CHAPTER-I
INTRODUCTION

1.1 Background

Various structural, functional and physiological factors of urinary tract establish it as ecological niche and plays important role in the genesis of infection. Urine can promote the growth of bacteria as its composition includes a variety of nutrients such as glucose, amino acids and uric acid and hence act as a good bacterial broth. Also, the physiological values of pH and osmolarity allow rapid bacterial growth (Schwan *et al*., 2002).

Urinary Tract Infection (UTI) is described as the microbial invasion of any tissues of the urinary tract and is one of the most common infectious diseases which have been most extremely studied in the field of clinical practice (Behzadi *et al*., 2010; Dulawa, 2004). Urinary tract infection is clinical condition ranging from asymptomatic presence of bacteria in the urine to severe infection of the kidney (Nguyen, 2004). It is one of the most common bacterial infections encountered by clinicians in developing countries (Tessema *et al*., 2007).

Urinary tract infection refers to organisms growing within and damaging the urinary tract. However, in clinical practice, it is defined in relation to the number of bacteria in a voided urine sample. Significant bacteriuria occurs if equal to or greater than 10^5 of the same organism is present per ml of urine. Symptomatic bacteriuria is defined as presence of 10^2 or more coliform organisms per ml of urine plus pyuria, 10^5 or greater number of other pathogens per ml, or any growth of pathogens from a supra pubic aspirate of urine (MacLean, 2001).

Physiological factors that help bacteria to thrive in urinary tract include dysfunctional voiding, infrequent voiding, incomplete bladder emptying and constipation. In constipation stool remains in the rectum for a long period of time and bacteria tends to colonize in the perineum that poses increased risk for UTI (Dulczak and Kirk, 2005). The pathogenicity of bacteria in UTI is

influence by bacterial factors like bacterial adhesion and motility and host factors like immune response and genetic factors (Heffner and Gorelick, 2008).

Despite the presence of several antibacterial factors such as pH, urea concentration, osmolarity, various organic acids, salt content of the urine, urinary inhibitors to bacterial adherence e.g. Tamm-Horsfall protein (THP), bladder mucopolysaccharide, low-molecular weight oligosaccharides, secretory IgA and lactoferrin, the uropathogenic bacteria are able to adhere, grow and resist against host defenses. This finally results in colonization and infection of the urinary tract (Cunha, 2009; Dulawa, 2004; Fihn, 2003).

Uropathogens causing UTI as well as asymptomatic bacteriuria (ASB) emanate from the distal gut, colonize the vagina and ascend to the bladder via the urethra (Foxman, 2002). The vast majority of uncomplicated UTI is caused by Gram negative bacillus (Blondeau, 2004).

UTIs are the second most common infection and a significant cause of morbidity in the United States accounting for about 8.1 million visits to health care providers each year (Langermann *et al*., 2000; Schappert *et al*., 2008). It has been estimated that there are 175 million cases of UTI worldwide each year (Johnson and Russo, 2003).

The most prominent bacterial pathogens causing urinary tract infection are *Escherichia coli, Enterococcus* spp., *Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae, Candida albicans, Enterobacter* spp., and coagulase-negative *Staphylococcus*. However, uropathogenic *E. coli* (UPEC) strains are the primary causative agents that cause upto 90% of UTIs (Horvath *et al*., 2012).

Uropathogenic bacteria are posing a serious threat to the safety and proper functioning of health care facilities, as they are being resistant to multiple antibiotics (Watts *et al*., 2010). Protracted use of antibiotics can damage periurethral flora allowing uropathogens to colonize and infect the urinary

tract (Nguyen, 2004). Fluoroquinolones are drugs of choice for UTI in the cases of resistant to other drugs (O'Donnell and Gelone 2000; Schaeffer, 2002). Ciprofloxacin is reliable antibiotic to treat uropathogens and is also available in both oral and intravenous formulations so it is the most frequently prescribed Fluoroquinolone. Ciprofloxacin has shown an excellent activity against pathogens commonly encountered in complicated UTIs (Astal, 2005).

Asymptomatic bacteriuria (ASB) is commonly defined as the presence of more than 100,000 organisms/ml in 2 consecutive urine sample in the absence of declared symptoms. Untreated ASB is a risk factor for acute cystitis (40%) and pyelonephritis (25-30%) in pregnancy. These cases account for 70% of all cases of symptomatic UTI among unscreened pregnant women (Johnson and Kim, 2012).

It is thought to be present in 3%–5% of young healthy women and is more common in patients with diabetes mellitus (DM) and elderly persons (Raz, 2003). In a study conducted by Renko *et al*. **(**2011), the prevalence of ASB was three times higher in all patients with DM compared with control subjects.

Eighty to ninety percent of women experience UTI more than once in their life time and 5-10% of these women experience recurrent UTI. Recurrent urinary tract infection is defined as greater than three UTI's per year. Most women with recurrent UTI's have reinfection, while a minority (5-10%) have relapse. About 20 percent of young women with a first UTI will have a recurrent infection (Tolkoff-Rubin *et al*., 2008).

Incidence of infection is elevated in diabetic patients (Meiland *et al*., 2002). An association between UTI and DM was noted in a autopsy series reported in 1940. UTI is more widespread in women with DM than in non diabetic women as a consequence of debilitated immune system (Mehvish and Betty, 2011).

Asymptomatic bacteriuria was more prevalent among women with DM (26%) than in women without diabetes (6%). Diabetic patients should be given

special attention for management of UTI since they are at high risk of developing UTI and other risk factors for UTI include sexual intercourse, age, duration of diabetes, glycemic control, and complications of diabetes (Janifer *et al*., 2009).

Women are especially prone to UTIs for anatomical reason that their urethra is shorter, allowing bacteria quicker access to the bladder. Also, a woman's urethral opening is near to the sources of bacteria from the anus and vagina posing life time risk of having UTI greater than 50% (Griebling, 2007). UTI is uncommon in men and contributes to have larger complications after initial infection (Mehvish and Betty, 2011).

