CHAPTER-I

1. INTRODUCTION

The term UTI refers to the invasion of the urinary tract by a non resident infectious organism. UTI encompasses a wide variety of clinical entities whose common denominator is microbial invasion of any tissue of the tract from the renal cortex to the urethral meatus. Urethritis caused by Chlamydiae and Gonococci is not included in the definition because of their unique characters and strict localization to the urethra and genital system (Pokharel, 2004).

Urinary tract infection (UTI) is one of the most important causes of morbidity in the general population, and it is the second most common cause of hospital visits. Recurrent infections are common and can lead to irreversible damage of kidneys, resulting in renal hypertension and renal failure in severe cases. In the community, women are more prone to develop UTI. About 20 % of women experience a single episode of UTI during their lifetime, and 3% of women have more than one episode of UTI per year. Pregnancy also makes them more susceptible to infections (Das *et al*, 2006)

The urinary tract consists of the kidneys, ureters, bladder, the urinary tract and urethra. All areas above the urethra in a healthy human are sterile (Forbes *et al*, 2002). Infection may be expressed predominantly at a single site, kidney i.e. pelvis and cortex (pyelonephritis), pelvis and ureter (pyelitis), ureter (ureteritis), bladder (cystitis), prostate (prostatitis) and urethra (urethritis) but the entire urinary tract is always at a risk of invasion of bacteria, once any one of its part is infected (Brooks *et al*, 2004).

The pathogens causing UTIs are almost always predictable, with *Escherichia coli* the primary etiologic agent among both outpatients and inpatients (Sahm *et al*, 2001). Other pathogens include *Proteus mirabilis*, *Klebsiella* spp., and other *e*nterobacteriaceae and *Staphylococcus saprophyticus*. In more complicated UTIs, particularly in recurrent infections, the relative frequency of infection caused by

Proteus, *Pseudomonas*, *Klebsiella* and *Enterobacter* spp. increases. Hospitalized patients are most likely to be infected by *E.coli*, *Klebsiella* spp, *Proteus mirabilis*, *Staphylococci* and other enterobacteriaceae, *Pseudomonas aeruginosa* and enterococci. In addition, UTIs are leading cause of Gram negative sepsis in hospitalized patients and are the origin for about half of all nosocomial infections caused by urinary catheters (Forbes *et al*, 2002).

The anatomy of the female urethra is of particular importance to the pathogenesis of UTI. The female urethra is relatively short compared to the male urethra and lies in the close proximity to the warm, moist, perirectal region, which is teeming with microorganisms because of shorter urethra, bacteria can reach the bladder more easily in the female host (Forbes *et al*, 2008)

The exact prevalence of UTI is age and sex dependent. The incident of UTI among males remains relatively low after one year of age and until 60 years of age. When enlargement of prostate interferes with emptying of the bladder. Therefore UTI is predominantly a disease of females. The association of UTI with sexual intercourse may also contribute to the increased incidence because sexual activity increases the chances of bacterial contamination of the female urethra, finally as a result of anatomic and hormonal changes that favor development of UTI. The incidence of bacteriuria increases during pregnancy (Forbes *et al*, 2008).

UTI are important complications of diabetes and renal diseases, renal transplantation and structural and neurological abnormalities that interfere with urine flow. In 40% to 60% of renal transplant recipient, the urinary tract is the source of bacteria and in these patients recurrence is about 40% (Forbes *et al*, 2008).

Kidney transplantation originated in the United States in 1954. The first successful renal transplant was done on identical twins by Dr. John Murray. In developed countries, approximately 75% of the transplants performed use organs from cadaveric donors while the developing countries transplant about 85 – 100% of the kidneys from living donors (Enns *et al*, 2011)

UTI is the most common post transplantation infection. Nearly, 80% of renal transplant recipient suffer at least one episode of infection during the first year after

transplantation and infection remains the leading cause of morbidity and mortality throughout the post transplant course (Charfeddine *et al*, 2002).

Prevention and treatment of infection in the transplant recipient is of major importance. The required immunosuppressive therapy must be judiciously administrated. Previous studies have shown that there is a typical timetable that characterizes the post transplantation infection pattern, particular types of infection being more likely to occur at different time period. Accordingly, in the first month of post transplantation, opportunistic (fungal, nocardial, and protozoal) infection are almost non-existent. (Charfeddine *et al*, 2002).

The greatest risk of life threatening infection is in the time period of one to six months of post- transplantation, when the immunosuppressive effects of anti-rejection therapy are at their peak. After six months of transplantation, the time when maintenance immunosuppressive therapy is at lowest level, three types of infection are commonly observed. These includes: chronic; particularly viral infections, an occasional opportunistic infection and usual infections prevailing in the general community such as influenza, pneumococcal pneumonia and urinary tract infection (Charfeddine *et al*, 2002). Febrile UTI play a crucial role in progression of chronic kidney disease even before and also after kidney transplantation. UTI may lead to kidney damage affecting long term graft survival by scarring and intestinal injury (Ulrike *et al*, 2007).

Multiple drug resistance (MDR) bacterial isolates have been frequently reported from different parts of the world as an emergence of treatment problem. The MDR strain is defined as the strain that showed resistance to three or four antibiotics among commonly six prescribed drugs. An antibiotic resistance is defined as the microbe which is sensitive to certain antibiotic start gaining resistance against it. Infections caused by MDR strains often lead to death (Tuladhar *et al*, 2003).

It is estimated that about 2.7 million people are suffering from kidney disease in Nepal and about two thousands add up to this number yearly. It is further estimated that nearly 750 kidney transplantation are needed in a year to meet the national needs, which accounts to about two transplantations a day. But due to various constraints

prevalent, less than ten transplantations altogether in the two institutions, are being performed in a month (Chalise *et al,* 2010).

Renal transplantation (RT) service was started successfully for the first time in Nepal at Tribhuvan University Teaching Hospital (TUTH), Kathmandu, on 8 th August 2008, immediately after obtaining a license from the government. Live donor RT has however, been legalized in Nepal only since 2000. Previous efforts at RT in Nepal had been unsuccessful (3 transplant in one of the nursing home in 1996 and at central government hospital in November 2004). This service was also started in December 2008 at Bir Hospital, Kathmandu, which became the second hospital in the country to successfully carry out kidney transplantations (Chalise *et al,* 2010). The National Kidney Foundation Nepal, an NGO that aims to provide quality and affordable healthcare services and education regarding kidneys to Nepalis, was founded in March 2010. Otherwise, NGOs supporting kidney patients are rare here.

Nepali law only permits the transplantation among close relatives and potential kidney donor's can be the father, mother, sister, brother, husband, wife, son, daughter, uncle, aunt, mother-in-law, fatherin-law, step father/mother or adopted children. This strict legislation is to prevent the possible organ trade and foul play in procuring the organ (Chalise *et al,* 2010).

This study aims to investigate the bacterial pathogens responsible for UTI in kidney transplanted patients and compare with UTI in other patients (other than kidney transplanted patients), and find out their antibiotic susceptibility pattern in patients visiting tertiary level reference labolatory, National Public Helalth labolatory (NPHL),Teku Kathmandu.

CHAPTER-II

2. OBJECTIVES

2.1 GENERAL OBJECTIVE

To isolate and identify bacterial isolates causing UTI in kidney transplanted patients and compare with other UTI suspected patients.

SPECIFIC OBJECTIVES

1. To determine the pathogenic bacterial isolates from urine specimens of patients of different age groups and gender visiting NPHL.

2. Comparative evaluation of UTI in kidney transplanted and other UTI suspected patients.

3. To evaluate the antibiotic susceptibility pattern of the isolates

4. To find out the prevalence of Multi Drug Resistance (MDR) isolates.

CHAPTER-III

3. LITERATURE REVIEW

3.1 URINARY TRACT INFECTION

UTI is defined as the detection of both bacteriuria 10^5cfu/ml and pyuria >10 leucocytes/HPF (Goya *et al*, 1997). The term UTI refers to the invasion of the urinary tract by a non resident infectious organism. UTI encompasses a wide variety of clinical entities whose common denominator is microbial invasion of any tissue of the tract from the renal cortex to the urethral meatus. Urethritis caused by chlamydiae and gonococci is not included in the definition because of their unique characters and strict localization to the urethra and genital system (Pokharel, 2004).

UTI is also defined as bacteriuria that is the multiplication of the organisms in urinary tract and the presence of more than a hundred thousand organisms per ml in the mid stream urine(Chakraborty,2001) The presence of bacteria in urine is called bacteriuria (Cheesbrough, 2000).

According to Griebling, (2009) Urinary tract infection is common condition in both male and female of all ages. The prevalence and incidence of UTI is found higher in females than in male which is likely due to several factors such as anatomic differences, hormonal effects and behavior patterns.The clinical manifestation of UTI depends on various factors such as portion of the urinary tract involved, the etiologic organisms, the severity of infection, and the patient's ability to mount an immune response to it.

Bacteria which may lead to the infection of prostate, epididymis or the testes are also included the definition of UTI (Fowler and Mariano, 1990).

Low count significant bacteria is may be due to collection of urine before the organisms reached the phase of growth after entry into urinary tract, patients under treatment, in younger females count is low also called honey moon cystitis, endocrine disorders like diabetes, chronic infection of kidney, obstruction of ureter, infection by relatively slow growing organisms like Streptococci, other than Enterococci, *Staphylococcus saprophyticus, Haemophilus influenza* (Pokherel, 2004).

Urinary Tract Infections are the most common bacterial infections in women and results in a significant morbidity and health care costs (Harding and Ronald, 1994). *Escherichia coli* is the primary urinary tract infection pathogens isolated from outpatients (Nicolle, 2001) while *Klebsiella spp* and *Staphylococcus spp* are less common pathogen (Khadri *et al*, 2009). It is estimated that 150 million urinary tract infections occur annually worldwide (Stamm and Norrby, 2001).

3.2URINARY SYSTEM

3.2.1 ANATOMY OF URINARY TRACT

The urinary tract consists of the kidneys, ureters, bladder and urethra.

Based on anatomic location urinary tract is divided into

a) Lower urinary tract

b) Upper urinary tract

The lower urinary tract encompasses the bladder and urethra and the upper urinary tract encompasses the ureters and kidneys.

The anatomy of the female urethra is of particular importance to the pathogenesis of UTIs. The female urethra is relatively short compared with the male urethra and also lies in close

proximity to the warm, moist, perirectal region, which is teeming with Q microorganisms. Because of the shorter urethra, bacteria can reach the bladder more easily in the female hosts (Forbes *et al,* 2002).

3.2.2 RESIDENT MICROORGANISMS OF THE URINARY TRACT

The urethra has resident micro flora that colonize its epithelium in the distal portion. Some of these organisms are:

Coagulase negative Staphylococci Excluding *Staphylococcus saprophyticus,* Viridians and Non-Hemolytic Streptococci, Lactobacilli, Diptheroides (*Corynebacterium* species) Non-pathogenic (Saprobic) *Neisseria* species, Anaerobic cocci, *Propinibacterium* species, Anaerobic gram negative bacilli, Commensal *Mycobacterium* species, *Mycoplasma* spp. Potential pathogens, including grarn negative aerobacilli (primarily enterobacteriaceae) and occasional yeast are also present as transient colonizer. All areas of urinary tract above the urethra in healthy human are sterile. Urine is typically sterile, but non-invasive method for collecting urine must rely on a specimen that has passed through a contaminated environment. Therefore, quantitative cultures for diagnosis of UTIs have been used to discriminate between contamination, colonization and infection (Forbes *et al*, 2002).

3.3 PATHOGENESIS RELATED TO UTI

The urinary tract system is the single anatomic structures that help to maintain proper water and salt balances throughout the body and excrete the liquid wastes from the body. It is made up of kidneys, ureters, bladder and urine (Simon and Shortlife, 2003; Griebling, 2009). Bacteria can cause infection of urinary tract by two routes. In the ascending route the bacteria migrates from the anus towards the urethra moving up to the kidneys causing UTIs which is the most common cause of bacterial infection. In descending route the infection of kidney occurs by the hematogenous spread of bacteria then descending downward toward the urethra (Simon and Shortlife, 2003; Honton, 2000).

3.4 RENAL TRANSPLANTATION

Chronic kidney disease is a pathophysiologic process with multiple etiologies. Later in its course, if uncontrolled by dietary and medical management, it progresses to end stage renal disease (ESRD) necessitating lifetime dialysis treatment or kidney transplantation. Transplantation of the kidney is frequently the most effective treatment of chronic renal failure. Virtually all end-stage renal disease patients who receive a transplant have a higher life expectancy than risk matched patients who remain on dialysis. Diabetics and older candidates, however, have a higher mortality rate than other transplant recipients. Their survival rates, however, are increased by transplantation than chronic dialysis. Graft rejection has been the most important area of concern in solid organ transplantation (Bomasang *et al,* 2006)

Urinary Tract Infection and Renal Transplantation:

Urinary tract infection (UTI) is the most common infections following renal transplantation. Some reports suggest that UTI are mostly benign, while other suggests that they induce graft loss. In one study, 80% of patients with cellular rejection had a UTI, suggesting that UTI might trigger a graft rejection (Takai *et al*, 1998). Urinary tract infection is an important cause of morbidity in renal transplant recipients. Around 50% of patients suffer from at least one episode of the infection during the first 6 months post transplant (Part *et al*, 1985).

Infections typically occur in the first few months post-transplantation following a time scheme. The most frequent infections seen are: urinary tract infection (61%), respiratory tract infections (8%), intra-abdominal infections (7%), and cytomegalovirus infections (8%) (Bomasang *et al,* 2006).

Lyerova *et al*, 2001, found that patients with urologic complications after renal transplantation had a worse renal function compared with patients without this complication. An earlier study done by Griffn *et al*, (1979) found that half of infections occured in the first six months and half occurred later. Graft pyelonephritis, however, was found in 13% of recipients over a 13-year period in a study done by Giral *et al* (2002) and infections occurring in the first three months to be associated with reduced graft survival. Avoidance of surgical complications and use of antimicrobial prophylaxis are the most important means of decreasing frequency of urinary infections. (Bomasang *et al*, 2006).

Transplantation and Infections complications

Infection is leading cause of morbidity and mortality in transplant recipients. Infection aureusand rejection is intimately linked through the immunosuppressive therapy. For example to combat a rejection episode, increased doses of immunosuppressive agents are needed, which in turn increases the recipient's risk of infection. On the other hand, if the immune-suppression is decreased to help combat on infection, the patient at a higher risk for rejection (Braunwald *et al*, 2001). The most common infections cause of death was pneumonia, sepsis, peritonitis and meningitis due to common gram negative organisms. *Staphylococcus aureus*, *Enterococcus spp*, *Candida spp*, *Aspergillus fumigatus*, *Pneumocystic carinii* and Mycobacterial infections is influenced by other factors: indwelling catheters, malnutrition, uremia, hyperglycemia and infection with immune suppressive viruses (Braunwald *et al*, 2001).

3.5 PREDISPOSING FACTORS TO UTI

a) **Gender and Sexual activity:** The female urethra appears to be particularly prone to colonization with colonic Gram negative bacilli because of its proximity to the anus, its short length (about 4 cm) and its termination beneath the labia. Sexual intercourse causes the introduction of bacteria into the bladder and is temporarily associated with the onset of cystitis; it thus appears to be important in the pathogenesis of UTIs in younger women. An important factor predisposing to bacteriuria in men is urethral obstruction due to prostatic hypertrophy (Stamm, 2003).

b) **Pregnancy:** This predisposition to upper tract infection during pregnancy results from decreased ureteral tone decreased ureteral peristalsis and temporary incompetence of the vesicoureteral valves (Stamm, 2003). UTIs during pregnancy pose particular risks for both mother and child. It increases the risk for premature birth, infant mortality and later chronic kidney disease. UTIs occurring in the first and third trimester of pregnancy increase the risk for mental retardation and developmental delay in the infant from 1.2% to 2.0%. Infants of women who harbor *Ureaplasma urealyticum* also have increased risk for respiratory infections (Todar, 2002). About 2.0-11.0% of pregnant women have asymptomatic bacteriuria in early pregnancy. The higher prevalence occurs in women of lower socioeconomic status and those with a past history of UTI. From 13.0% to 27.0% of women with asymptomatic bacteriuria in early pregnancy will experience acute pyelonephritis later in pregnancy (Nicolle, 1994).

c) **Bacterial Virulence Factors:** Not all strains of *E. coli* are equally capable of infecting the intact urinary tract. Bacterial virulence factors markedly influence the likelihood that a given strain once introduced into the bladder, will cause UTI. Most *E. coli* strains that cause symptomatic UTIs in non catheterized patients belong to a small number of specific O, K, and H serogroups (Stamm, 2003).

Numerous investigations suggest that the strains of *E. coli* that cause UTIs possess certain virulence factors that enhance their ability to colonize and invade the urinary tract. Some of these virulence factors include increased adherence to vaginal and uroepithelial cells by bacterial surface structures (adhesions, in particular, pili), a-hemolysin production and resistance to serum-killing activity (Forbes *et al*, 2002).

Uropathogenic *E. coli* (UPEC) causes 90.0% of UTI in anatomically-normal, unobstructed urinary tracts. The adhesion that has been most closely associated with uropathogenic *E. coli* is the P fimbriae. UPEC usually produce siderophores that probably play an essential role in iron acquisition for the bacteria during or after colonization. They also produce hemolysins which are cytotoxic due to formation of transmembranous pores in host cells.

Another factor thought to be involved in the pathogenicity of the uropathogenic strains of *E. coli* is their resistance to the complement-dependent bactericidal effect of serum (Todar, 2002).

