cephalosporins, Nalidixic acid, Cotrimoxazole showed high resistant. The finding is in harmony with the finding of Kenechukwu *et al*. (2006) in which the gram positives were highly resistant to Nalidixic acid, Cotrimoxazole etc. The reasons may be due to poor patient compliance, indiscriminate prescription (Arul Prakasam *et al*., 2012), transmissible

elements/plasmids (Nue, 1994), conjugation (Tomasz, 1994) etc. This finding is in harmony with many researchers- Aboderin *et al.* (2009) reported the Nitrofurantoin (80%), as high susceptibility and Nalidixic Acid (10%) and Cotrimoxazole (5%) as having high resistivity. In another study conducted by Yoon *et al.* (2011), in which also Imipenem (100%), Amikacin (97.7%), were shown high susceptible to *E. coli*. Incase of CoNS, the most effective drugs were Imipenem (100%), Nitrofurantoin (42.9%) and Cefotaxime (42.9%). Among these antibiotics, old generation Cephalosporin (Cephalexin) was ineffective.

These findings of resistance reported in these studies showed a tendency towards higher prevalence rates of resistance in recent years. This indicates a need for regular monitoring of antimicrobial susceptibility rates by standardized sampling and measurement procedures. Such monitoring would help identify relevant factors that contribute to the spread of resistant pathogens and would support the prudent use of antibiotics (Erb *et al*., 2007).

Overall, the seemingly mundane form of UTI involving different risk factors and affecting different age groups and gender in hemodialysis can be very life threatening disease. On the other hand, the recent development of new antibiotics and the appearance and increase of antibiotic-resistant strains, which are created because of antibiotic abuse and inappropriate choice of antibiotics, have lead to changes in the antibiotic susceptibilities of these UTI causing pathogens. Therefore, a need for a comprehensive study of the identification of the causative agent and its susceptibility to antimicrobial agents is important in selecting suitable drugs for treating the patient in early stage of the UTI developments in hemodialysis patients.

CHAPTER-VI
CONCLUSION AND RECOMMENDATIONS

6.1 **Conclusion**

The prevalence of UTI in hemodialysis was found to be one fourth. This gives the connotation that the hemodialysis patients are at a higher risk to UTI. The prevalence of UTI was higher in female and higher age groups of the hemodialysis patients. It was more in A Rh+ blood group patients. Since, the UTI is essentially the disease requiring antibiotic treatment; the infection has a large socio-economic impact and contributes to the emergence of antibiotic resistance in the hospital and the community. All these, put forth the hemodialysis in a forefront of the UTI infection.

6.2 Recommendations

a) The different risk factors like diabetes mellitus, hypertension, prolonged use of hemodialysis catheters, hospital indwelling catheters, etc. which are associated with frequent and serious UTI infections so understanding the increased risk and recognizing the earliest signs help to prevent and manage the UTI in time.

b) The antibiotics like Amikacin, Nitrofurantoin, Imipenem showed higher effectiveness towards UTI pathogens so these antibiotics can be the major drug of choices.

c) Our study showed a few potential directions for future research like establishing of antibiotic guidelines for UTI, prescribing pattern study in hemodialysis UTI in large samples and surveillance and monitoring of antibiotic resistance.

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