Nearly 50% of women experience at least one urinary tract infection (UTI) in their lifetime (Garofalo *et al*., 2007). Women with DM are about two to three times more likely to have bacteria in their bladders than women without DM. There also seems to be an increased risk of the infection spreading upwards into the kidneys in diabetic patients and diabetic women with urinary tract infections are also more likely to require hospitalization than non diabetic women (Harding *et al*., 2002).

The most common cause of UTI in men and women with or without DM is *E. coli.* The most common infecting organism in asymptomatic bacteruria women with diabetes is *E. coli* and other organisms include *Klebsiella* spp., *Enterobacter* spp. and Group B *Streptococcus* (Bonadio *et al*., 2006).

Not all the people have equal chances of catching UTI. Thus, peculiar group of people who are more liable to catch UTI include female, elderly peoples, postmenopausal women, diabetes patients etc. Apart from this age, level of education, use of insulin etc are the other risk factors for UTI. Dearth of information is accessible regarding the association between these risk factors and UTI. This study was hence brought up in mind with connotes to assess various risk factors associated with UTI. In this sense, this study will be beneficial to point out the predisposing factors of UTI and to alarm the people at high risk to take early precaution so as to avert critical outcomes. Also, this

surveillance of UTI will put forth appropriate therapy by purveying correct knowledge of the organisms that causes UTI and their antibiotic susceptibility pattern. Hence this study will also make possible to identify risk factors and to formulate strategies focused on particular risk factors.

1.2 OBJECTIVES

General objective

To assess the risk factors of UTI

Specific objectives

1. To determine the prevalence of UTI in diabetic patients
2. To identify microorganisms that causes UTI in diabetic patient and non diabetic patients
3. To perform antibiotic susceptibility pattern of the isolates
4. To describe relationship between UTI and demographic variables (age, sex, education etc)

CHAPTER-II
LITERATURE REVIEW

2.1 Urinary tract infection

Normally, the urinary tract is sterile but urinary tract infections can be caused by variety of conditions (Behzadi *et al*., 2010). UTI is a condition in which bacteria establish and multiply within the urinary tract and is most common infection occurring after respiratory and gastrointestinal tract infections. UTI may be either community acquired or hospital acquired (Najar *et al*., 201l). UTI is diagnosed if at least 10^5 organisms is present in 1 ml of urine in an asymptomatic patient or as more than 10^2 organisms in 1 ml of urine with accompanying pyuria (>7 WBC/ml) in a symptomatic patient (Johnson and Kim, 2012).

The diagnosis of UTI is done by testing a sample of urine for pus and bacteria. Patients are instructed to collect mid-stream clean catch urine after washing their genital area (Lafi, 2010). Since the organisms responsible for the UTI are the commensals of perianal and vaginal regions the important way to avert UTI is to focus on personal hygiene (Kolawole *et al*., 2009)

Clinically UTI can occur on different portion of the urinary tract. It can be caused by different etiologic organism(s) and with different severity of the infection ranging from dysuria, organ damage to death of the patient due to pyelonephritis (Foxman and Brown, 2003; Mehvish and Betty, 2011). Signs and symptoms may include fever, chills, dysuria, urinary urgency, frequency and cloudy or malodorous urine. Infections are almost always ascending in origin and caused by bacteria in the periurethral flora and the distal urethra. These bacteria inhabit the distal gastro-intestinal tract and colonize the perineal area (Sibi *et al*., 2011).

Symptomatic and Asymptomatic UTI

UTIs may be symptomatic (e.g., cystitis and pyelonephritis) or asymptomatic (Watts *et al*., 2010). Infection that clinically apparent are called symptomatic infection and such infection make patient to visit doctor. Asymptomatic

infections go unnoticed due to lack of any clinical symptoms and signs. Symptomatic and asymptomatic bacteriuria are almost equally distributed, ranging from 40% - 50% (Jha *et al*., 2009). Diabetic patients encounter urinary urgency and incontinence, during night (Mehvish and Betty, 2011).

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

Recurrent UTI

Recurrent UTI refers to 3 or more episodes of UTI within the 12 months, or 2 UTIs within the 6 months (UMHS Urinary Tract Infection Guideline, May 2005). Recurrent UTI is a serious and menacing problem, high proportion of which is caused by the same strain of bacteria as initial infection (Rosen, 2010). Recurrent UTI infection is present in women those who have greater adherence of uropathogens to uroepethelial cells (Hooton, 2001). It is estimated to affect 25% of women with a history of UTI (Garofalo *et al*., 2007).

Complicated UTI

Complicated UTI is a clinical syndrome with systemic and local signs and symptoms of fever, chills, malaise, flankpain, back pain, and CVA (costovertebral angle) pain or tenderness along with functional or anatomical abnormality of the urinary tract. UTIs are complicated in the cases of diabetes, or in the context of structural or functional abnormalities of the urinary tract including stents and catheter (Tabibian *et al*., 2008).

Cystitis and Pyelonephritis

UTI in lower tract i.e. in the bladder and/or urethra is called cystitis and urethritis respectively. Similarly upper tract infection, located in the ureters, collecting system, and parenchyma of kidney is called pyelonephritis (Heffner and Gorelick, 2008). Cystitis is recognized by dysuria, frequency, urgency, malodorous urine, enuresis, hematuria, and suprapubic pain and

pyelonephritisis recognized by fever over 38.5 °C, chills along with costovertebral angle or flank pain and tenderness with pyuria (Dulczak and Kirk, 2005; Ramadan, 2003).

2.2 Risk factors of UTI

The population which are at more risk of urinary tract infection includes newborn (including the premature), mature girls, sexually active females and elderly females (Salih, 2011). Various factor such as age, duration of being diabetic, glycemic control, and complications of DM are also associated with UTI (Janifer *et al*., 2009). In a study by Lafi *et al*. (2010), the majority of the UTI patients were females (74%) and minority were males (26%).