The adherence property has also been demonstrated with other species of bacteria. *Proteus* strains are able to facilitate their adherence to the mucosa of the kidneys. Also, *Proteus* spp. are able to hydrolyze urea via urease production, which results in an increase in urine pH that is directly toxic to kidney cells and also stimulates the formation of kidney stones. Similar findings have been made with *Klebsiella* spp. and *S. saprophyticus*. Motility may be important for organisms to ascend to the upper urinary tract against the flow of urine and cause pyelonephritis (Forbes *et al*, 2002).

d) **Genetic factors:** Increasing evidence suggests that host genetic factors also influence susceptibility to UTI. The number and type of receptors on uroepithelial cells to which bacteria may attach are at least in part genetically determined. Many of these structures are components of blood group antigens and are present on both erythrocytes and uroepithelial cells. For example, P fimbriae mediate attachment of *E. coli* to p-positive erythrocytes and are found on nearly all strains causing acute uncomplicated pyelonephritis (Stamm, 2003).

e) **Catheters and Hospitalizations:** Over 20.0% of hospital-acquired infections are of urinary tract and about 75.0% of these follow the use of catheters in the urinary tract. Catheterized patients who develop diarrhea are nine times more likely to develop UTIs than are patients without diarrhea (Leigh, 1996). Bacteriuria develops in at least 10.0 to 15.0% of hospitalized patients with indwelling urethral catheters. The risk of infection is about 3.0 to 5.0% per day of catheterization (Stamm, 2003).

f) **Kidney Stones**: Kidney stones, in some cases, can cause obstruction followed by infection, particularly pyelonephritis. Symptoms of severe UTI in people with a history of kidney stones may indicate obstruction of the urinary tract, which is a serious condition. Formation of infectious urinary calculi is the most common complication accompanying

UTI by members of the genus *Proteus* supported by other studies (Li *et al*, 2002; Torzewska *et al*, 2003). Recent studies have shown that men have higher risk of forming renal stone than women (Curhan *et al*, 1998; Yagisawa *et al*, 1999).

In a study on bacteriology of urinary calculi in relation to UTI, out of 52 patients, 37.0% patients had calculi associated UTI with *E. coli* and *P. mirabilis* being the most common causative microorganisms (Nass *et al*, 2001). Kumar (2003) found that the prevalence of Renal Stone (RS) was higher without UTI (44.4%) than those with UTI (27.8%) in males. In case of females, the result showed 17.6% and 5.1% in cases with and without UTI.

g) **Diabetes:** UTI is an important clinical problem for people with diabetes. UTI is 2-3 times more common in adult diabetic patients than in non-diabetics (Lehn *et al*, 1996). There is an increased prevalence of asymptomatic bacteriuria in diabetic women, but not in diabetic men (Zhanel *et al*, 1991). On a population basis, diabetic women, depending on age, are 6-24 times more likely than non-diabetic women to be admitted for acute pyelonephritis; and diabetic men are 3.4-17 times more likely than their non-diabetic counterparts to be admitted for the same condition (Nicolle *et al*, 1996).

The risk for symptomatic complicated UTIs may also be higher in people with diabetes. In fact, certain UTI-related abscesses are reported only in patients with diabetes. These patients are also at higher risk for fungal-related UTIs. The suggested mechanisms of an increased susceptibility to UTI are decreased antibacterial activity due to 'sweet urine', defects in neutrophil function and increased adherence to uroepithelial cells (Todar, 2002).

h) **Renal transplantation**: UTIs are the most common infections following renal transplantation. Some reports suggest that UTIs are mostly benign, while other suggests that they may induce graft loss. About 80.0% of patients with cellular rejection had a UTI, suggesting that UTI might trigger a graft rejection (Takai *et al*, 1998). UTI is an important

cause of morbidity in renal transplant recipients. Around 50.0% of patients suffer from at least one episode of the infection during the first 6 months post transplant (Part *et al*, 1985). About 20.0% of UTIs occurs during the first year of transplantation. Female recipients have significantly more UTI than males (Russel *et al*, 2000).

i) **Other factors:** These include obstruction to free flow of urine due to tumor, stricture, stone or prostatic hypertrophy, neurogenic bladder dysfunction, vesicoureteral reflux (VUR) etc. (Stamm, 2003).

3.6 ETIOLOGICAL AGENTS OF UTI

Bacteria of only a limited number of species are able to initiate infection in the normal urinary tract, but members of many other species cause infection in patients with an abnormality of the urinary tract, in catheterized patients and in those receiving antimicrobial treatment (Forbes *et al*, 2002).

Gram negative: *Escherichia coli, Klebsiella spp, Proteus mirabilis, Proteus vulgaris, Enterobacter spp, Citrobacter spp, Serratia spp, Morganella morganii, Pseudomonas aeruginosa.*
Gram positive: *Staphylococcus aureus, Staphylococcus saprophyticus, Enterococcus faecalis,* Group B streptococci.
Other pathogens: *Chlamydia trachomatis, Mycoplasma* (*Ureaplasma urealyticum), Candida spp, Mycobacterium tuberculosis.*
(Source: Cheesbrough, 2000; Fowler and Mariano, 1990)

Other less frequently isolated agents are other Gram negative bacilli, such as *Acinetobacter* and *Alcaligenes* spp., other *Pseudomonas* spp., *Citrobacter* spp. and beta-hemolytic streptococci. Gram positive pathogens such as *E. faecalis*, *S. saprophyticus* and group B streptococci can also infect the urinary tract. UTIs due to *E. faecalis* are usually associated with the use of instruments or catheterization. Novobiocin resistant *S. saprophyticus* is a true primary pathogen of the urinary tract, which is responsible for 20%

of urethritis and cystitis in sexually active but otherwise healthy young women (Forbes *et al*, 2002, Leigh, 1990).

Salmonella Typhi and *Salmonella* Paratyphi can be found in the urine of about 25% of patients with enteric fever from the third week of infection (Cheesbrough, 2000). *Candida* infection may occur in diabetic and immunocompromised patients. Rarer infecting organisms include *Streptococcus agalactiae*, *Streptococcus milleri*, other streptococci and *Gardnerella vaginalis* (Collins *et al*, 1986).

3.7 CATEGORIZATION OF UTI

UTI encompasses a broad range of clinical entities that differ in terms of clinical presentation, degree of tissue invasion, epidemiologic settings and requirements for antibiotic therapy (Forbes *et al*, 2002)

3.7.1 On the basis of symptoms, severity and inflammatory changes

a) **Uncomplicated UTI:**

Uncomplicated UTI occur in patients with urinary tracts that are normal from both a structural and functional perspective. In uncomplicated UTI microbial invasion of the urinary tract occurs but no any inflammatory changes are seen i.e, shows no structural and neurological lesions in the tract. The first episode in this type of UTI shows *E.coli* and has been found particular in female. Most common organisms encountered in uncomplicated UTI are *E.coli, Klebsiella spp, Proteus spp*, *Citrobacter spp, Enterobacter spp etc*.

b) **Complicated UTI :**

Microbial invasion of the urinary tract occurs along with structural and neurological lesions.the main surgical problem occurring is due to obstruction in the ureter by stone formation in the kidney and passage of it in ureter and tumor formation in the tract due to which patient cannot pass urine as normally. Complicated urinary tract infection

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3.7.2 Classification Based on Source of infection: On the basis of source of infection, UTI can be classified as community acquired UTI (non-catheter associated UTI) and hospital acquired UTI (catheter associated or nosocomial UTI).

a) **Community acquired UTI (non-catheter associated UTI):** This occurs in patients who are not admitted to the hospital at the time they become infected. *E. coli* is by far the most frequent cause of uncomplicated community acquired UTIs. Other bacteria frequently isolated from patients with UTIs include *Klebsiella* spp, other Enterobacteriaceae and *Staphylococcus saprophyticus* (Forbes *et al*, 2002).

b) **Hospital acquired UTI (catheter associated or nosocomial UTI):** Hospital acquired UTIs are those developing in patients after admission to the hospital, which were neither present nor in incubation at the time of hospitalization.As many as 20.0% of all hospitalized patients who receive short-term catheterization develop a UTI. Hospitalized patients are most likely to be infected by *E. coli, Klebsiella* spp, *Proteus mirabilis,* staphylococci, other Enterobacteriaceace, *Pseudomonas aeruginosa* and enterococci (Forbes *et al*, 2002). Studies have demonstrated the importance of the attachment and growth of bacteria on the surfaces of the catheter in the pathogenesis of catheter-associated UTI. The encrustations formed on the catheter surface provide a refuge for bacteria and may protect them from antimicrobial agents and phagocytes (Stamm, 2003).

3.7.3 Classification Based on Symptoms and Levels of Infection: UTIs can also occur without symptoms and with symptoms but very low bacterial levels.

a) **Asymptomatic Urinary Tract Infection (Bacteriuria):** When a person has no symptoms of infection but significant numbers of bacteria have colonized the urinary tract, the condition is called asymptomatic UTI (also called bacteriuria). The condition is harmless in most people and rarely persists, although it does increase the risk of developing symptomatic UTIs. Screening for asymptomatic bacteriuria is not necessary during most routine medical examinations except in pregnant women, immuno

compromised patients and people undergoing urologic surgery, in whom the condition can lead to serious infection.

b) **Acute Urethral Syndrome:** Patients with this syndrome are primarily young, sexually active women, who experience dysuria, frequency, and urgency but yields fewer organisms than 10^5 CFU/ml urine on culture. This condition is usually caused by *E. coli* or other bacteria that cause cystitis, but in lower numbers, or by a sexually transmitted disease such as Chlamydia or gonorrhea (Forbes *et al*, 2002).

3.7.4 On the basis of anatomic sites:

On the basis of anatomic structures UTI is subdivided in two general categories (Chakraborty, 2001).

1. Lower tract infection: Two type a.Urithritis b.Cystitis
2. Upper tract infection: Two type a. Acute pyelitis that is infection of pelvis and kidney b. Acute pyelonephritis that is infection of parenchyma of kidney

3.8 URINARY TRACT INFECTION IN RENAL TRANSPLANT RECEPIENT

Although significant advances have been made in the surgical technique, the immunologic aspects and the immunosuppressive protocols of renal transplantation infection continues to be a major problem. Nearly 80% of renal transplant recipients suffer at least one episode of infection during the first year after transplantation and infection remains the leading cause of morbidity and mortality throughout the post-transplant course (Sqalli *et al* 2008).

Prevention and treatment of infection in the transplant recipient is of major importance and the required immunosuppressive therapy must be judiciously administrated (Sqalli *et al* 2008).

Previous studies have shown that there is a typical timetable that characterizes the post-transplantation infection pattern; particular types of infection being more likely to occur at different time periods.Accordingly, in the first month of post- transplantation, opportunistic (fungal,nocardial and protozoal) infections are almost non-existent. Indeed, the major presentations of infection are the bacterial wound, pulmonary and urinary tract infection. The greatest risk of life threatening infection is in the time period one to six months post-transplantation, when the immunosuppressive effects of anti rejection therapy are at their peak. Cytomegalovirus is the most common opportunistic organisms encountered at this time interval. After six months of transplantation, the time when maintence immunosuppressive therapy is at its lowest level, three types of infections are commonly observed. These include: chronic, particularly viral, infections an occasional opportunistic infection and the usual infections prevailing in the general community such as influenza, pneumococcal pneumonia and urinary tract infection (Charfeddine et *al*, 2002).

3.9 EPIDEMIOLOGY OF UTI IN KIDNEY TRANSPLANT RECEPIENT

Although *Escherichia coli* remained the most frequently isolated microorganisms it is found up to 80% of UTIs, this may be due to underlying immunosupression and colonization. Also some patients receive antibiotic prophylaxis or treatment, which may have an impact on colonization. Surveillance urinary catheters may help, especially for patients with recurrent UTI and those with abnormal lower urinary tract and neurogenic bladder; not infrequently, bacteria with multiple antibiotic resistances, such as *Pseudomonas spp,* can be detected

Treatment of UTI after renal transplantation

Aggressive and specific treatment of fUTI is mandatory. In our opinion, in fUTI, parenteral antibiotics should be preferred, at least initially, to achieve fast tissue saturation.The optimal treatment duration has not been studied, but we favor 14 days in transplant pyelonephritis. In accordance with Benador et al., we sometimes continue with oral treatment when the clinical situation has improved. As *Enterococcus* and *Pseudomonas* spp. are more frequent, we currently use a combination of ceftazidime and ampicillin to cover for *Escherichia coli*, *Pseudomonas*, and *Enterococcus.* Others have recommended ampicillin and gentamycin for the same reason (Haller *et al*, 2004) Antibiotic resistance of urinary tract pathogens and rationale for empirical intravenous therapy. However, nephrotoxicity of the latter is a concern. Oral fluroquinolone medications such as ciprofloxacin are another alternative in this situation. Fungal UTIs may occur and require specific treatment; requires antifungal prophylaxis is given during high dose antibiotic treatment. Monitoring of blood levels is important during acute UTI, as antibiotic treatment may interfere with resorption. Steroid dose must be increased sometimes during fUTI, especially early after renal transplantation, to avoid symptoms of adrenal insufficiency (Urlike *et al*, 2009).

Symptomatic afebrile UTI may be treated with oral antibiotics unless specific risk factors are present (renal dysfunction etc) (Hansson *et al*, (1999). Urinary tract infection, in: Barret TM, Avner E, Harmon WE (eds). Again treatment should be specific and oral cephalosporin may be the first choice, especially if the patient was on antibiotic prophylaxis. Whether asymptomatic UTIs have to be treated remains controversial and are often an individual decision. In patients with abnormal bladder anatomy and catheterization such as in spina bifida, colonization is frequent and symptoms such as dysuria may be absent. There is no evidence and no consensus as to whether in these patients bacterial colonization needs treatment, including bladder washing with antibiotics (Urlike *et al*, 2009).

3.10 LABORATORY DIAGNOSIS OF UTI

A urine sample from patient with a suspected UTI is the most common type of specimen received by most clinical microbiological laboratories. The schedule for routine examination should therefore be carefully determined with a view to obtaining the necessary diagnostic information with the greatest possible economy of labour and resources (Collee *et al*, 2001).

3.10.1 Methods of Specimen Collection and Transport

Prevention of contamination by normal vaginal, perineal and anterior urethral flora is the most important consideration for collection of a clinically relevant urine specimen. (Cheesbrough, 2000), suggests that whenever possible, the first urine passed by the patient at the beginning of the day should be sent for examination. This specimen is the most concentrated and therefore the most suitable for culture, microscopy and biochemical analysis. For the isolation and identification of bacteria in urine sample, the sample collection is very important. Generally, four types of urine samples are made available for laboratory investigation depending upon the situation/condition of the patient. They are mid-stream urine (MSU), straight catheterized urine, suprapubic bladder aspirated urine and urine from indwelling catheter.

Most urine samples submitted for microbiological examination are in the form of MSU where it is hoped that the flushing action of urine will cleanse the urethra and limit contamination by urethral commensals (Gillespie, 1994). Although slightly more invasive, straight catheterization may allow collection of bladder urine with less urethral contamination but need a physician or other trained health professionals. If good aseptic techniques are used, suprapubic bladder aspiration can be performed with little risk to get contamination-free urine in premature infants, infants, small children, and pregnant women and other adults with full bladders. In catheterized patients, urine should be collected directly from the catheter and not from the collection bag because organisms can multiply there, obscuring the true relative numbers (Forbes *et al*, 2002).

Since urine itself is a good culture medium, all specimens should be processed by the laboratory within 2 hours of collection, or be kept refrigerated at 4^0C until delivery to the

laboratory and processed no longer than 18 hours after collection (Vandepitte *et al*, 2003). Transport medium that can be used for urine specimens are 1.8% boric acid, sodium chloride or polyvinylpyrolidine (Pokharel, 2004).

3.10.2 Screening Procedures

As many as 60.0-80.0% of all urine specimens received for culture may contain no etiologic agents of infection or contain only contaminants. A wide range of screening techniques has been developed for detection of urinary tract infection so that time, reagents and money of the laboratory is saved. Of these, the Gram stain is the easiest, least expensive, and probably the most sensitive and reliable screening method (Forbes *et al*, 2002).

3.10.3 Urinalysis

A urinalysis involves a physical and chemical examination of urine. In addition, the urine is centrifuged to allow sediments containing blood cells, bacteria, and other particles to collect. This sediment is then examined under a microscope. A urinalysis includes the observation of the urine for color, cloudiness, acidity and white blood cells (WBC) counts. A high WBC count in the urine is referred to as *pyuria*. Pyuria is usually sufficient for a diagnosis of UTI in nonhospitalized patients if other standard symptoms (or fever in small children) are also present(Vandepitte,2003).treatment can be started without the need of for further tests if the cell count is high with cloudy urine in patients with symptoms and sings of UTIs

Pus cells: These are round 10-15 µm in diameter cells that contains granules. In urinary infections they are often found in clumps. Normal urine may contain a few white cells (<5/HPF) (Cheesbrough, 1984). The visualization of leucocytes, principally neutrophils, is indicative of bacteriuria but may result from any inflammatory disorder of the urinary tract such as acute glomerulonephritis, renal tubular acidosis, and non-infectious irritation

to ureter, bladder or urethra or may be due to dehydration, stress and fever (Godkar, 2001). Pyuria is considered significant if ≥5 pus cells are seen per HPF in urine sediments.

Red blood cells (RBCs): Red blood cells are found in small numbers in normal urine. In normal male and female, occasional red cells (0-2/HPF) may be seen on microscopic examination of the sediment. The finding of RBC counts ≥3/HPF is considered as abnormal (Froom *et al*, 1986; Steward *et al*, 1985; Wargotz *et al*, 1987). Haematuria may be found in urinary schistosomiasis, bacterial infections, acute glomerulonephritis, sickle cell disease, leptospirosis, infective endocarditis, calculi (stones) in the urinary tract, malignancy of the urinary tract and hemorrhagic conditions. The number may exceed during renal disease, post streptococcal glomerulonephritis, lower urinary tract disease, other disease including appendicitis, salphingitis, malaria, sub-acute bacterial endocarditis etc (Cheesbrough, 1984).