2.2.1 Dietary factors

Dietary factors that alter the properties of the faecal bacterial flora may affect the risk of getting UTI. Cranberry and cranberry-lingoberry juice are found to be useful in treating UTI (Kontiokari *et al*., 2003).

2.2.2 Marital status

In a study by Lafi *et al*. (2010) majority (82%) of positive UTI cases were married and low percentage (18%) of positive UTI cases were single. In another study the proportion of UTI in married women were almost double (65.6%) than that of unmarried women. The high sexual activity, pregnancy and delivery predispose women of reproductive age group to urinary tract infection (Salih, 2011).

2.2.3 Postmenopause

Urinary tract infections (UTI) occur frequently in postmenopausal women (Hu *et al*., 2004). In postmenopausal women the level of estrogen hormone is abated due to which these women have less acidic pH of vagina that inhibits vaginal flora (*Lactobacillus*) and thus poses risk for recurrent UTI (Geerlings *et al*., 2003; Hooton, 2001).

2.2.4 Post transplant

UTI is the most common infection following renal transplantation (approximately 44–47%) (Alangaden *et al*., 2006; Dantas *et al*., 2006). The predisposing factors of UTI in post transplant include immunosuppressive medication, diabetes, bladder catheters and ureteral stents (Papasotiriou *et al*., 2011).

2.2.5 Pregnancy

All women should be screened twice during pregnancy for asymptomatic bacteriuria since 1-4% women will develop acute cystitis for the first time whilst pregnant and 1-2% will develop pyelonephritis and all bacteriuric patients should be treated for 7 days, with follow up cultures to identify relapses (Najar *et al*., 2011; Olen *et al*., 2007). Various hormonal and physiological changes in pregnant women increases urine statist and renders them susceptible to UTI. Apart from this they are also considered immunologically compromised (Ciesla, 2007; Johnson and Kim, 2012).

2.2.6 Blood Group

Genetic circumstances such as belonging to blood groups AB or B, constitute independent risk factors in some but not all studies (Kinane *et al*., 1982). *Escherichia coli* strains that colonize the human urinary tract expresses lectins specific for globotetraosylceramide and globo-A and expression of the globo-A receptor was restricted to individuals with blood group A (Lindstedt *et al*., 1991). In the study conducted by Sakallioglu and Sakallioglu (2007), 36% cases with A positive blood group were revealed UTI positive.

2.2.7 Age

UTI is age dependent and bacteriuria is more common at the extremes of life, during the first year of life and during adolescence. Age had no significant relation with ASB (Boroumand *et al*., 2006). However, Salih (2011) concluded in his study that higher proportion (37.7%) of UTI cases belonged to age group 20-29 years, followed by 30-39 years (26.3%) and least proportion (18.03%) of UTI cases belonged to age group 11-19 years and >40.

Similar results were also shown in a study by Kolawole *et al*. (2009) where both male and female had high prevalence of UTI in age 21-25 years and 26-30 years. In a study by Shakya *et al*. (2012), female belonging to age group 20-40 years were more prone to UTI while high prevalence of infection was found in male of age group 60-80 years and above.

However, in case of diabetic patients, 42.1% infection was found in patients above 60 years in a study by Saber *et al*., 2010 and 67.8% and 61% infection was found in male and female >55 years respectively in a study by Janifer *et al*. (2009).

2.2.8 DM and its duration

UTIs are a common burden in patients with DM (Hakeem *et al*., 2009). In addition, Boyko *et al*. (2002) found no association between risk of getting UTI and whether DM was present for ≤10 or ≥10 years. However, higher risk of UTI and asymptomatic bacteriuria were seen only among women with a longer duration of DM (Boyko *et al*., 2005).

In another study, no significant relation between bacteriuria and duration of being diabetic was found. Among patients with less than 10 years of diabetes, 10.1% and among patients with 10-20 years diabetes, 20.6% (n = 7) had bacteriuria but among the 13 patients who had diabetes of more than 20 years duration, none had bacteriuria (Boroumand *et al*., 2006).

2.2.9 Insulin therapy

According to a study by Boyko *et al*. (2005), diabetic patients with insulin therapy are at significantly higher risk of UTI compared to those with oral medication (relative risk = 2.9) since diabetic patients treated with insulin are at higher risk of urge incontinence than their counterparts treated with oral medications or diet. In other words, patients treated with insulin are suffering from complicated diabetes and thus are at higher risk of UTI (Al-Rubeaan *et al*., 2012).

2.2.10 $HbA1_c$

Boroumand *et al*. (2006) found no significant association between bacteriuria and HbA1c levels (p = 0.75). 57.1 % (n = 12) of the patients with HbA1c levels of less than 8 had bacteriuria, and 42.9% (n = 9) of the patients whose HbA1c levels were 8 or more, had also developed bacteriuria. However, according to another study by Geerlings *et al*. (2002), *E. coli* with type 1 fimbriae has increased adherence to uroepethelial cells in patients with poorly controlled $HbA1_{c.}$

Other predisposing factors of UTI includes genetic predisposition, behavioural factors, urologic structural abnormalities, immune-suppression, hypertension, stone formation, nosocomial acquired infections and instrumentation like catheterization (Behzadi, *et al*., 2010; Behzadi and Behzadi, 2008; Dulawa, 2004; Hooton, 2000).

Host factors such as the epithelial cell receptivity are also important in the onset of infection (Moura *et al*., 2009). *E. coli* binds to vaginal epithelial cells from healthy controls less avidly than to vaginal epithelial cells from women with recurrent UTI. Vaginal cell receptivity also varies as a function of hormonal status. Bacterial adherence tends to be higher earlier in the menstrual cycle and in postmenopausal women as compared with the pre-women or postmenopausal women who are on estrogen replacement therapy predisposing them to recurrent UTI (Cohn and Schaeffer, 2004; Franco, 2005).