Epithelial cells: It is normal to find a few epithelial cells in urine. These cells are nucleated and vary in size and shape. When seen in large number, however, they usually indicate inflammation of the urinary tract or vaginal contamination of the specimen (Cheesbrough, 1984). Wargotz *et al* (1987) reported that greater than or equal to five squamous epithelial cells per high power field is considered as abnormal. Normally few cells (3-5/HPF) from genitourinary tract can be found in urine due to sloughing off of old cells. Increased number of tubular epithelial cells suggests tubular damage. It can occur in pyelonephritis, acute tubular necrosis, salicylate intoxication and kidney transplants rejection (Godkar, 2001).

3.10.4 Chemical Examination of Urine

Chemical tests for bacteriuria are of value in large population-screening programmes. These include detection of protein and glucose in the urine, nitrate reductase (Greiss) test, leukocyte esterase test and triphenyltetrazolium-chloride reduction test (Leigh, 1990).

3.10.5 Bacteriological Examination of Urine

Bacteriological culture of the urine is the only accurate way of diagnosing bacteriuria. Quantitative or semi-quantitative techniques depending upon the resources of the laboratory are to be preferred. The accurate methods of counting bacteria e.g. the pour-plate technique or the surface-viable count are time-consuming and expensive in use of materials. Most of the laboratories use a semi-quantitative technique. The standard loop, filter-paper strip, dip-spoon and dip-slide are all useful means of examining large numbers of urine specimens, but they differ considerably in the amount of medium used and in performance time (Leigh, 1990).

Standard Loop Method: An inoculating loop of standard dimensions is used to take up a small, approximately fixed and known volume of mixed uncentrifuged urine and inoculate it over a plate of agar culture medium. The plate is incubated, the number of colonies is counted and this number is used to calculate the number of viable bacteria per ml of urine. Thus, if a 0.004 ml loopful of urine yields 400 colonies, the count per ml will be 10^5, or just indicative of significant bacteriuria (Collee *et al*, 1996).

3.10.6 Antibiotic Susceptibility Testing

Antibiogram is performed in vitro to assess the antimicrobial susceptibility of the isolated pathogens which also guide the clinician in selecting an effective antimicrobial against the infection. The primary goal of antibiotic susceptibility test is to determine whether the bacterial pathogens of concern have develop any resistance to the antimicrobial agents which is the potential choice of drugs in the management of disease (Greenwood *et al*, 2001)

World Health Organization (WHO) recommended modified Kirby- Bauer disk diffusion technique is used by most laboratories to test routinely for antimicrobial susceptibility. Using this test, antimicrobial resistance is detected by allowing the antibiotics to diffuse from a point source, commonly in the form of an impregnated filter paper disc, into an

agar medium that has been seeded with the test organism. Visible growth of bacteria occurs on the surface of the agar where the concentration of antibiotic has fallen below its inhibitory level for the test strain. Following incubation, the diameter of the zone of inhibition around each disc is measured in millimeters (Collee *et al*, 1996).

3.11 BACTERIAL RESISTANCE TO ANTIBIOTICS

Antibiotic resistance is an emerging problem worldwide. It is true to say that early treatment failures with antibiotics did not represent a significant clinical problem because other classes of agents, with different cellular targets, were available. It is the emergence of multiple resistances, i.e. resistance to several types of antibiotic agent that is causing major problems in the clinical practice today. Several factors drove this situation in the 1970s and 1980s, including the introduction of extended-spectrum agents and advances in medical techniques, for example, in organ transplantation and cancer chemotherapy. The net result has been a huge selective pressure in favor of multiple resistant species. Notable Gram positive organisms include methicillin-resistant *Staphylococccus aureus* (MRSA) and coagulase-negative staphylococci (CoNS), glycopeptide-moderate sensitive *S. aureus* (GISA), vancomycin-resistant *Enterococcus* (VRE) species and penicillin non-susceptible *Streptococcus pneumoniae* (PNSSP). Concerns among the Gram negative organisms include multidrug-resistant *Pseudomonas aeruginosa*, *Stenotrophomonas maltophila* and *Acinetobacter baumannii* and members of the Enterobacteriaceae with extended-spectrum beta-lactamases (ESBLs) (Smith, 2004).

The primary concerns for resistance among the enteric Gram negative bacilli have been the declines in susceptibility for the fluoroquinolones and the third-generation cephalosporins. Resistance mechanisms compromising the fluoroquinolones are the mutations in the topoisomerase II and IV targets. The ESBLs are generally encoded by mobile genes that can be highly prevalent among some Enterobacteriaceae such as *E. coli* and *K. pneumoniae*. First detected in the early 1980s, ESBLs have diverse geographic distributions and remarkably variable substrate affinities that can produce confusing susceptibility testing results (Smith, 2004).

Inherent (Natural) Resistance: Bacteria may be inherently resistant to an antibiotic. For example, a streptomycete has some gene that is responsible for resistance to its own antibiotic; or a Gram negative bacterium has an outer membrane that establishes a permeability barrier against the antibiotic; or an organism lacks a transport system for the antibiotic; or it lacks the target or reaction that is hit by the antibiotic (Todar, 2002).

Acquired resistance: Acquired drug resistance may result from mutation, adaptation or gene transfer. Spontaneous mutations occur at low frequency. Rapid mutation can occur and there is clearly a heavy selective pressure resulting from the overuse of antibiotics in medical practice. The probability that a mutation arises will be proportional to the number of target sites within the gene. In *E. coli*, mutations in the *gyr*A gene, encoding the Gyr A subunit of topoisomerase II and leading to fluoroquinolone resistance have been identified in at least seven locations in the *par*C gene, encoding a subunit of topoisomerase IV, have been observed. As a consequence, the prediction that the mutation rate would be higher in *gyr*A than *par*C is correct. Indeed, the opposite is true for fluoroquinolone resistance in *S. pneumoniae* (Smith, 2004; Todar, 2002).

Genetic resistance may be chromosomal or transferable on plasmids or transposons. Chromosomal Resistance develops as a result of spontaneous mutation in a locus that controls susceptibility to a given antimicrobial drug serves as a selecting mechanism to suppress susceptible organisms favor the growth of drug-resistant mutants. Spontaneous mutation occurs with a frequency of 10^{-12} to 10^{-7} and thus is an infrequent cause of the emergence of drug resistance in the clinical practice (Smith, 2004)

Bacteria often contain extra chromosomal genetic elements called plasmids. Genetic material and plasmids can be transferred by transduction, transformation and conjugation. By the process of conjugation, resistance plasmids may be transferred between and within different species and genera; and can code for multiple antibiotic resistance. Plasmid-

mediated resistance has been increasingly recognized among Gram negative enteric pathogens. Some plasmids carry genes for resistance to one and often several antimicrobial drugs. Plasmid genes for antimicrobial resistance often control the formation of enzymes capable of destroying the antimicrobial drugs. Thus, plasmids determine resistance to penicillins and cephalosporins by carrying genes for the formation of beta-lactamases. Plasmids code for enzymes that acetylate, adenylate, or phosphorylate various aminoglycosides; for enzymes that determine the active transport of tetracyclines across the cell membrane and for others (Smith, 2004).

Transposons are small pieces of DNA, which, unlike plasmids, cannot replicate themselves, but can 'jump' between different plasmids, and between plasmids and chromosomes. An example of an important gene carried by antibiotic resistance transposon is known as TEM-1. It controls the production of beta-lactamase and is incorporated into plasmids which then mediate resistance to beta-lactam antibiotics in some strains of *E. coli, Klebsiella* spp., *H. influenzae* and *N. gonorrhoeae*. The resistance transposon can be transferred from one strain to another. (Smith, 2004)

Mechanism of Antimicrobial Resistance

There are many different mechanisms by which microorganisms might exhibit resistance to drugs.

1. Microorganisms produce enzymes that destroy the active drug. Examples: Staphylococci resistant to penicillin G produce a beta-lactamase that destroys the drug. Other beta-lactamases are produced by Gram negative rods.
2. Microorganisms change their permeability to the drug. Examples: Tetracyclines accumulate in susceptible bacteria but not in resistant bacteria. Streptococci have a natural permeability barrier to aminoglycosides.
3. Microorganisms develop an altered structural target for the drug. Examples: Erythromycin-resistant organisms have an altered receptor on the 50S subunit of the ribosome, resulting from methylation of a 23S ribosomal RNA. Resistance to some penicillins and cephalosporins may be a function of the loss or alteration of Penicillin binding proteins (PBPs).

4. Microorganisms develop an altered metabolic pathway that bypasses the reaction inhibited by the drug. Example: Some sulphonamide-resistant bacteria do not require extracellular para-amino benzoic acid (PABA) but, like mammalian cells, can utilize preformed folic acid.
5. Microorganisms develop an altered enzyme that can still perform its metabolic function but is much less affected by the drug. Example: In trimethoprim-resistant bacteria, the dihydrofolic acid reductase is inhibited far less efficiently than in trimethoprim-susceptible bacteria (Brooks *et al*, 2004).

Multiple drug resistance (MDR)

In recent years, multidrug resistance (MDR) has increased among certain pathogens. These include *S. aureus*, enterococci and *M. tuberculosis*. These strains are resistant to many antibiotics and have been responsible for major epidemics worldwide, usually in hospitals where they affect patients in high-dependency units such as intensive care units, burn units and cardiothoracic units (Brooks, 2004; Smith, 2004)

R-factors: One of the earliest examples was in Japan in 1959. Previously sensitive *E. coli* became resistant to multiple antibiotics through acquisition of a conjugative plasmid (R-factor) from resistant *Salmonella* and *Shigella* isolates. A number of R-factors have now been characterized including RP4, encoding resistance to ampicillin, kanamycin, tetracycline and neomycin, found in *P. aeruginosa* and other Gram negative bacteria; R1, encoding resistance to ampicillin, kanamycin, sulphonamides, chloramphenicol and streptomycin, found in Gram negative bacteria and pSH6, encoding resistance to gentamicin, trimethoprim and kanamycin, found in *S. aureus.*(Brooks, 2004; Smith,2004)

Mobile gene cassettes and integrons: Many Gram negative resistance genes are located in gene cassettes. One or more of these cassettes can be integrated into a specific position on the chromosome termed as integron. Thus, integrons are genetic elements that

recognize and capture multiple mobile gene cassettes (Smith, 2004). Although integrons by themselves are not mobile, due to their presence in plasmids and transposons, they can be transferred horizontally. Integrons for these reasons a major mechanism for the spread and maintenance of MDR (Brooks, 2004; Smith, 2004).

Chromosomal multiple-antibiotic resistance (Mar) locus: The multiple-antibiotic resistance (*mar*) locus was first described in *Escherichia coli* by Stuart Levy and colleagues at Tufts University and has since been recognized in other enteric bacteria. The locus consists of two divergently transcribed units, *mar*C and *mar*RAB (Smith, 2004).

Chromosomal multiple-antibiotic resistance (Mar) locus: The multiple-antibiotic resistance (mar) locus was first described in *E.coli* by Stuart Levy and colleagues at Tufts University and has since been recognized in other enteric bacteria. The loss consists of two divergently transcribed units, marc and marRAB (Smith, 2004)

3.12 EPIDEMIOLOGY OF UTI

In world context

An estimated 11.0 % of women in the US report at least one physician-diagnosed urinary tract infection per year, and the lifetime probability that a woman will have a urinary tract infection is 60.0 % (Foxman *et al*, 2000).

Although UTI is not usually thought of as a disease associated with community-wide outbreaks, certain multidrug-resistant, uropathogenic lineages of *E. coli* have exhibited epidemic behavior. *E. coli* O15:K52:H1 caused an outbreak of community-acquired cystitis, pyelonephritis, and septicemia in South London in 1987 and 1988 (Phillips *et al*, 1988) and is an endemic cause of urinary tract infection in Barcelona, Spain (Prats *et al*, 2000).

Urinary tract infection (UTI) is the most common infection diagnosed in outpatients as well as in hospitalized patients. Thus a prospective study was carried out in the Government Medical Colleage and Hospital at Anantapur, India from July to December, 2007 in order to determine the frequency of multidrug resistance (MDR) and Extended spectrum beta lactamase (ESBL) producing uropathogens from community acquired

urinary tract infection (CAUTI) of the 410 samples tested in this study 222 isolates showed significant bacterial growth among which *Escherichia coli* strain was most prevalent (45%), followed by *Klebsiella spp* (18%). Among gram negative bacterial isolates, high prevalence of antibiotic resistance was observed against Ampicillin, Norfloxacin, Cotrimoxazole, 26% of strain included in this study was found to be ESBL producer where MDR is higher compare to non ESBL producer. Moreover Imipenum showed high potency and widest coverage against gram negative isolates (100%). This presented study showed a high frequency of antimicrobial resistance and ESBL production in *Enterobacteriaceae* isolated from CAUTI patients in India (Khadri et *al*, 2009).

A study carried out in Korea, a total 2,312 patients older than 25 years and diagnosed from January 2007 to December 2009 as having urinary tract infection were studied. The prevalence of ESBL producing microorganisms including *E.coli* was examined. Univariate analysis were performed with age, gender inpatient status, previous hospitalization, recent history of urinary catheterization, recent exposure to antibiotics, and past history of urogenital organ operation as risk factor for the emergence of ESBL producing microorganisms. In this study, the antimicrobial susceptibility of *E.coli* to each of the third generation cephalosporin; cefotaxime, ceftazidime, and ceftriaxone was 87.6%, 93.4% and 87.7 respectively, and the prevalence of ESBL producing E. coli was 12.1. In inpatient urinary tract infection, the susceptibility of *E.coli* was 78%, 84.5% and 76.9% respectively. And prevalence was 23.1%. In this study, risk appeared to be increased in case of ESBL producing microorganisms increased in case with a previous hospitalization, a recent history of urinary catheterization, in patient status cefaclor medication, cefminox administration, and female gender (Lee *et al*, 2010).

A Canadian National Surveillance Study showed that ampicillin, cotrimoxazole, mecillinam, nitrofurantoin and ciprofloxacin mean resistance rates for 2,000 urinary tract isolates collected from outpatients across Canada in 1998 were 41.1%, 19.2%, 14.7%,

5.0% and 1.8% respectively. For *E. coli* isolates alone (n=1,681), comparable rates were 41.0%, 18.9%, 7.4%, 0.1% and 1.2 % respectively. The majority of *E. coli* isolates resistant to ampicillin, cotrimoxazole or ciprofloxacin were susceptible (MIC, <16µg/ml) to mecillinam (Zhanel *et al*, 2000).

A study done among uropathogens in 14 medical centres in the Asia-Pacific region between 1998 and 1999 found that over 50.0% of the 958 pathogens were *E. coli* and *Klebsiella* spp. followed by *P. aeruginosa, Enterococcus* spp. and *Enterobacter* spp. Susceptibility for the three enteric bacilli was high for carbapenems (100.0%), 'fourth generation' cephalosporins (cefepime 94.9-98.6%) and amikacin (≥93.0%) (Turnidge *et al*, 2002).

A study conducted in the United Kingdom (UK) during 1999-2000 showed that *E. coli* was the predominant pathogen causing UTI followed by *Enterococcus faecalis, Klebsiella pneumoniae* and *Proteus mirabilis*. Nitrofurantoin was very active against isolates of *E. coli* (96.3% susceptible) and *E. faecalis* but not against *K. pneumoniae, P. mirabilis* or *Pseudomonas aeruginosa*. Overall susceptibility to trimethoprim ranged from 58.1% to 84.5% for the most prevalent pathogens. Ciprofloxacin was highly active against the uropathogenic bacteria examined in this study with susceptibilities of between 88.6% and 97.7% for the most prevalent pathogens (Farrell *et al*, 2003).

A retrospective study on all of the bacterial strains isolated from the urine of outpatients who attended the Pasteur Institute of Bangui with a suspected UTI between January 2000 and April 2002 found that more than 84.0% of isolates were Enterobacteriaceae: *E. coli* (55.6%), *K. pneumoniae* (16.9%), *Citrobacter diversus* (4.2%), *Salmonella* spp. (3.5%) and other Enterobacteriaceae (4.2%). Other Gram negative bacteria (*P. aeruginosa* and *Acinetobacter* spp) accounted for 3.5% of the isolates. Only 10.2% of the isolates were Gram positive: *S. aureus* (4.5%), *Streptococcus agalactiae* (3.8%) and *Enterococcus faecalis* (0.6%). A high percentage of the Enterobacteriaceae were resistant to amoxicillin and cotrimoxazole although most remained susceptible to ciprofloxacin (Hadiza *et al*, 2003).

The study performed with isolates from community-acquired UTIs collected from 15 centers representing six different geographic regions of Turkey showed that *E. coli* was the causative agent in 90.0% of the uncomplicated UTIs and in 78.0% of the complicated UTIs ($p<0.001$). About 17.0% of *E. coli* strains isolated from uncomplicated cases and 38.0% of *E. coli* strains isolated from complicated UTI were found to be resistant to ciprofloxacin (Arslan *et al*, 2005).

A study done in various geographic regions in the US and Canada revealed that the most common organisms were *E. coli* (57.5%), *K. pneumoniae* (12.4%), *Enterococcus* spp. (6.6%), *P. mirabilis* (5.4%), *P. aeruginosa* (2.9%), *Citrobacter* spp. (2.7%), *S. aureus* (2.2%), *Enterobacter cloacae* (1.9%), Coagulase-negative staphylococci (1.3%), *S. saprophyticus* (1.2%), *Klebsiella* spp. (1.2%), *Enterobacter aerogenes* (1.1%) and *Streptococcus agalactiae* (1.0%). Among all 1990 isolates, 45.9% were resistant to amp, 20.4% to cotrimoxazole, 14.3% to nitrofurantoin, 9.7% to ciprofloxacin and 8.1% to levofloxacin. Fluoroquinalone resistnce was highest in patient's ≥65 years of age. For the 1142 *E. coli* isolates, resistance rates were: ampicillin (37.7%), cotrimoxazole (21.3%), ciprofloxacin (5.5%), levofloxacin (5.1%) and nitrofurantoin (1.1%). This study reported higher rates of antibiotic resistance in US versus Canada outpatient urinary isolates (Zhanel *et al*, 2005).

A study done at Mexico, 52 patients with kidney transplantation were evaluated for UTI at 3-145 days (mean 400 days) after surgrry. 42 received a graft from a live donor and 10 from a deceased donor. There were 22 females and 30 male patients, aged 11-47 years.UTI developed in 19/52 (37%) patients at 3-75 (mean 19.5 days) after transplantation. Recurrent infection was observed in 7/52 (13.4%) patients at days 17-65. UTI was more frequent in patients who received deceased grafts compare with live grafts (7/10, 70%vs 12/42, 28%; $p<0.007$). Female patients were more susceptible than male

(11/22, 50%vs 8/22, 30.35%; p<0.042). Five year survival rate was 94.5 %(45/52 patients).Kidney grafts exit update is 47/52(90.2%), and there were no significant differences between graft rejection and UTI (p<0.2518). Isolated bacteria were *Escherichia coli* (31.5%), *Candida albicans* (21%) and *Enterococcus spp* (10.5%), followed by *Pseudomonas aeruginosa, Klebsiella pneumoniae, Morganella morganii*, *Enterobacter cloaceae* and *Micrococcus spp*. Secondary in infection were produced by (7/19.36.8%). *Enterococcus spp* (57%), *E. coli* (28%) and *Micrococcus spp* (14.20%). Antibiotic resistance was 22% for ciprofloxacin and 33% for ampicillin. Therapeutic alternatives were azteonam. Trimethoprim, sulphamethoxazole, netimicin and fosfomycin (Sanchez *et al*, 2010)

This study was done at Morocco to describe the epidemiological pattern and evaluate the favoring factors of UTI in renal allograft recipients. This study evaluated all the UTIs in 47 kidney recipients transplanted from living- related kidney donors in Rabat University Hospital, Morocco, from January 1998 to December 2005.The mean follow up was 28^{+}_{-} 19 months. The mean age of the patients was 32 $-^{+}$ 10 years with a male /female ratio of 1.35/1.20 (42%) suffered at least one episode of UTI. UTIs were asymptomatic in 70% of the patients, while manifested as acute pyelonephritis in 17% and uncomplicated acute bacterial cystitis in 13%.UTI episodes occurred in 68% of the patients during the first 3 months post kidney transplantation with recurrence rate of 55%, and all the patients experienced a favorable course. Gram-negative bacilli were principally isolated agents; *E.coli* was found in 60% the patients and *Klebsiella* in 30%. UTI was more common in females (p<0.04) and cases of post transplantation vesicourethral reflux (p<0.03). The graft survival rate at the end of the study was comparable for both UTI and and non-UTI groups (Tarik *et al*, 2008)

UTI in Nepal

In a study done at Maternity Hospital, Thapathali, it was found that 15.9% of the urine samples showed significant bacteriuria among pregnant women whereas it was only 5.0% among non-pregnant women. The prevalence of *E. coli* was found to be much higher (52.5%), followed by *Klebsiella* spp. (40.7%) and *Proteus* spp. (6.8%). Among the isolated *E. coli*, 100.0%, 50.0%, 30.0%, 25.0% and 5.0% of the organisms were found to

be resistant to ampicillin/amoxicillin, cephalexin, tetracycline, cotrimoxazole and ciprofloxacin respectively. And 94.5%, 60.0%, 38.0%, 44.0% and 0% of the isolated *Klebsiella* spp. were found to be resistant to same antibiotics respectively (Ghimire *et al*, 1994).

E.coli was found as the most predominant pathogen (57.0%) followed by *Klebsiella pneumoniae* (24.0%), *Proteus* spp (10.0%), *Pseudomonas aeruginosa* (1.7%), *Salmonella typhimurium* (1.7%), *Shigella boydii (*1.7%), *Streptococcus faecalis* (1.7%) and *S. aureus* (1.7%). In vitro susceptibility test of these pathogens showed that almost all isolates were susceptible to nitrofurantoin (88.0%), followed by ciprofloxacin (81.0%), nalidixic acid (69.0%) and chloramphenicol (60.0%) whereas cotrimoxazole and amoxicillin were least effective antibiotics against these bacterial isolates (Gautam *et al*, 1997).

Rai *et al* (2001) found that *E. coli* (61.8%) was the most predominant pathogen followed by *Klebsiella pneumoniae* (12.2%) and *S. aureus* (12.2%). With regards to antibiotic susceptibility pattern, cephalexin (100.0%) was the most effective drug for Gram positive bacteria, followed by nitrofurantoin (93.8%), ciprofloxacin (85.7%), cotrimoxazole (50.0%) and norfloxacin (50.0%). Likewise, nitrofurantoin (77.3%) was the drug of choice in UTI for Gram negative bacteria, followed by gentamicin (59.1%) and cotrimoxazole (40.9%).

In a study done at NPHL, it was found that urine samples of kidney transplant patients showed 15.0% positive growth. *E. coli* (46.7%) was the most predominant bacteria causing UTI followed by *Klebsiella* spp. (13.3%), *Pseudomonas* spp. (13.3%), *S. aureus* (13.3%), *Proteus* spp. (3.3%), *Citrobacte*r spp. (3.3%), *Streptococcus faecalis* (3.3%) and *Morganella morganii* (3.3%). Gentamicin and amikacin (100.0%) were the most effective drugs against Gram negative bacteria (Ghimire *et al*, 2004).

In a retrospective study conducted in five hospitals of Kathmandu, the most common organisms causing UTI was found to be *E. coli* (49.0%), followed by *S. aureus* (23.0%), *Klebsiella* spp. (9.7%), *Proteus* spp. (3.6%), *Pseudomonas* spp. (0.8%) and *Citrobacter*

spp. (2.8%). All the organisms causing UTI were found to be susceptible to nitrofurantoin and amoxycillin whereas ciprofloxacin was found to be most effective (Jha and Bapat, 2005).

In a study done at Kathmandu Model Hospital, it was found that the predominant bacteria causing UTI were the Gram negative isolates constituting 88.2% among them 67.9% were MDR strains whereas Gram positive bacteria constituted only 11.8% out of which 38.9% were MDR strains (Shrestha *et al*, 2005).

In a study done at NPHL, *E. coli* was the most common isolate accounting for 43.3% of all bacterial isolates followed by *S. aureus* (23.3%) and *Klebsiella* spp. (16.6%). Amoxycillin was found to be most effective antibiotic against *E. coli* followed by nalidixic acid and nitrofurantoin. Similarly, ampicillin was found to be the most effective antibiotic against *S. aureus* whereas amoxycillin and norfloxacin were equally effective against *Klebsiella* spp. (Jha and Bapat, 2005).

A study was done at Manipal Teaching Hospital, Manipal College of Medical Sciences, Pokhara, Nepal; it was 1,680 clinically suspected cases of urinary tract infections from inpatients of various clinical departments during one year. In this study significant bacteriuria was found in 71.7 % of the samples, 17% were sterile, 4.8% showed insignificant bacteriuria, and 6.5% non- pathogenic bacteria. The most common pathogens isolated were *Escherichia coli* (59.4%), *Klebsiella spp* (15.7%) and *Enterococcus faecalis* (8.1). The mean susceptibility was high for amikacin (87.2%), ciprofloxacin (74.8%), ceftazidime (71.5%) and gentamycin (70.4%) but low for nitrofurantoin (35%), cephalexin (49.7%), and ampicillin (50.5%).*Escherichia coli* was found to be most susceptible to amikacin (98%), followed by gentamycin (87.9%), ceftazidime (80.8%), norfloxacin (78.4%) and cotrimoxazole (77.9%) (Das *et al*, 2006).

A retro specific study was carried out from march 2007 to august 2007 at TUTH,a total of 6580 urine samples from patients attending both inpatient and outpatients departments of TUTH were included in this study, the urine samples is subjected to culture and sensitivity test with the use of standard bacteriological techniques. As described by American Society of Microbiology (ASM) 26% of total urine samples showed significant bacterial

growth.14 different bacterial species were isolated, among these *Escherichia coli* (59.59%), was significantly the most predominant one ($p<0.05$) followed by *Staphylococcus aureus* (12.58%), *Klebsiella spp* (10.78%), *Enterococcus faecalis* (7.95), *Pseudomonas aeruginosa* (5.01%), *Acinetobacter calcoaceticus* (1.09%) and others.majority of gram negative bacteria showed susceptibility towards amikacin. *Escherichia coli* was found to be sensitive towards amikacin (81.5%), followed by nitrofurantoin (79.2%), and gentamycin (65%).Staphylococcus aureus showed susceptibility towards gentamycin (75.5%), cloxacillin (67.5%) (Kattel *et al*, 2008).

A retro specific study was conducted to find out urinary tract infection (UTI) in children and their antibiotic sensitivity pattern among Nepalese children. This was at Kanti Children Hospital in Kathmandu (Nepal), by analyzing the records of urine samples collected for culture and sensitivity tests over a period of six months (April to November, 2007). Of the total 1878 mid –stream urine samples collected from a suspected cases of UTI, 538 (28.6%) were positive for pathogenic organisms. There were no positive differences in growth positive rate in two genders (M: 51.7% and F: 48.3%). Of the various pathogenic organisms isolated, *Escherichia coli* constituted for 93.3% followed by Proteus spp, *Klebsiella spp, Citrobacter spp, Staphylococcus aureus* and others. *E. coli* was found to be most sensitive to amikacin, chloramphenicol, nitrofurantoin and ofloxacin and least sensitive to most commonly used drugs like cephalexin, nalidixic acid, cotrimoxazole and norfloxacin (Rai *et al*, 2008).

CHAPTER-IV

4. MATERIALS AND METHODS

This study was conducted at National Public Health Laboratory, Teku from April 2010 to January 2011. During this period, a total of 1233 urine samples from patients suspected of UTI were collected and processed according to the standard laboratory methods (Vandepitte *et al*, 2003, Collee *et al*, 2004).

4.1 MATERIALS

All the materials used during study are listed in the Appendix-III**.**

4.2 METHODS

4.2.1 Data Collection

Patients visiting NPHL for urine culture was interviewed (Questionnaire- Appendix I) for clinical history during sample collection. The gathered information includes patient's lab number, age, sex, immunosuppressive disease, signs and symptoms (dysuria, frequency, urgency, fever, stomach pain etc.), date of onset, antibiotics used.

4.2.2 Specimen collection

Each patients were instructed for proper collection of sample. The patients were given a clean, dry and sterile and leak proof container and requested for 5-10 ml mid-stream urine sample. The samples were processed as soon as possible. In case of delay the sample was refrigerated at 4ºC. Detailed guidelines for collection of clean catch mid-stream urine are mentioned in Appendix-VI

4.2.3 Specimen evaluation

Before proceeding, the urine specimens were evaluated in terms of their acceptability. Considerations included proper labeling(full name, age, sex, serial number, date and time of collection) visible signs of contamination(turbidity, particles, blood cells) and any transportation delays in getting the specimen to the laboratory. A properly labeled specimen contained patient's full name, date and time of collection. Single urine

specimen was collected from each patient so bacteriological culture was performed and then routine microscopic observation was done after inoculation.

4.2.4 Sample processing

a) Routine macroscopic examination

Macroscopic examination of the urine sample collected was conducted by observing its color and appearance and reported accordingly (Vandepitte *et al*, 2003, Collee *et al*, 2004).

b) Routine microscopic examination

About 5 ml (about half) of urine sample was taken in a clean sterile centrifuge tube and centrifuged at 3000 rpm for 10 min. The supernatant was discarded. The sediment was then examined by wet mount preparation (Vandepitte *et al*, 2003, Collee *et al*, 2004).

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. number of objects seen in40X objective of microscope (Vandepitte *et al*, 2003, Collee *et al*, 2004).

4.2.5 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, 1984). An inoculating loop of standard dimension (4 mm) was used to take up approximately fixed (±10% error was accepted) and known volume (0.001ml) of mixed uncentrifuged urine was inoculated on the surface of 5% Blood Agar (BA) and MacConkey Agar (MA). Urine specimen was thoroughly mixed to ensure uniform suspension of bacteria before inoculating the agar plates. The inoculated MA and BA plates were aerobically incubated overnight at 37º C. The bacterial count was reported as described by Vandepitte *et al*, (2003)

The bacterial count was reported as:

- Insignificant: Less than 10^4 organisms/ml.
- Repeat specimen: 10^4-10^5 organisms/ml, doubtful significance.
- Significant bacteriuria: More than 10^5 organisms/ml.

4.2.6 Identification of isolates

The isolated colony from plates showing significant growth was further preceded for identification. Plate showing no growth, mixed growth, and bacterial growth of insignificant number was excluded from the study. Identification of the bacteria isolated from the urine sample was conducted according to the protocol provided by the clinical hand book of medical microbiology and Collee *et al*, (2001). The single distinct colony was gram stained. A single distinct colony from MA for both the gram negative and gram positive bacteria was picked by using sterile straight wire loop and inoculated on NA. It was incubated at 37ºC for 24 hours. After the overnight incubation, the culture was used to perform biochemical test and antibiotic susceptibility test.

4.2.7 Antibiotic susceptibility testing

The antimicrobial susceptibility testing of the isolates were done by modified Kirby-Bauer disk diffusion method as recommended by CLSI (Clinical and Laboratory Standards Institute) using Mueller Hinton agar (MHA).

4.2.8 Quality control

Strict quality control was maintained to obtain reliable microbiological results. The quality of each agar plate prepared was mainted by inocubating one plate of each batch in the incubator. Control strains of ATCC were used for the identification test and for the standardization of Kirby- Bauer test and also for correct interpretation of inhibition zones of diameter. Quality of sensitivity test was maintained by maintaining the thickness of MHA at 4mm and the p^H 7.2-7.4. Similarly antibiotics disks having correct amount as indicated was used. Strict asceptic condition was maintained while carrying out all the procedures.

4.2.9 Data analysis

All the data obtained was statistically analysed by using Statistical Package for Social Sciences (SPSS) version 16. The Chi-square test was used as per need to determine significant association between different factors for the causation of UTI.

CHAPTER-V

5. RESULTS

This study was conducted among patients suspected of urinary tract infection visiting National Public Health Laboratory (NPHL), Kathmandu, Nepal. Twelve hundred and thirty three mid-stream urine samples were collected for urine culture, among them 175 were culture positive and they were subjected to antimicrobial susceptibility test.

5.1. Distribution of patients requested for urine culture

Table1. Age and gender wise distribution of patients whose urine were analysed

Age group	Sex of patients				Total	%
	Female		Male			
	Number	%	Number	%		
0-10	15	1.22	22	1.78	37	3
10-20	28	2.27	21	1.70	49	3.97
20-30	196	15.90	195	15.82	391	31.72
30-40	153	12.41	222	18	375	30.41
40-50	78	6.32	100	8.11	178	14.43
50-60	60	4.87	57	4.62	117	9.49
60-70	25	2.03	30	2.43	55	4.46
>70	17	1.38	14	1.14	31	2.52
Total	572	46.40	661	53.60	1233	100

It was found that highest number of patients 31.72 % (391/1233), were from age group (20-30) years, followed by age group (30-40) years i.e 30.41 % (375/1233).

5.2. Sexwise distribution of uropathogens

Table 2.Age and gender wise distribution of culture positive results

Age group	Male		Female		Total	
	Number	%	Number	%	Number	%
0-10	1	0.57	1	0.57	2	1.14
10-20	4	2.29	4	2.29	8	4.58
20-30	17	9.71	40	22.86	57	32.57
30-40	24	13.71	24	13.71	48	27.43
40-50	12	6.86	11	6.29	23	13.15
50-60	10	5.71	14	8	24	13.71
60-70	5	2.86	4	2.29	9	5.15
>70	1	0.57	3	1.71	4	2.28
Total	74	42.29	101	57.71	175	100

Out of 175 positive cases, highest number of growth was obtained from age group 20- 30 [32.57% (57/175)]. Highest percentage 22.86% (40/175) was observed in female of age group 20-30, similarly in male patients highest percentage of growth was 13.71% (24/175) in age group 30-40, least number of growth was in age group (0-10), which was 1.14% (2/175). Higher number of growth was obtained in productive age group that is (20-40), which was 60 % (105/175).

5.3. Sexwise distribution of culture positive cases in Kidney transplanted patients

Table 3: Sexwise distribution of patients among kidney transplanted patients

Sex	Infection status		Total
	Infected (Culture positive)	Non infected (Culture negative)	
Male	14(63.64%)	319(72.67%)	333(75.85%)
Female	8(36.36%)	98(22.32%)	106(24.15%)
Total	22(5.01 %)	417(94.99%)	439(100)

Among 439 sample from kidney transplanted patients, 94.99% (n=417) were noninfected where as only 5.01% (n=22) patients were infected. Among 22 culture positive cases 63.64% (n=14) were male patients, whereas 36.36% (n=8) were female patients.

5.4. Distribution of culture positive isolates among Kidney transplanted patients

Table 4: Transplantation status of patients and their culture positivity

Age group	KidneyTransplanted		Non kidney transplanted		Total	%
	Positive	%	Positive	%		
0-10	0	0	2	1.31	2	1.31
10-20	1	4.56	7	4.58	8	9.14
20-30	4	18.18	53	34.64	57	52.82
30-40	5	22.73	43	28.10	48	50.83
40-50	5	22.73	18	11.76	23	34.49
50-60	5	22.73	19	12.42	24	35.15
60-70	2	9.09	7	4.58	9	13.67
>70	0	0	4	2.61	4	2.61
Total	22	100	153	100	175	

Out of 175 culture positive cases; 12.57 % (22/175) samples were from kidney transplanted patients and 87.43 % (153/175) were from other UTI suspected patients. More growth was seen in after 30 years of age in kidney transplanted patients, which was 22.73% (5/22).