2.3 Epidemiology

In a study by Adeyeba *et al*. (2007) in Ibadan Nigeria, prevalence of UTI in diabetic patients was 21%, much higher than that of the healthy controls (5%). Out of diabetic patients 61.9% female and 38.1% male were infected. While out of healthy volunteers 58% male and 62% female were infected.

Among 636 elderly diabetic patients, in a study conducted by Jha *et al*. (2009), bacteriuria was seen in 60 patients. The prevalence of ASB among diabetics above 40 years age in Chitwan seemed to be 9.43% with higher incidence ASB in female (12.7%) than that in male diabetic patients (5.8%).

Probability of UTI is 70-80% with symptoms of either urgency or frequency. There is probability of 25% of having UTI either if dysuria is present alone as symptom of UTI or if vaginal symptoms is present along with urinary symptoms. Back pain and previous history of UTI have also been shown to increase the likelihood of UTI. Other symptoms which probably increase likelihood of UTI include urinary urgency, new urinary incontinence, voiding of small volumes, suprapubic pain, and nocturia. Generally, UTI symptoms are of abrupt onset (< 3 days) (UMHS Urinary Tract Infection Guideline, 2005).

2.4 DM and UTI

Diabetic mice have increased susceptibility to UPEC infection and higher bacterial burden than non diabetics. Also, diabetics are infected with a broader range of uropathogens, and more commonly develop serious UTI sequel than non diabetics (Rosen *et al*., 2008).

Asymptomatic bacteriuria, acute pyelonephritis and complications of UTIs are reported to be more common in patients with DM (Lukman *et al*., 2009). Various factors have been implicated in the higher prevalence of asymptomatic bacteriuria and incidence of UTIs in patients with DM compared with patients without DM. These factors include differences in host responses between DM and non diabetic patients, difference in the infecting bacterium itself, the presence of glucosuria and impairment of granulocyte function and higher postvoid residual bladder volume among women with diabetes (Geerlings, 2008; Yu *et al*., 2004). Presence of DM has been shown to enhance the frequency of recurrent UTI by two to threefold (Franco, 2005).

UTIs are more common among women with diabetes and are routinely associated with incontinence (Jackson *et al*., 2005). All the case of ASB was detected from the patients having fasting blood sugar (FBS) more than 126 mg/dl (Jha *et al*., 2009). In the absence of confounding factors, insulin-treated diabetes occurs as a risk factor for UTI (Boyko *et al*., 2002; Brown *et al*., 2001).

2.5 Physiology and immunity of diabetic patient

There are several reasons for an increased frequency of UTIs in diabetic patients (Lukman *et al*., 2009). Due to less urinary leukocyte cell count in diabetic patients, they have lower urinary concentration of cytokines (IL8 and IL6) which may contribute to the increased incidence of UTIs in this patient group (Geerlings *et al*., 2000; Hoepelman *et al*., 2003).

In addition, abnormal intracellular calcium metabolism that results into lowered Tamm Horsfall protein and granulocyte dysfunction in diabetic patients causes increased adherence of uropathogens to uroepithelial cells (Baqui *et al*., 2008; Sahib, 2008).

Poor circulation of blood in diabetes reduces ability of infection fighting WBC cells either to get to their targer site or to ingest offending bacteria and kill them than the normal WBC (Adeyeba *et al*., 2007). In addition, a higher glucose concentration in the urine may create a culture medium for pathogenic microorganisms (Boyko *et al*., 2005).

2.6 Pathogenesis and virulence factor of organisms causing UTI

The genesis of urinary tract infection occurs from the pathogens in the bowel or sometimes from the vagina during sexual intercourse. These pathogens reach bladder through urethra and in some cases they ascend more upward through ureters to reach kidney (Hooton, 2012).

Studies with mice have revealed that uropathogenic *Escherichia coli* (UPEC) isolates invade cells that line the bladder and get protected from innate immunity. They are not only protected but are also replicated rapidly forming distinctive intracellular bacterial communities (IBCs) with each IBCs comprised of ~10^4 bacteria (Garofalo *et al*., 2007; Schwartz *et al*., 2011).

E. coli expressing type-1 fimbriae (a virulence factor) have increased adherence to uroepithelial cells of diabetic women hence plays important role in the pathogenesis of UTI, especially if diabetes is poorly controlled (Geerlings *et al*., 2002).

UTI in women develops after colonization of the vaginal and periurethral epithelium by the infecting organisms (Wullt *et al*., 2003). Motility mediated by flagella and pili appears to be important for the ascension of bacteria in urinary tract (Lane *et al*., 2007; Wright *et al*., 2005). Also, a study conducted by Wullt *et al*. (2002) suggested that fimbrial expression in the human urinary tract plays a key role for bacterial establishment and disease induction.

E. coli has different virulence determinants such as adhesin (type 1, P and S fimbriae and a fimbrial adhesin), which bind to specific molecules in the uroepithelium. Additional virulence factors of *E. coli* associated with UTI include hemolysin, polysaccharide, cytotoxic necrotizing factor, siderophores and others (Johnson, 2003). Strain pathotypes characterized by sets of virulence factors facilitate the pathogenesis processes and are important determinants of uropathogenicity (Bekal *et al*., 2003; Guy, 2006; Johnson and Russo, 2005; Marrs *et al*., 2005).

On comparison to faecal isolates, UPEC (Uropathogenic *E. coli*) isolated from patients with UTI possess more virulence factors (Johnson *et al*., 2005). UPEC typically carry large blocks of genes, called pathogenicity-associated islands (PAI), not generally found in commensal faecal isolates and are known to contribute to the pathogenicity of bacteria and their resistance to antibiotics (Guyer *et al.*, 2002; Hacker and Kaper, 2000; Johnson and Russo, 2005).

Organisms achieve resistance by producing beta lactamases, which destroys the antibiotics by blocking the entry of these antibiotics, or by efflux pump which actively pump out these antibiotics (Prais *et al*., 2003).

2.7 Organisms causing UTI

As Gram positive organisms play lesser role in UTIs, the predominant number of pathogens isolated were Gram negative bacilli. Among Gram negative bacilli 71.3% patients had *E. coli*, 13.5% had *Klebsiella* spp. and 8.8% had *Pseudomonas* spp. *Enterobacter* spp. and *Citrobacter* spp. were present in only 2% of Gram negative bacilli–infected patients. Both non-fermenting Gram negative bacilli and *Proteus* spp. were found only in 1% of the patients.

Among Gram positive cocci 59% had *Enterococci* spp. followed by coagulage-negative *Staphylococcus* (25%), β-hemolytic *Streptococcus* (8%), Non-hemolytic *Streptococcus* (6%) and *Staphylococcus aureus* (2%) (Janifer *et al*., 2009).

In a two years surveillance conducted by Behzadi *et al*. (2010), the Gram negative bacteria, *E. coli* and *K. pneumoniae* were the most predominant bacterial agents causing UTI. Also, the statistical tests showed significant association between female gender and UTIs caused by *E. coli* ($p< 0.05$). In a study by Edward (2002), similar microbiological pathogens were seen in diabetic and non diabetic women whereby *E. coli* was the predominant organism.

In the similar study by Adeyeba (2007), frequent causative agents of UTI was found as *E. coli* accounting for 46% of the isolates followed by *Klebsiella* spp. 30%, *Candida albicans* 11%, *Proteus* 5%, *Staphylococcus aureus* 5% and *Pseudomonas aeruginosa* 3%.

In contrast to above studies, Salih (2011) concluded that *Staphylococcus aureus* was the most common microorganisms causing UTI in about 36.0%, *Pseudomonas aeruginosa* was the second most common microorganism that caused urinary tract infection in about 19.7% cases. *Escherichia coli* caused UTI in about 14.8, *Proteus mirabilis* in about 9.8%, *Klebsiella aerogenes* in about 3.3% and *Enterobacter* in about (1.6%) cases.

Mehvish and Betty (2011) found in a study that UTI isolates in diabetic males and females included *E. coli* 46 (32.9%) 28 (25.5%), *E. faecalis* 44 (31.4%) 32 (29.1%), *P. aeruginosa* 2 .4%) 10 (9.1%) and *S. aureus* 26 (18.6%) 22 (20%). Also, no prevalence of *P. aeruginosa* was found in the non diabetic group. *Pseudomonas* spp. was prevalent in 1.4% males and 9.1% females. There was incidence of opportunistic pathogen *P. aeruginosa* in diabetic patients due to immune suppression, which never cause any symptoms of UTI in the non diabetic subjects.

The proportion of UTI caused by *Candida albicans* was 283/4136 i.e. 6.8%. 123/4136 (43.5%) cases of candiduria belonged to men and 160/4136 (56.5%) cases belonged to female. The remaining patients (93.2%) were infected from bacteria (Behzadi *et al*., 2010).

Uropathogens observed in UTI did not differ by diabetes status (diabetic women vs. non diabetic women): *E. coli*, 74.4% vs. 75.8 %; *Klebsiella* spp., 7.0% vs. 6.3%; *Proteus* spp., 7.0% vs. 5.3%; group B *Streptococcus*, 2.3% vs. 3.2%; *Enterococcus* spp., 0% vs. 5.3%; other organisms, 9.3% vs. 9.3% (Boyko *et al*., 2005).

2.8 Antibiotics susceptibility pattern

The choice of antibiotic and duration of treatment depends on the history of patient and bacterial agent identified. For uncomplicated UTI disease is cured with 1 or 2 days of treatment and to ensure that the infection is cured antibiotics should be taken within a week or two week (Lemone *et al*., 2004). The use of antimicrobial agent should not be generalized but should be based on patient's allergy and compliance history, local community, availability and cost (Hooton, 2012).

The treatment of UTI is complicated by resistance to antibiotics which cause higher patient morbidity, higher costs of re-evaluation and re-treatment, higher rates of hospitalization and greater use of broader-spectrum antibiotics (Hooton *et al*., 2004). Strains of ESBL causing UTI are often multidrug-resistant, which complicates treatment and are often not detected early (Paterson, 2006).

Janifer *et al*. (2009), found Gram negative bacilli to be more sensitive than Gram positive cocci to Aminoglycosides such as Netillin (67 *vs* 42%), Amikacin (65 *vs* 29%), and Tobramycin (30 *vs* 14%) whereas Gram positive cocci (50%) were found to be more sensitive to Ofloxacin than Gram negative bacilli (23%). Similarly, Gram negative bacilli (62%) were more sensitive than Gram positive cocci (33%) to Ciprofloxacin.

Not much difference in sensitivity was observed between Gram positive cocci (35%) and Gram negative bacilli (33%) to Cefoperazone. Gram positive cocci (63%) were found to be more sensitive to Cefotaxime than Gram negative bacilli (51%), whereas Gram negative bacilli (62%) were more sensitive than Gram positive cocci (48%) to Ceftizoxime (Janifer *et al*., 2009)

The antibiotics to which *E. coli* strains were most commonly resistance were Cephalothin (57%), Ampicillin (49%), Trimethoprim, and Trimethoprim-Sulfamethoxazole (Rebecca *et al*., 2010). Quinolones were the least active drug against uropathogens in the study conducted by Eshwarappa *et al*. (2011) and resistance rate for Ciprofloxacin has been increasing over decades with their resistance rate ranging from 47% to 69% among Gram negative organisms (Akram *et al*., 2007).