5.5. Sexwise distribution of uropathogens according to Gram reaction

Table 5 Distribution of culture positive cases and their gram reaction

Sex of patients	Gram reaction		Total	%
	Gram Negative bacteria	Gram Positive bacteria		

Male	71	3	74	42.29
Female	94	7	101	57.71
Total	165(94.29%)	10(5.71%)	175	100

Out of total 175 bacterial growth, 94.29% (165/175) were due to gram negative bacteria whereas only 5.71% (10/175) were due to gram positive bacteria. Among 175 cultures positive cases 42.29 % (74/175) were from male patients, similarly 57.71% (101/175) were from female patients

5.6. Distribution of uropathogens in urine isolates

Table 6: Distribution of urine isolates:

S.N	Organisms	Organisms	
		Total Number of Isolates	**%**
1.	*Enterobacter spp*	3	1.71
2.	*Acinetobacter spp*	6	3.43
3.	*Alkaligenes spp*	1	0.57
4.	*Citrobacter freundii*	3	1.71
5.	*Escherichia coli*	107	61.14
6.	*Edwardsiella spp*	1	0.57
7.	Enterococci spp	4	2.29
8.	*Klebsiella oxytoca*	9	5.14
9.	*Klebsiella pneumoniae*	22	12.57
10.	*Proteus mirabilis*	4	2.29
11.	*Proteus vulgaris*	2	1.14
12.	*Pseudomonas aeruginosa*	6	3.43
13.	*Staphylococcus aureus*	2	1.14
14.	*Staphylococcus saprophyticus*	2	1.14
15.	*Streptococcus spp*	2	1.14
16.	*Providencia spp*	1	0.57
	Total	175	100

Out of 175 different organisms, *E.coli* was most predominant i.e, 61.14 % (n=107) followed by *Klebsiella pneumonia* 12.57% (n= 22), *Klebsiella oxytoca* 5.14% (n=9) and *Pseudomonas aeruginosa* and *Acinetobacter spp* 3.43% (n= 6)

5.7. Distribution of isolated organisms in urine culture

Table7: Distribution of isolated organisms in urine culture:

S.N	Organisms isolated	Non-Kidney transplanted	%	Kidney Transplanted	%	Total	%
1.	*Enterobacter spp*	3	1.96	0	-	3	1.71
2.	*Acinetobacter spp*	5	3.27	1	4.55	6	3.43
3.	*Alkaligenes spp*	1	0.65	0	-	1	0.57
4.	*Citrobacter freundii*	3	1.96	0	-	3	1.71
5.	*Escherichia coli*	98	64.05	9	40.91	107	61.14
6.	*Edwardsiella spp*	1	0.65	0	-	1	0.57
7.	*Enterococci spp*	3	1.96	1	4.55	4	2.29
8.	*Klebsiella oxytoca*	7	4.58	2	9.09	9	5.14
9.	*Klebsiella pneumoniae*	17	11.11	5	22.73	22	12.57
10.	*Proteus mirabilis*	3	1.96	1	4.55	4	2.29
11.	*Proteus vulgaris*	1	0.65	1	4.55	2	1.14
12.	*Pseudomonas aeruginosa*	4	2.61	2	9.09	6	3.43
13.	*Staphylococcus aureus*	2	1.31	0	-	2	1.14
14.	*Staphylococcus saprophyticus*	2	1.31	0	-	2	1.14
15.	*Streptococcus spp*	2	1.31	0	-	2	1.14
16.	*Providencia spp*	1	0.65	0	-	1	0.57
	Total	153		22		175	100

Out of 175 positive cases, 16 different types of organisms were isolated. In other UTI suspected patients *Escherichia coli* 64.05 % (98/153) was most predominant, followed by *Klebsiella pneumoniae* 11.11% (17/153) and *Klebsiella oxytoca* 4.58% (7/153).

Similar results obtained in kidney transplanted patients, isolation of *Escherichia coli* was 40.91% (9/22), followed by *Klebsiella pneumoniae* 22.73% (5/22), *Klebsiella oxytoca* and *Pseudomonas aeruginosa* 9.09% (2/22).

5.8. Antibiotic susceptibility pattern of uropathogens

Table8. Antibiotic susceptibility pattern of isolated organisms:

Antibiotics	Gram negative			Gram positive			Total	%
	Susceptible	%	Total	Susceptible	%	Total	Susceptible	Susceptible
Amoxicillin	43	26.06	165	6	60	10	49	28
Ceftriaxone	112	67.88		8	80		120	68.57
Ofloxacin	91	55.15		2	20		93	53.14
Ciprofloxacin	86	52.12		4	40		90	51.43
Norfloxacin	85	51.52		4	40		89	50.86
Cotrimoxazole	67	40.61		8	80		75	42.86
Nitrofurantoin	101	61.21		4	40		105	60
Second generation antibiotics								
Antibiotics	Susceptible	%	Total	Susceptible	%	Total	Susceptible	Susceptible
Ceftazidime	29	43.94	66	0	-	-	29	43.94
Ceftazidime clavunic acid	60	90.91		0	-		60	90.91
Amikacin	60	90.91		0	-		60	90.91
Gentamycin	31	46.97		0	-		31	46.97
Cephipime	22	33.33		0	-		22	33.33
Penicillin	0		-	3	50	6	3	50

Oxacillin	0	-	-	5	83.33		-	83.33
Total	675			39			844	

Out of total 175 isolates, 67.88% (112/165) were susceptible to Ceftriaxone followed by 61.21% (101/165) Nitrofurantoin, 55.15% (91/165) Ofloxacin and 52.12% (86/165) Ciprofloxacin, among 1st line antibiotics. Similarly in 2nd line antibiotics 90.91% (60/66) were susceptible to Ceftazidime-clavunic acid and Amikacin in case of gram negative isolates. In case of gram positive isolates, 80% (8/10) were susceptible to Ceftriaxone and Cotrimoxazole, followed by 60% (6/10) Amoxicillin, 40% (4/10) Ciprofloxacin and Norfloxacin. Similarly in 2nd line antibiotics 83.33% (5/6) were susceptible to Oxacillin and 50% (3/6) and Penicillin.

Table 9: Antibiotic susceptibility pattern of *E.coli*

Escherichaia coli (N=107)					
First generation antibiotics					
Antibiotics group	Antibiotics	Resistant		Susceptible	
		Number	**%**	Number	**%**
Beta-lactam	Amoxicillin	77	71.96	30	28.04
	Oxacillin	-	-	-	-
	Penicillin	-	-	-	-
Quinolone/Fluroquinolones	Ofloxacin	51	47.66	53	49.53
	Norfloxacin	60	56.07	47	43.92
	Ciprofloxacin	57	53.27	50	46.73
Sulfamazole	Cotrimoxazole	68	63.55	39	36.45
Cephalosporins	Ceftriaxone	36	33.64	71	66.36
Nitrofurans	Nitrofurantoin	34	31.76	73	68.22
Second generation antibiotics					
Cephalosporins	Ceftazidime	26	54.17	22	45.83
	Ceftazidme clavunic acid	4	8.33	44	91.67
	Cephipime	25	52.08	23	47.91
Aminoglycosides	Gentamycin	22	45.85	26	54.17
	Amikacin	4	8.33	44	91.67

Among total 107 *E.coli* isolates, 68.22% (73/107) were susceptibile towards Nitrofurantoin (Nitrofurans group), followed by Ceftriaxone (Cephalosporins groups) 66.36% (71/107), Ofloxacin, Ciprofloxacin and Norfloxacin (Quinolone/Floroquinolone) were susceptible to 49.53%, 46.73% and 43.92% respectively in first generation antibiotics. Least susceptible were Amoxicillin 28.03% (30/107) in Beta-lactam groups of antibiotics. But in second generation antibiotics Ceftazidme- clavunic acid and Amikacin

91.67% (44/48) showed high susceptibility towards *E.coli* and Cephipime 47.91% (23/48) were least susceptible antibiotics.

Table 10: Antibiotic susceptibility pattern of *Klebsiella pneumoniae*

Klebsiella pneumoniae (N=22)					
First generation antibiotics					
Antibiotics group	Antibiotics	Resistant		Susceptible	
		Number	**%**	Number	**%**
Beta-lactam	Amoxicillin	18	81.82	4	18.18
	Oxacillin	-	-	-	-
	Penicillin	-	-	-	-
Quinolone/Fluroquinolones	Ofloxacin	7	31.82	15	68.18
	Norfloxacin	9	40.91	13	59.09
	Ciprofloxacin	8	36.36	14	63.64
Nitrofurans	Nitrofurantoin	14	63.64	8	36.36
Sulfamazole	Cotrimoxazole	10	45.45	12	54.55
Cephalosporins	Ceftriaxone	7	31.82	15	68.18
Second generation antibiotics					
Cephalosporins	Ceftazidime	5	83.33	1	16.67
	Ceftazidime clavunic acid	1	16.67	5	83.33
	Cephipime	4	66.67	2	33.33
Aminoglycosides	Gentamycin	4	66.67	2	33.33
	Amikacin	0	0	6	100

Out of 22 *Klebsiella pneumonie* isolates, 68.18% (15/22) were susceptible to Ceftriaxone (Cephalosporins group) and Ofloxacin (Quinolone/Floroquinolone groups), followed by Ciprofloxacin 63.64% and Norfloxacin 59.09% (Quinolone/Floroquinolone groups) of first generation antibiotics. Amoxicillin (Beta-lactam groups) was 18.18% (4/22). But in second generation antibiotics, Amikacin showed hundred percent susceptibility and Ceftazidme clavunic acid 83.33% showed second high susceptibility test towards *Klebsiella pneumonia*e.

Table 11: Antibiotic susceptibility pattern of *Klebsiella oxytoca*

Klebsiella oxytoca(N=9)					
First generation antibiotics					
Antibiotics group	Antibiotics	Resistant		Susceptible	
		Number	**%**	Number	**%**
Beta-lactam	Amoxicillin	5	55.55	4	44.44
	Oxacillin	-	-	-	
	Penicillin	-	-	-	
Quinolone/Fluroquinolones	Ofloxacin	6	66.67	3	33.33
	Norfloxacin	2	22.22	7	77.78
	Ciprofloxacin	4	44.44	5	55.55
Sulfamazole	Cotrimoxazole	3	33.33	6	66.67
Cephalosporins	Ceftriaxone	2	22.22	7	77.78
Nitrofurans	Nitrofurantoin	1	11.11	8	88.89
Second generation antibiotics					
Cephalosporins	Ceftazidime	1	50	1	50
	Ceftazidime clavunic acid	1	50	1	50
	Cephipime	0	0	2	100
Aminoglycosides	Gentamycin	1	50	1	50
	Amikacin	0	0	2	100

Out of 9 *Klebsiella oxytoca*, it was found highly susceptible to Nitrofurantoin in 88.89% (8/9) in Nitrofurans groups of antibiotics. Ceftriaxone and Norfloxacin showed 77.78% (7/9) same susceptibility towardas *Klebsiella oxytoca*, followed by Cotrimoxazole 66.67% (6/9), in first generation antibiotics. Least susceptible was

ofloxacin 33.33% (3/9). But in second generation antibiotics Amikacin and Cephipime showed 100% susceptibility towards *Klebsiella oxytoca*.

Table 12: Antibiotic susceptibility pattern of *Acinetobacter spp*

Acinetobacter spp (N=6)					
First generation antibiotics					
Antibiotics group	Antibiotics	Resistant		Susceptible	
		Number	**%**	Number	**%**
Beta-lactam	Amoxicillin	3	50	3	50
	Oxacillin	-	-	-	-
	Penicillin	-	-	-	-
Quinolone/Fluroquinolones	Ofloxacin	0	0	6	100
	Norfloxacin	0	0	6	100
	Ciprofloxacin	0	0	6	100
Sulfamazole	Cotrimoxazole	3	50	3	50
Cephalosporins	Ceftriaxone	1	16.67	5	83.33
Nitrofurans	Nitrofurantoin	2	33.33	4	66.67
Second generation antibiotics					
Cephalosporins	Ceftazidime	1	100	0	0
	Ceftazidime clavunic acid	0	0	1	100
	Cephipime	1	100	0	0
Aminoglycosides	Gentamycin	1	100	0	100
	Amikacin	0	0	1	100

Out of total 175, 6 *Acinetobacter spp* was isolated; Ofloxacin Norfloxacin and Ciprofloxacin (Quinolone/Fluroquinolones group of antibiotic) showed 100% susceptibility towards *Acinetobacter spp*. Similarly in second generation antibiotics, Amikacin, Ceftazidime clavunic acid and Gentamycin also showed 100% susceptibility towards *Acinetobacter spp.*

Table 13: Antibiotic susceptibility pattern of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa (N=6)					
First generation antibiotics					
Antibiotics group	Antibiotics	Resistant		Susceptible	
		Number	**%**	Number	**%**
Beta-lactam	Amoxicillin	6	100	0	0
	Oxacillin	-	-	-	-
	Penicillin	-	-	-	-
Quinolone/Fluroquinolones	Ofloxacin	4	66.67	2	33.33
	Norfloxacin	5	83.33	1	16.67
	Ciprofloxacin	4	66.67	2	33.33
Sulfamazole	Cotrimoxazole	4	66.67	1	16.67
Cephalosporins	Ceftriaxone	5	83.33	1	16.67
Nitrofurans	Nitrofurantoin	3	50	3	50
Second generation antibiotics					
Cephalosporins	Ceftazidime	4	80	1	20
	Ceftazidime clavunic acid	2	40	3	60
	Cephipime	2	40	3	60
Aminoglycosides	Gentamycin	5	100	0	0
	Amikacin	1	20	4	80

Out of total 175, total isolation of *Pseudomonas aeruginosa* was resistant over most groups of antibiotics. Out of 6 antibiotics, nitrofurantoin showed 50% (3/6), i.e maximum susceptibility towards *Pseudomonas aeruginosa*. Among second generation antibiotics, Amikacin 80% (4/5) showed high susceptibility towards *Pseudomonas aeruginosa*.

5.10. Antibiotic susceptibility pattern of *Proteus mirabilis*

Out of total 175 isolates, total isolation of *Proteus mirabilis* was 2.29% (n=4). Which was hundred percent resistant to Cotrimoxazole and Amoxicillin. Similarly 50% resistant to Ciprofloxacin Ofloxacin, Nitrofurantoin while, 25% *Proteus mirabilis* were resistant over Ceftriaxone, Amikacin, Gentamycin, Norfloxacin and Ceftazidime. Out of these antibiotics Ceftazidime clavunic acid didnot show any resistivity.

5.11. Antibiotic susceptibility pattern of *Proteus vulgaris*

Out of total 175 isolates, total isolation of *Proteus vulgaris* was 1.14% (n= 2). Which were hundred percent resistant Amoxicillin, Cotrimoxazole and Ciprofloxacin while 50% organisms were resistant to Ofloxacin, Norfloxacin and Ceftadidime, whereas *Proteus vulgaris* were hundred percent sensitive to Ceftriaxone.

5.12. Antibiotic susceptibility pattern of *Enterococci spp*

Out of total 175 isolates, total isolation of *Enterococci spp was* 2.29% (n=4). Ofloxacin, Ciprofloxacin showed resistivity of 75%, whereas Ceftriaxone and Norfloxacin showed resistivity of 50%. Cotrimoxazole, Nitrofurantoin, Penicillin, Oxacillin and Amoxicillin showed the resistivity of 25%.

5.13. Antibiotic susceptibility pattern of *Enterobacter spp*

Out of total 175 isolates, total isolation of *Enterobacter spp* was 1.71% (n=3). 33.33% of organism shows resistivity to first generation antibiotics such as, Amoxicillin Ceftriaxone, Ofloxacin, Ciprofloxacin, Norfloxacin, Cotrimoxazole and Nitrofurantoin.

5.14. Antibiotic susceptibility pattern of *Citrobacter freundii*

Out of total 175 isolates, total isolation of *Citrobacter freundii* was 1.71% (n=3). Amoxicillin, Nitrofurantoin were hundred percent resistant, whereas Cotrimoxazole and ofloxacin showed 50% resistivity. Gentamycin, Norfloxacin, Ciprofloxacin showed resistivity of 33.33%, but hundred percent sensitive to Ceftriaxone and Amikacin.

5.15. Antibiotic susceptibility pattern of *Staphylococcus aureus*

Out of total 175 isolates, total isolation of *Staphylococcus aureus* was 1.14% (n= 2).Which was hundred percent sensitive towards Ceftriaxone and Cotrimoxazole, whereas they were 50% resistant to Amoxicillin, Ofloxacin, Ciprofloxacin, Norfloxacin, Nitrofurantoin and Penicillin.

5.16. Antibiotic susceptibility pattern of *Staphylococcus saprophyticus*

Out of total 175 isolates, total isolation of *Staphylococcus saprophyticus was* 1.14% (n= 2) Antibiotics Amoxicillin and Ofloxacin showed hundred percent resistivity, whereas Nitrofurantoin, Norfloxacin, Ciprofloxacin and Penicillin showed 50% percent resistivity towards *Staphylococcus saprophyticus.*

5.17. Antibiotic susceptibility pattern of *Alkaligenes spp*

Out of total 175 isolates, total isolation of *Alkaligenes spp was* 0.57% (n=1) which was only showed resistivity to Amoxicillin i.e, Beta lactam group of antibiotics, while other antibiotics showed hundred percent sensitivity towards *Alkaligenes spp.*

5.18. Antibiotic susceptibility pattern of *Streptococccus spp*

Out of total 175 isolates, total isolation of *Streptococccus spp* was 1.14% (n= 2).Which was 100% sensitive to Cotrimoxazole, Penicillin, Oxacillin, Amoxicillin, and Ceftriaxone, but hundred percent resistant to Ofloxacin, whereas 50% sensitive to Norfloxacillin, Ciprofloxacin, and NItrofurantoin.

5.19. Antibiotic susceptibility pattern of *Edwardsiella spp*

Out of total 175 isolates, total isolation of *Edwardsiella spp* was 0.57% (n=1).Which was sensitive to all classes of antibiotics.