2.9 Antibiotic resistance pattern

Irrational and repeated use of antibiotics is the main cause of increasing resistant organisms of UTI. Uropathogens resistant to 3rd generation Cephalosporin are increasing (Acharya *et al*., 2011).

Antibiotic resistance varies from one country to another depending on antibiotic use. Relatively inexpensive and highly effective drug Trimethoprim-sulphamethoxazole which is used to treat many UTIs, except those caused by *Enterococci* and *Pseudomonas* spp. interferes with the bacterial metabolism of folate but use of Trimethoprim-Sulphomethoxazole (TMP-SMX) has declined due to the increased incidence of bacterial resistance (Nguyen, 2004).

In the findings of Kolawole *et al*. (2009), majority of the isolates from the urine sample were not sensitive to the commonly used drugs Nitrofurantoin, Ampicillin and Co-trimoxazole. Resistance of Gram negative pathogens to Trimethoprim-Sulphamethoxazole is 6.5% in a study in the Department of Emergency Medicine, University of Florida in the USA while appeared to be 55.2% in another study done in Taiwan (McLoughlin and Joseph, 2003; Wu *et al*., 2004).

TMP-SMX resistance is high among older children and those with a history of antibiotic use (McLoughlin and Joseph, 2003). Resistance to Ampicillin and Sulfamethoxazole/Trimethoprim tends to increase year after year (Wu *et al*., 2004).

In a study conducted in the hospital of Fattouma Bourguiba by Ghedira *et al*. (2004), 96% of uropathogenic strains were resistant to Ampicillin, Amoxicillin and Cefalotin, 67% of strains were resistant to Amoxicillin with Clavulanic acid and only 34% of them were resistant to Cotrimoxazole (a combination of Sulphamethoxazole/Trimethoprim). Third generation Cephalosporin and Aminoglycosides were still active on the majority of strains.

There is 20% increase in resistance rates to Cotrimoxazole and 1st generation Cephalosporins (Pape *et al*., 2004). Ampicillin has the highest resistance rate against *E. coli* (74.2%) followed by Co-trimoxazole (61.3%); whereas Nitrofurantoin has the lowest resistance rate against *E. coli* (2.2%), followed by Amikacin (4.9%), Ceftriaxone (7.5%) and Ciprofloxacin (12%) (Yuksel *et al*., 2006). In another study by Adeyeba *et al*. (2007), most isolates were found to be sensitive to Oflaxacin, Gentamycin, Nitrofurantoin, Nalixidic acid and Cotrimoxazole while they were resistant to Tetracyclines, Ampicillins, Cefuroxime and Ceftazidine.

Imipenem, Ticarcillin-Clavuanate, and Piperacillin-Tazobactum are effective drug for seriously ill patients infected with *Pseudomonas*. Sulfactum/Cefoperazone and Pipercillin/Tazobactum were highly sensitive to both Gram positive cocci and Gram negative bacilli. Gram negative bacilli were found to be highly sensitive to Ciprofloxacin (62%) than to Oflaxcin (23%). Gram positive cocci (63%) were found to be more sensitive to Cefoxatimethan than Gram negative bacilli (51%) (Janifer *et al*., 2009).

Of the 3-day regimens, TMP/SMX is more effective and less expensive than Nitrofurantoin, Cefadroxil, or Amoxicillin for treatment of uncomplicated cystitis in women. Quinolones have also been shown to be effective in 3-day

courses, however cost is increased significantly over TMP combinations. Ciprofloxacin, 100 mg BID for 3 days, appears to be the most cost effective Quinolone regimen/SMX. Longer courses of therapy should be used in women who are diabetic, pregnant (Quinolones contraindicated), have had symptoms longer than 7 days, or have other evidence for complicated UTI (UMHS Urinary Tract Infection Guideline, May 2005)

2.10 Pattern of organism causing UTI in Nepal

In a study conducted in Bharatpur, Nepal by Acharya *et al*. (2011), UTI was more common in young females. Also, 24.94% sample was positive with UTI causing organisms. *E. coli* were the predominant (68.77%) isolates followed by *Enterobacter* spp. (13.92%).

E. coli is most commonly encountered uropathogens covering up to 41.66 % of renal infections. The higher prevalence of *E. coli* may be due to poor hygienic condition of the patients and it is especially higher among females due to contamination of perineum through faecal flora (Jha *et al*., 2009).

Raza *et al*. (2011) concluded from their study that *E. coli* was the major isolate (75.7%), followed by *K. pneumonia* (75.7%) and *Acenitobacter* (5.2%). The minor isolates in their study were *C . albicans* (0.6%) and *S . aureus* (0.2%).

Out of the 40 cases in a study by Sharma *et al*. (2011), *E. coli* was isolated in 27 (67.5%) followed by *Klebsiella* spp. in 8 (20.0%), *Proteus* spp. in 4 (10.0%) and *Pseudomonas* spp. in 1 (2.5%). In a study conducted by Kumari *et al*. (2005) in eastern Nepal, *E. coli* was again the predominant organism (59.0%) followed *by Klebsiella* spp. (12.6%), *P. aeruginosa* (6.9%), *Acinetobacter* spp. (5.9%), *Enterococcus* spp. (4.2%) and others (2.6%).

2.11 Antibiotic susceptibility and resistance pattern of uropathogens in Nepal

In developing countries the emergence of antibiotic resistance uropathogens due to poverty, ignorance, poor hygienic practices, use of spurious drug and

treatment without knowledge of resistance has caused a serious health problem (Shakya *et al*., 2012).

Chloramphenicol, Cefotaxime and Nitrofurantoin are most effective antibiotics for cases of ASB. Erythromycin and Nalidixic acid appeared to be moderately sensitive to the bacterial isolates (Jha *et al*., 2009).