5.20. Antibiotic susceptibility pattern of *Providencia spp*

Out of total 175 isolates, total isolation of *Providencia spp* was 0.57% (n=1).Which was sensitive to all classes of antibiotics.

5.21. Distribution pattern of MDR isolates

Table 14: Distribution of MDR isolates

S.N	Organisms isolated	Total organisms isolated	Multidrug resistant	%
1.	*Enterobacter spp*	3	1	33.33
2.	*Acinetobacter spp*	5	1	0.2
3.	*Alkaligenes spp*	1	-	-
4.	*Citrobacter freundii*	3	1	33.33
5.	*E.coli*	107	57	53.27
6.	*Edwardsiella spp*	1	-	-
7.	*Enterococcus spp*	4	3	75
8.	*Klebsiella oxytoca*	8	2	25
9.	*Klebsiella pneumonia*	18	8	44.44
10.	*Proteus mirabilis*	6	4	66.67
11.	*Proteus vulgaris*	2	2	100
12.	*Pseudomonas aeruginosa*	6	5	83.33
13.	*Staphylococcus aureus*	2	-	-
14.	*Staphylococcus saprophyticus*	2	1	50
15.	*Streptococcus spp*	2	-	-
16.	*Providencia spp*	1	-	-
	Total	175	84	48

Out of total 175 isolates, multiple drug resistant (MDR) cases were found to 48 % (84/175). Among 107 *E.coli* samples, 53.27% (57/107) showed MDR positive; similarly *Proteus vulgaris* were 100% (2/2) MDR producers, whereas *Proteus mirabilis* were 66.67% (4/6) MDR positive. *Pseudomonas aeruginosa* were 83.33% (5/6) MDR positive. *Klebsiella pneumoniae* was 44.44% (8/18) MDR positive, similarly *Klebsiella oxytoca* were25% (2/8) MDR

positive. *Enterobacter spp* was 33.33% (1/3) MDR positive. *Citrobacter freundii* was 33.33% (1/3) MDR positive. Among Gram positive isolates *Enterococcus spp* was 75% (3/4) MDR positive, and *Staphylococcus saprophyticus* was 50% (1/2) MDR positive.

CHAPTER-VI

6. DISCUSSION AND CONCLUSION

6.1 Discussion

Out of total 1233 urine samples, 794 (64.31%) urine samples from other UTI suspected patients whereas 440 (35.69%) sample from kidney transplanted patients. In this study 53.61% were male patients whereas 46.39% were female patients.

Out of total 1233 urine samples 175 (14.19%) samples showed significant growth. A similar study carried out by Chhetri *et al* (2001) showed growth positivity of 21.8%. The low growth positive rate observed in this study might be due to inclusion of kidney transplant patients and others for routine check up only. This might also be due to inclusion of samples from patients under treatment.

In this study, age group (20-30) years had got high prevalence of UTI, a total of 32.57% (57/175) patients were UTI positive cases. In this study female of childbearing age group (20-40) had 36.57% (64/175) growth positive cases. Previous study did by Shrestha *et al*, (2005) at Kathmandu Model Hospital, Steenberg *et al*, (1985) Rajbhandari and Shrestha (2002) also the similar results. This result suggest that sexually active and women of childbearing age are more susceptible to UTI.

Females are more susceptible to UTI than males. Also in the present study, this fact was supported where the rate of growth positivity was found to be 57.38 % (101/176) in females and 42.62% (75/176) in males. This higher growth positivity seen in females was found to be statistically significant ($p<0.05$) and is attributed to their anatomical structure (short urethra and proximity to anal orifice) leading to easy access for coliform bacilli. This result confirms and expands the previous findings of Steenberg *et al* (1969) ein Denmark, Jha and Yadav (1992), Chhetri *et al* (2001), Jha and Bapat (2005) and Rajbhandari and Shrestha (2002) in Nepal.

In this study, age group of 20-40 years showed highest percentage of growth positivity. High-infected females also belonged to the same group. This finding correlates to the

results of Steenberg *et al* (1969), Manandhar *et al* (1996), Rajbhandari and Shrestha (2002), Regmi *et al* (2003), Shrestha *et al* (2005) and Jha and Bapat (2005). The females of this age group are sexually active and are of childbearing age. A number of studies suggest that sexual activity is an important factor in the pathogenesis of UTI in women. Prevalence of UTI in nuns and unmarried women is considerably lower than in married women (Leigh, 1990). These studies also support the fact that the sexual activities predispose an increase in incidence of UTI in sexually active ages. Among males, highest growth positivity was found among age group of 30-40 years. The similar study done by Jha, Bapat in 2005 also showed highest percentage of infected male were fall in age group 31-40.

Among 1233 total urine sample, 64.40% (794/1233) were from non kidney transplanted patients, whereas 35.60% (439/1233) were from kidney transplanted patients. The total of sample from kidney transplanted patients, significant bacteriuria was seen among only 22 cases (12.57%). Among total sample from kidney transplanted patients, 75.85% (333/439) were male patients, whereas only 24.15% (106/439) were female. Among sample from male patients 63.64% (14/22) were infected, whereas 36.36% (8/22) were from female. The study done by Ghimire *et al* (2004), 73.0% males and 27.0% females and significant bacteriuria was seen only in 30 cases (15.0%).

Sample from kidney transplanted patients had low growth might be due to prior use of antibiotics. Approximately 80% of total patients were on antibiotic prophylaxis and another reason for low positivity may be due to small samples size (439). In study carried out in Japan by *Takai et al*, 1998 reported that 26% of renal transplanted has UTI during the first year of transplantation. The majority of organisms cultured were gram negative bacterial isolates. The bacterial spectrum was not different than that of our study.

A research done by Mostapha *et al*, 1998 has reported that UTI is common following renal transplantation and was mainly due to Gram negative bacteria. The incidence has been reported to vary between 20 to 88%. One reason for this wide variation is the difficulty in distinguishing between asymptomatic and symptomatic infection in immune suppressed patients who may be symptomatic for many reasons. UTI diagnosed most frequently in the first month after transplantation however in our study; all transplant

surgery cases were performed outside Nepal. None of the patients studied were in this period (one month of surgery), which might be reason for low UTI positive cases. Bacteria reported in this study were similar to those causing UTI in the general population (Non transplant cases).

Among the total 175 bacterial isolates, 94.29% (165/175) were Gram negative bacilli and only 5.71% (10/175) were found to be Gram positive cocci. In a study done by Karki *et al* (2004), 91.1% of the isolates were Gram negative bacilli and 8.8% of them were Gram positive cocci. The higher incidence of UTI by Gram negative bacteria was also accounted in the study done by Dhakal *et al* (1999), Manandhar *et al* (1996), Shrestha *et al* (2003) and Talukder *et al* (1987), Habeeb *et al* (2009). From this study and the study done by other researchers, we can conclude that high incidence of UTI is caused by gram negative bacteria in comparison to gram positive bacteria.

Altogether 16 different bacterial isolates were found in this study. Among the isolates, *E. coli* (61.14%) was found to be the most predominant organism followed by *Klebsiella pneumoniae* (12.57%), *Klebsiella oxytoca* (5.14%) *Acinetobacter spp* (3.43%) *and* Enterococci (2.29%) were predominating amog others. Higher prevalence of *E. coli* seen in this study also resembled the study done by various other workers viz: Chhetri *et al* (2001), Sharma *et al* (1983), Tuladhar *et al* (1989), Jha and Yadav (1992), Manandhar *et al* (1996) and Dhakal *et al* 1999 in Nepal. The result is also in harmony with the study done at international context: Steenberg *et al* (1969), Kahlmeter (2000), Farrell *et al* (2003), Leigh (1990), Fowler (1990) and Kosakai (1990), Farrel *et al*, (2003).

In kidney transplanted patients also *E.coli* is predominating among others. In kidney transplanted patients, 5.14% (n=9) *E.coli* was isolated. Similarly in kidney transplanted patients, 2.86% (n=5) *Klebsiella pneumonia*e was isolated. But isolation of other organisms is comparatively very low. The low growth might be due to prior use of antibiotic prophylaxis and another reason for low positivity may be due to low sample size for kidney transplanted patients (Takai *et al,* 1998). A research done by Mostapha *et*

al, 1998 reported that UTI is common following renal transplantation and was mainly due to gram negative bacteria.

E. coli can bind to the glycoconjugate receptor (Gal α 1| 4 Gal) of the uroepithelial cells of human urinary tract so it can initiate infection itself. *E. coli* is isolated in 90.0% of infections and strains are characterized by unique virulence determinant, the p pilus (Gal-Gal receptor) (Johnson, 1991). *E. coli* is the most predominant organism to colonize the urethral meatus (Schaeffer and Chmiel, 1983) and perineum (Leigh, 1990) before ascending to the bladder. Strains of *E. coli* appear well adapted to invade urinary tract (serogroups 02, 04, 06, 07, 08, 09) which forms the majority of isolates of UTI (Chakraborty, 1995). This ability of *E. coli* may be the reason to be the most frequent organism to cause UTI in both sexes all over the world.

E. coli infection is high in female as compared to male. In this study also, *E. coli* infection was found to be 63.55% in females whereas 36.45 % in males (out of total *E. coli* isolates). Similar type of result was found by Dhakal *et al* (1999), Gautam *et al* (1998), Kosakai *et* al (1990) and Vorland *et al* (1985). Bacteriuria in ambulatory adult women is caused primarily by Gram negative bacilli derived from the faecal flora (Fowler, 1990). Pathogenic *E.coli* expresses specific adhesions such as P fimbriae and produce alpha and beta hemolysins. In a study performed by Petrof *et al*, (1999) in USA and Leonid *et al*, (2006) in Russia concluded that these factors are thought to play a role in attachment, ascent and colonization of different tissues surface during progression of steps involved in urinary tract.

Klebsiella pneumoniae was isolated as the second commonest pathogen in frequency causing UTI. *K. pneumoniae* 12.57% (n=22) accounted for second most common isolates. *Klebsiella oxytoca* was isolated as the third most common pathogens in frequency causing UTI. *Klebsiella oxytoca* accounted for 4.57% (8/175), which follows the statement of Fowler and Mariano (1990)-"*K pneumoniae* is the primary pathogens in the genus *K. oxytoca* may also cause bacteriuria." This finding of this study is harmony with the study done by Astal *et al* (2002), Das *et al* (2006), Gautam *et a*l (1998), Ghimire *et al* (1995), Hadiza *et al* (2003), Kumari *et al* (2005), Manandhar *et al* (2005), Sharma (1983) and Zhanel *et al* (2005).

Pseudomonas aeruginosa and *Acinetobacter spp* were isolated 3.43% (n=6) among total, fourth most predominating isolates. *Pseudomonas aeruginosa* is opportunistic pathogens. It is one of the primary causes of nosocomial infection. Mohamad *et al* (2007). In many other studies it has been found that *Pseudomonas aeruginosa* plays an important role in bladder infection and is considered as primary pathogens in compromised host (Dolan *et al,* 1989; Jones *et al,* 1999) and also in uncomplicated urinary tract infection (Kosakai *et al,* 1990). Similar study done by Kattel *et al* 2008, Department of microbiology, TUTH, Institute of medicine was found among gram negative isolates *Acinetobacter spp* were fourth most common pathogens of causing UTI.

Among gram positive isolates Enterococci spp were common pathogens causing UTI, 2.29% (n=4) organisms were isolated. Among total 75% organisms was multidrug resistant. 2.29% (n=4) species of Staphylococci were isolated. 1.14% (n=2) were *Staphylococcus aureus*, and 1.14% (n=2) were *Staphylococcus saprophyticus*. 1.14% (n=2) Streptococci were isolated.

Nepal is developing country most of the people are illiterate and do not know antibiotics and its mechanisms against bacteria and resistant developing mechanisms. So clinicians are not aware of effect of irrational use of drugs, wrong dose and dose taken insufficient length of time. It is now generally accepted as major public health issue and has significant implication on health and patient care. Resistance to antimicrobial drugs is associated with high morbidity and mortality, high health-care cost and prolonged hospitalization. The problem antimicrobial resistance is more troublesome to developing countries. WHO and the European Commission (EC) have recognized the importance of studying the emergence and determinants of resistance and the need for strategies for its control.

In this study, In case of first generation antibiotics Ceftriaxone (68.57%) was effective against isolated organisms followed by Nitrofurantoin (60%) of susceptibility. In second generation antibiotics Ceftazidime-clavunic acid amd Amikacin (91.91%) showed similar

susceptibility towards gram negative isolates which were resistant to first generation antibiotics. Similar study performed by Jha and Bapat (2005) at Sukraraj Tropical Hospital, 92.5% of urinary isolates were susceptible to Aminoglycosides groups of antibiotics.

On the other hand, (72%) organisms were resistant to Ampicillin.Which was found to be the least effective drug against Gram negative bacteria, followed by Cotrimoxazole (42.56%). Quinolone/Fluroquinolones groups of antibiotics showed susceptibility in similar manner, Ofoxacin, Ciprofloxacin and Norfloxacin showed susceptibility of 53.14%, 51.43%and 50.86% respectively.

In the urine isolates, Amoxicillin (28.04%) was found the least susceptible towards *E.coli* followed by Cotrimoxazole (36.36%). Nitrofurantoin (68.22%) was found to be most efficient antibiotics followed by Ceftriaxone (66.36%). Ofloxacin (49.53%), Ciprofloxacin (46.73%), and Norfloxacin (43.92%) showed susceptibility in Fluroquinolones group of antibiotics. The results found in this study is strongly supported by different other researchers.The study conducted by Karki *et al,* (2004) among outpatient and inpatient of Kathmandu Medical College Teaching Hospital, the *E.coli* isolates were most susceptible to Nitrofurantoin. The similar study conducted by Arosio *et al,* (1978) and Obi *et al,* (1996) resistant to Amoxicillin was observed. Similar type of result was found by Modi and Erch (2006). Resistance of *E. coli* to Quinolones has remained rare until recently, until their use increased (Oteo *et al*, 2001).

In second generation antibiotics *E.coli* were most susceptible to Ceftazidime -clavunic acid and Amikacin (91.67%).

K. pneumoniae was found to most susceptible to Ceftriaxone and Ofloxacin (68.18%) and least susceptible to Amoxicillin (18.18%) followed by Nitrofurantoin (36.64%). In Floroquinoles groups of antibiotics Ofloxacin, Ciprofloxacin and Norfloxacin showed susceptibility of 68.18%, 63.64% and 59.09% respectively. *K. pneumoniae* was found to most susceptible to Amikacin (100%) followed by Ceftazidime clavunic acid (83.33%) in second generation antibiotics. A similar result was found by Das *et al,* (2006) in which

Amikacin was found to be most effective against *K. pneumonia*e. Similar results obtained in case of *K. oxytoca* which was also hundred percent sensitive to Amikacin.

Pseudomonas aeruginosa was found to be resistant to most of antibiotics. Amoxicillin was found to be 100% resistant. Ofloxacin, Ciprofloxacin and Norfloxacin showed sensitivity of 33.33%, 33.33% and 16.67% respectively. Nitrofurantoin showed the sensitivity of 50% and (80%) in case of Amikacin. Ceftazidime -clavunic acid and Gentamycin also were less effective against *Pseudomonas aeruginosa.* Similar study done by Abubakar (2009), *Pseudomonas aeruginosa* was found most resistant to Amoxicillin. Repeated use of Quinolone therapy for complicated UTI, particularly *Pseudomonas aeruginosa* infection results in emergence of quinolone –resistant organisms (Ena *et al*, 1995)

Acinetobacter spp which were 100% sensitive to Quinolones/Floroloquinolens groups, they were sensitive to Ofloxacin, Ciprofloxacin, and Norfloxacin. First line drugs were sensitive to *Acinetobacter spp*. In second generation antibiotics, Aminoglycosides group of antibiotics were effective against *Acinetobacter spp*. Gentamycin and Amikacin showed the susceptibility of 100%.

Total isolation of *Citrobacter freundii* was 1.71% (n=3). Amoxicillin, Nitrofurantoin was 100% resistant, whereas Ceftriaxone and Amikacin were 100% sensitive antibiotics. Abubakar (2009) least susceptible antibiotics were found to be Amoxicillin (15.4%) and Gentamycin (15.4%).

Total isolation of *Proteus mirabilis* was 2.29% (n=4), which was 100% resistant to Cotrimoxazole; most efficient antibiotics were Amoxicillin and Ceftriaxone. Similarly 50% were resistant to Ciprofloxacin, Ofloxacin, Nitrofurantoin, Ceftazidime, and 25% *Proteus mirabilis* were resistant over Amikacin and Gentamycin. Out of these antibiotics Ceftazidime-clavunic acid didn't show anyresistant pattern. Total isolation of *Proteus vulgaris* was 1.14% (n=2). Which were 100% resistant to Amoxicillin, Cotrimoxazole and Ciprofloxacin. Ceftriaxone was effective against *Proteus vulgaris.*

Thus in UTI with *Proteus*, the urine becomes alkaline promoting stone formation and making acidification virtually impossible. The rapid motility of *Proteus* may also contribute to its invasion of the urinary tract (Brooks *et al,* 2004). *Proteus spp* is a common cause of UTI in boys and men and is associated with renal abnormalities, particularly calculi. In hospitalized patients, it may cause chronic UTI in association with obstruction or use of instrument (Leigh, 1990). Formation of infectious urinary calculi is the most common complication accompanying UTI by members of the genus *Proteus* supported by earlier studies (Li *et al,* 2002 and Torzewska *et al,* 2003).

In Gram positive isolates, among the *Staphylococcus aureus* most effective drug was found to be Ceftriaxone, and Cotrimoxazole. Whereas 50% susceptibility towards Ciprofloxacin, Ofloxacin, Norfloxacin, Nitrofurantoin and Penicillin.

Total isolation of *Enterococci spp* was 2.29% (n=4). 75% organisms were susceptible to Cotrimoxazole, Nitrofurantoin, Penicillin, Oxacillin and Amoxicillin. 50% organisms were susceptible towards Ceftriaxone and Norfloxacin and 25% organisms were susceptible towards Ofloxacin, Ciprofloxacin.

Total isolation of *Staphylococcus saprophyticus was* 1.14% (n=2) which was 100% resistant to Amoxicillin, Ofloxacin, whereas 50% organisms were susceptible to Nitrofurantoin, Norfloxacin, Ciprofloxacin and Penicillin. Ceftriaxone was effective antibiotics against *Staphylococcus saprophyticus* infection.

Total isolation of *Streptococccus spp* was *was* 1.14% (n=2). This genus were 100% susceptible to Cotrimoxazole, Penicillin, Oxacillin, Amoxicillin, and Ceftriaxone, but 100% resistant to Ofloxacin and 50% sensitive to Norfloxacin, Ciprofloxacin, and Nitrofurantoin.

MDR isolates were defined as those isolates resistant to three or more group of antimicrobial agents used in the study (Rijal *et al,* 2004). Among the 175 isolates that were evaluated against 14 antimicrobials, 7 were first line antibiotics whereas remaining were second line antibiotics according to laboratory policy. In this study, 48 % (84/175) bacteria were found out to be Multidrug resistant (MDR). Out of total 80 Gram negative isolates, 48.48% (80/165) were found to be multidrug-resistant and 40.0% (4/10) were

from Gram positive isolates. The lower prevalence of MDR, 13.9 % was found in the study done by Oteo *et al* (2001) when MDR criterion was resistance to 3 or more antibiotics.

In this study, total multiple drug resistant (MDR) cases were 48 % (84/175). Among total cases, MDR in *E. coli* were found to be 53.27% (57/107), *Proteus vulgaris* 100%, *Proteus mirabilis* 66.67%, *Pseudomonas aeruginosa* 83.33%, *Klebsiella pneumoniae* 44.44%, *Klebsiella oxytoca* 25%, *Enterobacter spp* 33.33% and *Citrobacter freundii* were 33.33% MDR positive.

Among Gram positive isolates *Enterococcus spp* was 75% and *Staphylococcus saprophyticus* was 50% MDR positive. The study done by Tuladhar *et al* (2003) at TUTH, MDR bacterial strains were detected in 35.2% cases in which the most predominant was *E. coli* (22.2%) followed by *Klebsiella* spp (6.1%) and *Staphylococcus aureus* (2.2%).

Outcome of prevalence of MDR depends on various factors, MDR criterion being the chief one followed by the types of antibiotics used in antibiogram and study population. The emergence of MDR is clearly related to the quantity of antibiotics and how they are being used (Levy, 1997).

We found that there was strong association between UTI and sex of patients. In this study, high number of patients who were diagnosed UTI positive belongs to the age group 20-40 years which were 60%% (105/175). In female age group 20-30 had highest number of growth, which was 22.86% (40/175), but 9.71% (17/175) in male as compared to female. This result supported by the study conducted by Steenberg *et al* (1985), Manandher *et al* (1996), Rajbhandari and Shrestha (2002), Regmi *et al* (2003), Shrestha *et al* (2005) and Jha and Bapat (2005), risk of developing UTI is high in females who are sexually active and are of childbearing age. Sexual activity has been considered as the major factor in the causation of UTI in women.

We found that there was also strong association between UTI and kidney transplantation status. It verifies the UTIs are the most common infections following renal transplantation. About 80.0% of patients with cellular rejection had a UTI, suggesting that UTI might trigger a graft rejection (Takai *et al*, 1998). UTI is an important cause of morbidity in renal transplant recipients. Around 50.0% of patients suffer from at least one episode of the infection during the first 6 months post transplant (Part V *et al*, 1985). About 20.0% of UTIs occurs during the first year of transplantation. Female recipients have significantly more UTI than males (Russel *et al*, 2000).

Higher rate of MDR was found in sample from Kidney transplanted patients 59.09% (13//22) than in Non kidney transplanted patients 46.41% (71/153). However, the association of MDR and non-MDR strains in Kidney transplanted patients and Non kidney transplanted patients was found statistically insignificant ($p>0.05$).

Thus from present study indicate large portion of bacteria were exposed to the antibiotics. Besides, large number of the bacterial isolates in the study showed multiple antibiotics resistance. The present study data gives idea about the common trend increased antibiotics resistance of uropathogens in UTI in this region, which may be due to geographic variation or indiscriminate or sublethal use of antibiotic. Previous reports have indicated that the high resistance of uropathogenic bacteria to antimicrobial agents in developing countries (Lester *et al*, 1990) is often due to self-medication, the suboptimal quality of antimicrobial drugs, and poor community and patient hygiene (Walson *et al*, 2001). Second, inappropriate use of antimicrobial agents is widespread as many people can easily buy antibiotics from some pharmacy stores and patent medicine stores, with or without prescriptions. This widespread and inappropriate use of antibiotics is recognized as a significant contributing factor to the spread of bacterial resistance and the development of resistance to antimicrobial agents (Mincey and Parkulo, 2001). Third, there is evidence that for most bacteria, increased usage of a particular antimicrobial agent correlates with increased levels of bacterial resistance to that agent (Granizo *et al*, 2000).

6.2 Conclusions

The study was carried out to isolate and compare the bacteria causing UTI in kidney transplanted and non transplanted patients and find out their antibiotic susceptibility pattern at National Public Health Laboratory, Teku.

The main findings of this study, 14.19% of the patients of urine showed growth of bacteria. Among 1233 urine sample, a total of 175 uropathogens belonging to 16 different species were isolated. Gram negative uropathogens 94.29% (165/175) were found predominant. Among Gram negative, *E.coli* was the major isolates. Gram positive uropathogens were 5.71% (10/175). Enterococcus spp were predominat. There is significant difference of positive growth between male and female patients ($P<0.05$) and this study shows the significant association between infection among Kidney transplanted and Non-transplanted patients ($p<0.05$). But there was no any significant association between MDR status between Kidney- transplanted and Non- transplanted patients. Ceftriaxone showed the susceptibility of 68.57% (120/175) among total isolares, followed by Nitrofurantoin 60% (105/175) in 1st generation antibiotics. In second generation antibiotics, Ceftazidime-clavunic acid and Amikacin showed the susceptibility of 90.91% (60/66), and oxacillin showed the susceptibility of 83.33% (5/6) among gram positive isolates. Multiple drug resistant (MDR) cases were 48 % (84/175), and MDR in *E. coli* were found to be 53.27% (57/107). Among 107 *E.coli* samples 57(53.27%) MDR, similarly *Proteus vulgaris* were 100% MDR positive.

CHAPTER-VII

7. SUMMARY AND RECOMMENDATIONS

7.1 Summary

1. Out of 1233 mid-stream urine samples, 14.19% (n= 175) were growth positive with significant number of bacteria and 85.81 % (1058/1233) samples showed no growth.

2. The infection rate was found to be higher in females101 (57.71%) than in males74 (42.29%). Association of significant bacteriuria and gender of patients was found to be statistically significant ($P<0.05$).

3. Out of total 439 sample from Kidney transplanted patients only 22 (5.01%) showed significant growth.

4. Out of total 175 uropathogens, 165 (94.29%) were Gram negative isolates whereas 10(5.71%) were Gram positive isolates.

5. Altogether 16 different bacteria were isolated from growth positive urine samples. *Escherichia coli* 107 (61.14%) was found the most predominant organisms followed by *Klebsiella pneumoniae* 22 (123.57%), *Klebsiella oxytoca* 9 (5.14%), *Acinetobacter spp* and *Pseudomonas aeruginosa* 6 (3.43%), Enterococci spp and *Proteus mirabilis* were 4 (2.29%), *Enterobacter spp and Citrobacter freundii* were 3 (1.71%) *Staphylococcus aureus, Staphylococcus saprophyticus, Proteus vulgaris* were 2 (1.14%), *Providencia spp, Edwardsiella spp Alkaligenes spp* were 1 (0.57%).

6. The most efficient first line antibiotic for isolates was found to be Ceftriaxone 68.57%, followed by Nitrofurantoin 60% and in second line antibiotics Ceftazidime –clavunic acid and Amikacin showed susceptibility of 89.55%.

7. In the total 175 uropathogens,48% (84/175)isolate were found to be MDR.In gram negative *E.coli* 53.27%(57/107) showed highest MDR and in Gram positive Enterococci spp 75%(3/4)showed highest MDR.

8. For the different factors assessed for the UTI culture positive patients, in age group 20-30 years was found to have high uropathogens positive patients. Sex of patients and infection status have strong association ($p<0.05$). Female of age group 20-30 was found high uropathogens positive patients 40 (22.86%), whereas male of age group 30-40have 24 (13.31%).

9. Transplantation status and infection status were found to have strong association ($p<0.001$).

7.2 RECOMMENDATIONS

1. Women of childbearing age may have chances to urinary tract infection so women of this age group should have regular antinatal check ups.

2. Kidney transplanted patients should continue the screening of UTI and strictly follow the recommended antibiotic treatment based on culture and sensitivity.
3. MDR screening procedure may provide appropriate antibiotic therapy for complete cure of infection.

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APPENDIX I

Questionnaire

Name of the patient: …… Date: …….

Age: …… Sex: ……

Specimen type: …… Blood pressure: …….

Clinical History: ……

Microbiological Investigation: ……..

1. Educational level a. Illiterate b. literate

2. Frequency of urination……time

3. Kidney transplant patients a. Yes b. No

4. Back pain a. Yes b. No

5. Fever a. Yes b. No

6. Time of onset of problem

7. Transplantation status a. Yes b. No

8. Antibiotics used a. Yes b. No

9. Yellowish color of urine a. Yes b. No

10. Pain in urination: a. Yes b. No

11. **Midnight urination** a. Yes b. No

12. Amount of water that you drink per

Day……..Litres

13. Blood in Urine a. Yes b. No

14. Reasons for hospital visit

a. Urinary tract infection b**.** Routine checkup **c.** Pregnancy d. Others

15. Previous such complication a. Yes b. No

APPENDIX II

Clinical and Microbiological profile of patient

Clinical Profile

Name of Patients…… Sex……..Lab No……Age: ….Adress…

Clinical History:

Patients on Antibiotics: a. Yes b. No

Immunosuppressive disease present a. Yes b. No

If yes, what: a. Kidney transplant patients' b. Diabetes c. Others

Symptoms: a. Dysuria b. frequency c. Fever d. Stomach pain e.Others

Microbiological Profile

Day1. Time of sample collection ……Specimen……..Method of sample

Microscopic observation: Color……Appearance…….Others

Direct Microscopic Observation

Wet mount Preparation of Centrifuged Urine

Observation	Number per HPF	Comments
Pus cells		
RBCs		

Culture of specimen on a. MacConkey Agar b. Nutrient Agar c. Blood agar

Incubation: a. Aerobic b. Anaerobic c. Microaerophillic

Incubation temperature and period……..

Day2. Reading of Culture Plates

Colony Characteristics on MacConkey Agar/Nutrient Agar

Media used	Shape	Size	Color	Texture	Haemolysis on BA	Lactose fermentation	Growth
MacConkey							
Nutrient Agar							

Gram-staining test:……..Catalase test:…….Oxidase test……Coagulase test:….

Provisional Identification of Organisms……..

Day2

Biochemical Tests: Results:

a.TSI……b.SIM……c. Citrate…….d. Urea Hydrolysis…..

e.MR…….f. VP…….

Organism Identified as:……

Antibiotic Sensitivity Test (Kirby-Bauer Method)

Antibiotic used	Zone of inhibition	Interpretation

Comments on Drug Resistance Pattern: MDR/Non MDR

Resistance to……..Number of Antibiotics.

Performed by

…….

Checked by

…….

APPENDIX III

Equipments and Materials used during the study

Equipments

Autoclave:	Sternite, Japan
Centrifuge:	Heltich, Japan
Distillation plant:	India
Refrigerator:	Sanyo Japan, LG Korea
Hot Air Oven:	Memmert, Germany
Incubator:	Sakura, Japan
Water bath:	Boekel 148003, Japan
Weighing balance:	Choyo MP, Japan
Microscope:	Olympus, Japan

Media used

Nutrient Agar

MacConkey agar

Nutrient agar

Mueller Hinton agar and Mueller Hinton broth

MR-VP medium

Simon citrate agar

BloodAgar

O/F Agar

Urea broth

Triple sugar iron agar

Sulfide Indole motility agar (SIM)

The entire medium used was from

1. Mast Company Limited UK
2. Oxoid Unipath Limited Hampshire UK

Chemical

H_2O_2	Oxidase
Kovac's Reagent	Methyl red
Barritt's Reagent	Barritt's Reagent B
Alpha-napthalamine	Bengall chemicals, India
Crystal Violate	Dehydrated alcohal
Hydrochloric acid	Iodine
Phenol red	Safranin

Test Organisms

E. coli (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923), *Klebsiella pneumoniae ATCC, Enterococcus faecalis* ATCC

Antibiotic discs	Ceftazidime(30 µg)
Ampicillin (30 µg)	Ceftazidime clavunic acid (30 µg)
Ciprofloxacin (5 µg)	Penicillin G (10 units)
Ofloxacin (5 µg)	Oxacillin (5 µg)
Norfloxacin (5 µg)	Cephipime (30 µg)
Ceftriaxone (30 µg)	
Nitrofurantoin (300 µg)	
Cotrimoxazole (25 µg)	
Cefotaxime (30 µg)	
Amikacin (15 µg)	
Gentamicin (15 µg)	

These entire antibiotic discs used were brought from Mast Co. Ltd. U.K.

APPENDIX IV

A. Composition and preparation of different media

1. MacConkey agar

Composition	gm/ltr
Peptic digest of animal tissue	17.00
Proteose peptone	3.00
Lactose	10.00
Bile salt	1.50
Sodium chloride	5.00
Neutral red	0.03
Agar	15.00
Final pH at 25ºC	7.1±0.2

51.3 gram of the medium was dissolved in 1000 ml of distilled water and then boiled to dissolve completely. The media was autoclaved at 121ºC for 15 mins. Sterilized medium was then poured in to sterile petridishes and was allowed to cool.

2. Nutrient agar

Composition	grams/litre
Peptic digest of animal tissue	5.00
Beef extract	1.50
Yeast extract	1.50
Sodium chloride	5.00
Agar	15.00
Final pH at 25ºC	7.4±0.2

28 gram of medium was suspended in 1000 ml of the distilled water and boiled to dissolve completely. Then medium was autoclaved at 121 ºC (15lbs pressure) for 15 min. the sterilized medium was then poured in to sterilized petridishes and then was allowed to cool.

3. Blood agar base (Infusion agar)

Composition	gm/ltr

Beef heart infusion form	500
Tryptose	10.00
Sodium chloride	5.00
Agar	15.00
Final pH at 25ºC	7.3±0.2

42.5 gram of the medium was suspended in 1000 ml of distilled water, dissolved by boiling and sterilized by autoclaved at 121ºC for 15 mins. After cooling to about 50-55 ºC, 5% v/v defibrinated sheep blood was added aseptically, then mixed with gentle rotation and poured in to sterilized petridishes and was allowed to cool.

4. Nutrient broth (NB)

Composition	gm/ltr
Peptic digest of animal tissue	5.00
Sodium chloride	5.00
Beef extract	1.50
Yeast extract	1.50
Final pH at 25ºC	7.4±0.2

13 gram of the medium was dissolved in 1000ml of water and then boiled to dissolve completely. The medium was then dispensed in to the tubes about 3ml in each and autoclaved at 121ºC for 15 minutes. The sterilized medium was then cooled to room temperature.

5. Mueller Hinton agar (MHA)

Composition	gm/ltr
Beef Infusion	300.00

Casein Acid Hydrolysate	17.50
Starch	1.50
Agar	17.00
Final pH at 25ºC	7.3±0.2

38 gram of the medium was dissolved in 1000ml of distilled water and then boil to dissolve completely. The medium was autoclaved at 121ºC for 15 mins. The sterilized medium was then poured in sterilized petridishes and was allowed to cool.

B. Composition and preparation of different biochemical test media

1. Simon citrate agar

Composition	gm/ltr
Magnesium sulfate	0.20
Mono ammonium dihydrogen phosphate	1.00
Dipotassium phosphate	1.00
Sodium citrate	2.00
Sodium chloride	5.00
Bromothymol blue	0.08
Agar	15.00
Final pH at 25ºC	6.8±0.5

24.2 gram of the medium was dissolved in 1000 ml of distilled water and boiled to dissolved completely.3 ml of medium was dispensed in each tube and autoclaved at 121ºC for 15 minutes. The sterilized mediums are allowed to settle at slant forming position.

2. Urea agar base (Christensen urea agar)

Composition	gm/ltr
Peptic digest of animal tissues	1.00
Dextrose	1.00
Monopotassium phosphate	0.8

Dipotassiu phosphate	1.20
Sodium chloride	5.00
Agar	15.00
Phenol red	0.012
Final pH at 25ºC	6.8±0.2

24 gram of the medium was suspended in 950 ml of water and dissolved by boiling and autoclaved at 121ºC for 15 minutes. After cooling to 50 ºC, 50 ml of sterile 40% urea solution was poured in to the medium and mixed with gentle rotation. Then 5 ml of the medium was dispensed in each tube and slant was prepared.

3. Sulfide indole motility (SIM) agar

Composition	gm/ltr
Peptic digest of animal	30.00
Beef extract	3.00
Peptonized iron	0.20
Sodium thiosulfate	0.025
Agar	3.00
Final pH at 25ºC	7.3±0.2

36.23 gram of the medium was dissolved in 1000 ml of distilled water and boiled to dissolve completely. Then it was dispensed in the test tube about 4 ml and autoclaved at 121 ºC for 15 minutes. Then it was cool down.