Most of the urinary isolates showed hundred percent resistant to Ampicillin and high degree of resistance to Nalidixic acid, Nitrofurantoin, Cotrimoxazole followed by Ciprofloxacin and Gentamicin in a study by Acharya *et al*. (2011). Higher sensitivity was noted with antibiotics Cephotaxime, Amikacin, Ofloxacin and Norfloxacin.

Shakya *et al*. (2012) showed increased rate of resistance against Amoxicillin and 49%-63% resistance rate of *E. coli* towards first line antibiotic Trimethoprim-Sulfamethoxazole concluding that use of these antibiotic as single agent for treatment of UTI is not appropriate. In addition, widely accepted drug Fluoroquinolones was moderately effective against *E. coli* (49%-52%) and low resistance was reported to Nitrofurantoin. Ceftriaxone had better activity as compared to Ceftixime, Cefotaxime and Ceftazidine among third generation Cephalosporin. Gentamicin showed moderate activity against *E. coli* (45-85%).

CHAPTER-III
MATERIALS AND METHODS

3.1 MATERIALS

The list of materials used throughout this work are given in Appendix-III.

3.2 METHODS

3.2.1 Study design and setting

This was descriptive cross-sectional study conducted in National Kidney Center, Kathmandu from November 2011 to May 2012. In this study 232 mid stream urine samples were collected from the patients visiting the hospital. One hundred and thirteen samples were collected from diabetic patients and 119 samples were collected from non diabetic patients. The urine samples were processed immediately after collection for routine examination, culture and antibiotic susceptibility pattern. Patients were also requested to fill questionnaire so as to collect information about demographic variables (age, sex, education, use of insulin etc.) and informed consent was obtained. The questionnaire is given in Appendix I.

3.2.2 Patient and patients characteristic

Patients with and without diabetes were included in this study while patients with chronic kidney diseases were excluded from the study as they have difficulty in urination.

3.2.3 Sample collection

Samples were randomly collected using simple random sampling method. Each patient was given a dry, sterile and wide-necked leak-proof container for the collection of 10- 20 ml of the clean catch mid-stream urine. Patients were well instructed for the collection of Clean Catch-Midstream Urine (CC-MSU). Male patients were instructed to wash the glans thoroughly and collect the mid portion of urine after passing initial small amount of urine, in a provided wide mouthed sterile container. On the other hand, females were instructed to cleanse the vulva and labia thoroughly and collect the mid portion of urine in provided wide mouthed leak proof sterile container. The urine samples were

tested immediately after collection and those samples that could not be tested and cultured within 4 hours of collection were refrigerated or preserved with boric acid. The refrigerated samples were processed within 24 hours.

3.2.4 Macroscopic examination of urine

The urine specimen obtained in the laboratory was macroscopically observed and its color and turbidity was noted.

3.2.5 Microscopic examination of urine

Five to ten ml of urine sample was taken in a clean sterile centrifuge tube and centrifuged at 3000 rpm for 10 minutes. The supernatant was discarded and the deposit was then mixed properly and examined by wet mount preparation. The number of WBC and RBC were estimated as number per HPF i.e. 40 X objective of microscope. WBCs in excess of 10^4 cells/ml (> 10cells/ml) of urine will indicate significant pyuria. Other observations done in microscopic examination were casts, crystals, epithelial cells and bacteria.

3.2.6 Chemical examination of urine specimens

Albumin, pH and sugar were detected in the urine sample by using uristix. The uristix was dipped into the urine specimen and the change in color in the test area was noted after 30 seconds. The change in color of the test area was compared to that of standard color and the result was interpreted.

3.2.7 Culture of urine specimens

Culture of each uncentrifuged urine specimens were done on 5% Blood Agar (BA), MacConkey Agar (MA) and SDA (Sabouraud Dextrose Agar) using semi-quantitative culture method (Cheesbrough, 2000). Fixed and known volume (0.001 ml) of urine sample was streaked into the culture media using calibrated loop. The urine samples were thoroughly mixed before inoculating into the agar media. The inoculated MA, BA and SDA plates were incubated in an inverted position at 37°C for 24 hours.

3.2.8 Examination of the plates

The culture plates were observed after 24 hours. If the significant growth had occurred the isolates were further proceeded for identification but if growth was not observed or if the growth was unidentifiable or if only tiny colonies were observed, the plates were reincubated for additional 24 hours. After sufficient incubation the number of colonies on each plate were counted. The bacterial count was reported according to criteria given Kass, Marpal and Sandford as below:

1. Less than 10^4/ml organisms, not significant
2. 10^4-10^5/ml organisms, doubtful (suggest repeat specimen)
3. More than 10^5/ml organisms, significant bacteriuria

The plates were also observed for type of culture whether it was single or mixed. However, the culture indicating the appearance of ≥3 organism types with no predominating organism was interpreted as due to possible contamination of the specimen and asked for another specimen. In addition to the previously described guidelines pure culture of *S. aureus* was considered significant regardless of the number of CFU. The presence of yeast in any number was also considered to be significant (Forbes *et al*., 2007; Ramana and Chaudhury, 2012).

3.2.9 Identification of the isolates

Identification of significant isolates was done by using standard microbiological techniques as described in the Bergey's Manual which involves morphological appearance of the colonies, staining reactions and biochemical properties.

Pure culture for identification

For obtaining the pure culture to perform biochemical tests, small quantity of organism from isolated colony of agar plates, MA and BA, was subcultured into nutrient agar plates and incubated at 37°C for 24 hours. Isolated colony from the nutrient agar was transferred into the nutrient broth and incubated at 37°C for 4 hours. Gram staining of the organism was performed from the nutrient agar.

Biochemical tests

After gram staining biochemical tests were performed for the identification of the genera and species of the organisms.