4. MR-VP medium

Composition	gm/ltr
Buffered peptone	7.00
Dextrose	5.00
Di- potassium phosphate	5.00
Final pH at 25ºC	6.9±0.2

17 gram of medium was dissolved in 1000 ml of distilled water and boiled to dissolve completely. 3 ml of medium was dispensed in each tube and autoclaved at 121ºC for 15 minutes.

5. **Triple sugar iron (TSI) agar**

Composition	gm/ltr
Peptic digest of animal tissue	10.00
Casein Enzymatic Hydrolysate	10.00
Yeast extract	3.00
Beef extract	3.00
Lactose	10.00
Sucrose	10.00
Dextrose	1.00
Sodium chloride	5.00
Ferrous sulphate	0.20
Sodium thiosulfate	0.30
Agar	12.00
Phenol red	0.024
Final pH at 25ºC	7.4±0.2

65 gram of the medium was dissolved in 1000ml of distilled water and dissolved completely. Then it was dispensed in to the tubes and autoclaved at 121ºC for 15 minutes. The sterilized medium in the test tube was then allowed to set in slant with a butt of 1inch thickness.

6. **Preparation of decarboxylation broth**

Composition	gm/liter
Yeast extract	5.00
Dextrose	1.00
Bromocresol purple	0.02
Final pH at 25ºC	6.8±0.2

Dissolve 14 gram of decarboxylation base powder in 1000 ml and 5 gram of required amino acid was added to it and boiled to dissolve. Then the broth was

dispensed in each tube about 5 ml and autoclaved at 121 ºC for 15 minutes. Then it was allowed to cool down and stored at 2-8 ºC until use.

C. Composition and preparation of different staining reagent

1. Gram stain

a. Crystal violet solution

Crystal violet	20.00
Ammonium oxalate	9.00
Ethanol or Methanol	95.00ml
Distilled water	1000ml

Preparation: 20 grams of crystal violet was weighed in a clean piece of paper and transferred to a clean brown bottle. Then 95 ml of ethanol was added and mixed until the dye is completely dissolved. To the mixture, 9 grams of ammonium oxalate dissolved in 200ml of distilled water was added. Finally the volume was made 1000ml by addition of distilled water.

b. Lugol's Iodine

Potassium iodide	20 gm
Iodine	10gm
Distilled water	1000 ml

Preparation: to 250 ml of distilled water, 20 gm of potassium iodide was dissolved and 10 gm of iodine was mixed to it until it was dissolved completely. Finally the volume was made 1000mlby addition of distilled water.

c. Acetone alcohol decolorizer

Acetone	500ml
Ethanol (Absolute)	475ml
Distilled water	25ml

Preparation: 475 ml of ethanol was added to 25 ml of distilled water and mixed and kept in a clean bottle. Then immediately 500ml of acetone was added to the bottle and mixed well.

d.Safranin (Counter stain)

Safranin (2.5% in 95% ethanol)	10.00 ml
Distilled water	100 ml

Preparation: 2.5% of Safranin solution was prepared in 95% ethanol and 10 ml of prepared suspension was mixed in 100 ml of distilled water.

e. Normal saline

Sodium chloride	0.85gm
Distilled water	100ml

Preparation: o.85 gram of sodium chloride was weighed and added to a bottle containing 100ml of distilled water and mixed well to dissolve the salt completely and autoclaved. Then it was stored.

2. Biochemical Test Reagents

a. For catalase test

Catalase reagent (3% H_2O_2)

Hydrogen peroxide	1ml
Distilled water	9ml

Preparation: To the 9ml of distilled water, 1ml of hydrogen peroxide was added and mixed well so as to make 3% solution of hydrogen peroxide.

b. For oxidase test

Oxidase strip soaked in oxidase reagent

Tetra methyl para-phenylene diamine dihydrochloride(TPD)

1gm

Distilled water

100ml

Preparation: 1 gram of TPD was dissolved in 100 ml of distilled water and strips of Whatmann no. 1 paper was soaked and drained for anout 30seconds. Then the strip was freeze dried and stored in dark bottle tightly.

c. For indole test

Kovac's indole reagent

Para Dimethyl amino benzaldehyde	2.00gm
Isoamyl alcohol	30.00ml
Concentrated hydrochloric acid	10.00ml

Preparation: in 30 ml of isoamyl alcohol, 2 gram of para amino bemzaldehyde was dissolved and transferred to clean brown bottle. Then to this solution,10 ml of concentrated hydrochloric acid was added and mixed well.

d. For methyl red test

Methyl red solution

Methyl red	0.05gm
Ethyl alcohol	28.0ml
Distilled water	22.0ml

Preparation: 0.05 gm of methyl red was dissolved in 28 ml of ethanol and transferred to a clean brown bottle. To this, 22 ml of distilled water was added and mixed well.

e. For Voges Proskauer test

Barritt's reagent

Solution A

Alpha-Napthol	5.0gm
Ethyl alcohol	100ml

Preparation: 5gm of α-Napthol was dissolved in 25 ml ethanol and transferred in to clean bottle. Then final volume was made 100ml by adding ethanol.

Solution B

Potassium hydroxide (KOH)	40.0gm
Distilled water	100ml

Preparation: 40 gram of KOH was dissolved in 25 ml of distilled water and transferred in to the clean bottle and final volume was made 100ml by adding distilled water.

f. Turbidity standard equivalent to McFarland 0.5

1% V/V solution of sulphuric acid was prepared by adding 1ml of concentrated sulphuric acid to 99 ml of distilled water. 1% W/V solution of barium chloride was prepared by dissolving 0.5 gram of dehydrate barium chloride in 50 ml of distilled water. Then to the 99.5ml of 1% sulphuric acid solution, 0.5 ml of barium chloride solution was mixed and stirred continuously. Then the solution was transferred in to the clean screw capped tube and stored at dark place until use. The test tube for the broth preparation should be of same size as of McFarland tube. The tubes can be stored and used for six months.

APPENDIX V

A. Procedure for gram staining (Forbes et al., 2007)

Gram staining is differential staining that differentiates all the bacterial species in to two large groups: gram positive and gram negative. Following steps are involved during gram staining.

1. A thin film of material to be examined was prepared and dried.
2. The material on the slide was heat fixed and allowed to cool before staining.
3. The slide was flooded with crystal violet stain and allowed to remain without drying for 10-30 second.
4. The slide was rinsed with tap water, shaking off excess.

5. Then the slide was flooded with iodine solution and allowed to remain on the surface without drying for twice as long as the crystal violet was in contact with the slide surface.
6. The slide was rinsed with tap water, shaking off excess.
7. The slide was flooded with acetone alcohol decolorizer for 10 seconds and rinsed immediately with tap water until no further color flows from the slide with the decolorizer. Thicker smear require more aggressive decolorization.
8. The slide was flooded with counter stain Safranin for 30 seconds and washed off with tap water.
9. The slide was blotted between two clean sheets of bibulous paper and examined microscopically under oil immersion at 100X.

B. Procedure for Antibiotic Sensitivity Testing (AST) by Disc Diffusion Method.

In the treatment and control of infectious disease, AST is done to select effective antimicrobial drugs against suspected organisms. Disc diffusion method is widely used technique for susceptibility testing and done by Kirby Bauer disc diffusion method.

The following steps are involved in AST by Kirby Bauer disc diffusion method.

1. An isolated colony of organism was suspended in the nutrient broth and incubated at 37ºC for 4 hours. The turbidity was matched with 0.5 McFarland turbidity standards.
2. A sterile cotton swab was taken and introduced in to the tube taken out the organism and swabbed uniformly on the surface of Mueller Hinton agar medium.
3. The plate was allowed to dry and antibiotic disc were placed on the agar surface and incubated for 18-24 hours.
4. After incubation the zone size was measured and results were interpreted according to the standard guidelines.

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 subculture 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 test.

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.

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

APPENDIX VI

Method of collection of mid-stream urine

It cannot be overemphasized that considerable importance is attached to the method of collection of urine specimens, transport to the laboratory and the initial efforts by the laboratory to screen and culture the urine. It is the responsibility of laboratory to provide patient with sterile, wide mouthed, glass or plastic jars, beakers or suitable receptacles. They should have tight-fitting lids or be covered with papers or foils prior to sterilization by dry heat or autoclaving.

Whenever possible, the first urine passed by the patients at the beginning of the day should be sent for examination. This specimen is the most suitable for culture. Microscope and biochemical analysis.Mid-stream

Women who are ambulatory should:

Wash her hands thoroughly with soap and water and dry them with clean towel.

Undress in suitable room, spread the labia and cleanse the vulva and labia thoroughly using sterile cotton gauze pads and warm soapy water wiping from front to rear. Rinse thoroughly with warm water and dry with a sterile cotton gauze pad. During the entire process the patient should keep the labia separated and not touch the cleansed area with fingers. Pass urine, discarding the first part of the stream. Collect the remaining urine in the sterile container, closing the lid as soon as the urine has been collected. Hand the clean-catch midstream urine, in the closed container, to the health personnel for delivery to the laboratory.

Men who are ambulatory should:

1. Wash his hands thoroughly with soap and water and dry them with a clean towel

2. Pull back the fore skin (if not circumcised) and wash the glans thoroughly using sterile cotton gauze pads and warm soapy water.

3. Rinse the glans thoroughly with warm water and dry with a sterile gauze pad.

4. Pass a small amount of urine. Still holding back the foreskin, the patients should pass most of remaining urine into a sterile container. This is midstream urine specimen.

5. Place the cover on the container and hand to the nursing staff for prompt delivery to the laboratory.

Infants and children

Collection of a clean-catch urine specimen from infants and children who are ill in bed or uncooperative can be a problem. Give the child water or other liquid to drink. Clean the external genitalia. The child can be seated on the lap of the mother, nurse or ward attendant, who should then encourage the child to urinate and collect as much urine as possible in sterile container. The container should then be covered and delivered to the laboratory for immediate processing.

For bedridden patients

The same procedure is followed, except that a nurse must assist the patient or, if necessary do the entire cleansing procedure before requesting the patients to pass the urine.

In both situations every effort must be made to collect a clean-catch urine specimen in a sterile container and to ensure that it is delivered promptly to the laboratory together with information on the patient, clinical diagnosis and requested procedures.

APPENDIX VII

Antibiotic susceptibility pattern of uropathogens

Organisms isolated	Total Resistance														
	Total	A	CRO	OF	CIP	NOR	TS	NF	CTX	CTX-CV	AK	G	P	OX	CPM
Enterobacter spp	3	1	1	1	1	1	1	1	0	0	0	0	0	0	0
Acinetobacter spp	6	3	1	0	0	0	3	2	1	1	0	1	0	0	1
Alkaligenes spp	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0
Citrobacter freundii	3	3	0	2	1	1	2	3	0	0	0	1	0	0	0

E.coli	107	77	36	54	57	60	68	34	26	5	4	22	0	0	15
Edwardsiella spp	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Enterococci spp	4	1	2	3	3	2	1	1	0	0	0	0	1	1	0
Klebsiella oxytoca	9	5	2	3	4	2	3	1	1	0	0	1	0	0	0
Klebsiella pneumoniae	22	18	7	7	8	9	10	14	5	1	0	4	0	0	4
Proteus mirabilis	4	4	1	2	2	1	4	2	1	0	1	1	0	0	0
Proteus vulgaris	2	2	0	1	2	1	2	1	0	0	0	0	0	0	0
Pseudomonas aeruginosa	6	6	5	4	4	5	4	3	4	1	1	5	0	0	2
Staphylococcus aureus	2	1	0	1	1	1	0	1	0	0	0	0	1	0	0
Staphylococcus saprophyticus	2	2	0	2	1	1	0	1	0	0	0	0	1	0	0
Streptococcus spp	2	0	0	2	1	1	1	1	0	0	0	0	0	0	0
Providencia spp	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	175	125	55	82	85	85	100	65	38	8	6	35	3	1	22

APPENDIX VIII

MORPHOLOGY AND CULTURAL CHARACTERISTICS OF BACTERIA ISOLATED FROM URINE SAMPLE

Bacteria	Morphological Characteristics	Cultural Characteristics
Escherichia coli	Gram negative rod of 1-3µm×0.4-0.7µm size, aerobic and anaerobic, nonsporing, motile, noncapsulated	On BA: Large 1-4 mm in diameter, grayish white, moist, smooth, convex and opaque. The colonies may appear mucoid and some strains are hemolytic. On MA: Bright pink colonies due to lactose fermentation, smooth, glossy and translucent.
Klebsiella spp.	Gram negative, short and thick rods of 1-2µm × 0.8 µm in size, nonsporing,	Large dome shaped moist and usually viscid or mucoid colonies when

	nonmotile and capsulated.	cultured on BA and MA. Most Klebsiella species are lactose fermenting.
Enterobacter spp	Gram negative rods, non sporing, non capsulated.	About 2 to 3 mm in diameter, moist, yellowish coloured, LF, motile organism.
Pseudomonas aeruginosa	Gram negative slender rod of 1.5-3µm×0.5 µm size, nonsporing, motile with a single polar flagellum, most strains produce slime, strict aerobe.	Six different colonial types of *Pseudomonas aeruginosa* are encountered(Philips, 1969).Type 1 is the most common: colonies are large, low convex, rough in appearance and often oval. Type 2 colonies are small; dome shaped and is described as coliform like. Colony 3 and 4 are small and appear rough and rugose respectively. On BA: Large flat colonies showing haemodigestion. On MA: Pale, nonlactose fermenting, colourless translucent colonies.
Proteus spp	Gram negative rod of 1-3 µm×0.4-0.6µm size, noncapsulated.	On BA: when cultured aerobically, most strains are swarming type and have a characteristics fishy odour. On MA: *Proteus spp* produces individual non lactose colonies.
Enterococcus *spp*	Gram positive, spherical cocci, occurring in pairs or short chains. They are non capsulated and majority are non motile	
Citrobacter freundii	Gram negative, non sporing, noncapsulated, motile rods.	Smooth, convex 2-4 mm colonies, sometimes rougher mucoid forms occur. On BA: Non-hemolytic On MA: Non-lactose fermenting colonies.
Staphylococcus aureus	Gram positive, spherical cocci, 0.8-1 µm in diameter, non sporing,	On BA: Large, 2-4 mm diameter, circular, smooth with glistening surface,

	facultative anaerobe, non-motile, except for rare strains, non capsulated.They are arranged in characteristics grape like clusters or in small groups, pairs, singles and short chain(less than five cocci in line)	entire edge, soft butyrous consistence and opaque and pigment appearance. The pigmentation is golden yellow to cream coloured.Some strains are Beta haemolytic when grow aerobically. On MA: Small(pin head size), 0.1-0.5 mm,pink or pink orange due to lactose fermentation. Some strains are non Lactose fermenting.

APPENDIX IX

Interpretation charts for antibiotics

A. Zone size interpretative chart (CLSI interpretation)

Antibiotic used	Concentration (µg)	Diameter of zone size(mm)			ATCC culture *E.coli* 25922 target zone size (mm)
		Resistant	intermediate	Sensitive	
Ampicillin	10	≤ 13	14-16	≥ 17	16-22
Ciprofloxacin	5	≤ 19	20-21	≥ 22	30-40
Ofloxacin	5	≤ 19	20-21	≥ 22	29-33
Nalidixic acid	30	≤ 13	14-18	≥ 19	22-28
Chloramphenicol	30	≤ 12	13-17	≥ 18	21-27
Ceftriaxone	30	≤ 13	14-20	≥ 21	29-35
Cefotaxime	30	≤ 14	15-22	≥ 23	29-35
Erythromycin	15	≤ 13	14-22	≥ 23	22-30
Amikacin	15	≤ 13	14-15	≥ 16	19-26
Gentamicin	15	≤ 14	15-16	≥ 17	19-26
Furazolidone	100	≤ 18		≥ 18	22-26

Cotrimoxazole	25	≤ 10	11-15	≥ 16	24-32
Tetracycline	30	≤ 14	13-18	≥ 19	18-25
Polymyxin B	300	≤ 11		≥ 11	13-19

B. Zone size interpretative chart (EUCAST interpretation)

Antibiotic used	Concentration (µg)	Diameter of zone size(mm)			ATCC culture *E.coli* 25922 target zone size (mm)
		Resistant	intermediate	Sensitive	
Ampicillin	10	≤ 14			16-22
Ciprofloxacin	5	≤ 19	20-21	≥ 22	30-40
Ofloxacin	5	≤ 19	20-21	≥ 22	29-33
Nalidixic acid	30				
Chloramphenicol	30	≤ 17		≥ 17	21-27
Ceftriaxone	30	≤ 20	21-22	≥ 23	29-35
Cefotaxime	30	≤ 18	19-20	≥ 21	29-35
Erythromycin	15				
Amikacin	15	≤ 13	14-15	≥ 16	19-26
Gentamicin	15	≤ 14	15-16	≥ 17	19-26
Furazolidone	100				22-26
Cotrimoxazole	25	≤ 13	11-15	≥ 16	24-32
Tetracycline	30				
Polymyxin B	300				

APPENDIX X

5.22. Association between sex of patients and infection status:

A. Chi- square test of sex of patients and infection status

Infection status	Sex of Patients		Total	p-value
	Male	Female		
Non infected	587	471	1058	p=0.001
Infected	74	101	175	
Total	572	661		

5.23. Association between transplantation status and infection status of patients:

B. Chi- square test of transplantation status and infection status of patients

Infection status	Transplantation status	Total	p-value

	Transplanted	Non transplanted		
Non infected	417	641	1058	p=less
Infected	22	153	175	than
Total	439	794	1233	0.001

5.24. Association between MDR and sex of patients' status:

C. Chi- square test of Transplantation status and MDR status

MDR	Sex of patients		Total	p-value
	Transplanted	Non transplanted		
Yes	13	71	84	p=0.265
No	9	82	91	
Total	22	153	175	

Between sex of patients and infection status, strong association was found (p=0.001), similarly between Transplantation status and infection status, there was also strong association was found (p=less than 0.001), but no association was found between MDR and transplantation status of patients (p=0.265).