1. Gram positive organisms were identified primarily on the basis of their response to Gram's staining, catalase, oxidase and coagulase tests.
2. Gram negative organisms were identified using Catalase test, Oxidase test, Indole test, Methyl Red test (MR), Voges Proskauer test (VP), Citrate utilization test, Triple Sugar Iron (TSI) test, Urease test, Sulphide Indole Motility test.
3. Germ tube test was performed for the yeast isolated (Appendix IV)

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

3.2.10 Antibiotic susceptibility testing

The antimicrobial susceptibility testing of the isolates was done by modified Kirby-Bauer disk diffusion method as recommended by Clinical Laboratory Standards Institute (CLSI) using Mullen Hinton Agar (MHA). The antibiotic disks used were Amikacin (30 µg), Cefotaxime (30 µg), Cephalexin (30 µg), Cefoxitin (30µg), Cotrimoxazole (25 µg), Erythromycin (15µg), Gentamicin (10µg), Ofloxacin (5 µg), Norfloxacin (10µg), Nalidixic acid (30µg) and Nitrofurantoin (300µg).

The isolated colonies were transferred to nutrient broth and were incubated for 4 hours, until the turbidity of bacterial growth was similar to that of 0.5 McFarland standard. Then the sterile cotton swab was dipped into the tube containing culture and inoculated over dried MHA by carpet culture technique. Using a sterile forceps, appropriate antimicrobial discs (6 mm diameter) was evenly placed on the inoculated plates, not more than 6 discs were placed on a 90 mm diameter plate. The plates were left at room temperature for the diffusion of antibiotics from disc. Then it was incubated at 37°C for 18 hours. After overnight incubation, the plates were examined to ensure confluent growth and the diameter of each zone of inhibition in mm was measured and results were interpreted.

3.2.11 Purity plate

The purity plate was used to ensure that the inoculation used for the biochemical tests is pure culture and also to see whether the biochemical tests are performed in an aseptic condition or not. Thus, before and after performing biochemical tests, the same inoculum was subcultured in nutrient agar medium and incubated. The media was then checked for the appearance of pure growth of organisms.

3.2.12 Quality control for test

Standard protocol was followed to maintain the quality of each test. All the agar plates were incubated at 37ºC for 24 hrs before use so as to examine for any contamination that occurred during media preparation and storage. Antibiotic Susceptibility test of the isolates was standardized by using control strains of *E. coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923). All the procedures were carried out strict aseptic condition.

3.2.13 Data Analysis

Data analysis was done using Statistical package for Social Sciences version 16. Simple descriptive analysis and chi square test were used to determine the association between risk factors and UTI. $p<0.05$ was considered significant.

CHAPTER-IV
RESULTS

This study was conducted among the patients visiting National Kidney Center (NKC), Banasthali, Kathmandu. Two hundred and thirty-two samples were collected from the patients visiting the hospital and the samples were processed in Microbiology Laboratory of Pathology Department of NKC.

Out of the total 232 patient 67 (28.9%) patients had UTI (Figure 2).

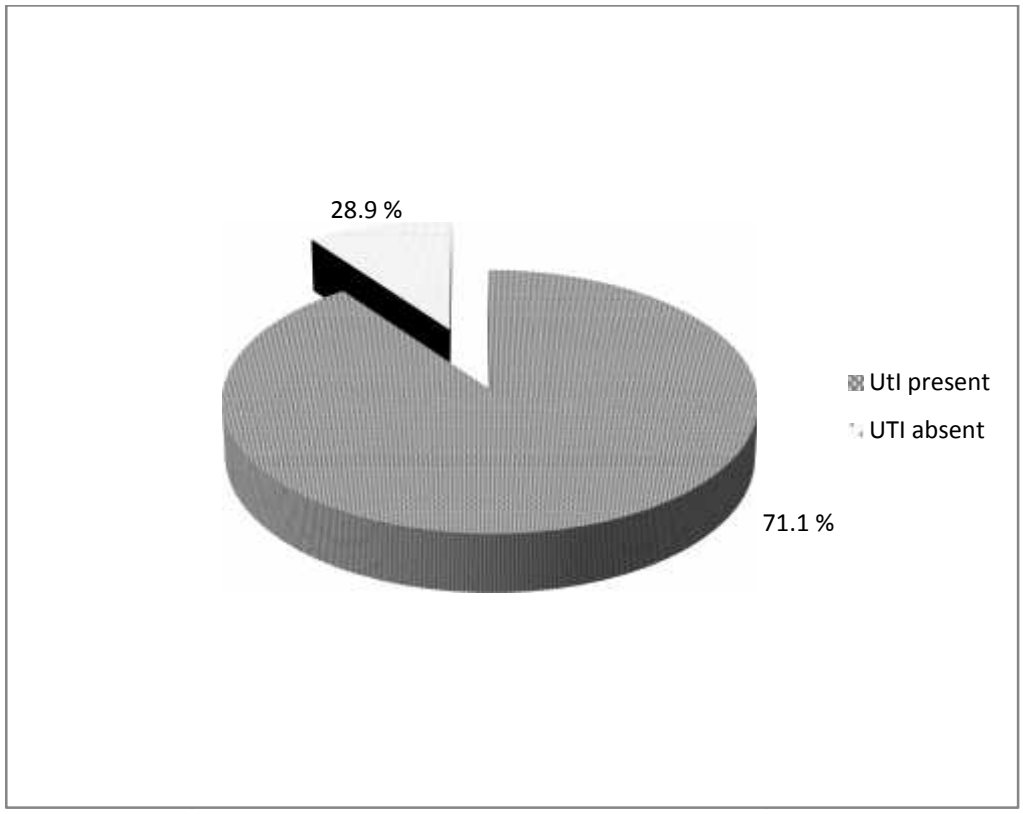

Figure 2: Infection pattern among the patients

4.1 Association between various risk factors and UTI

Patients belonging to age group 21-30 years were more infected (26.9%) than other age groups whereas least infected ones (2.9%) belonged to age group <20 years (Figure 3). There was no significant association between age of the patient and presence of UTI (p=0.075).

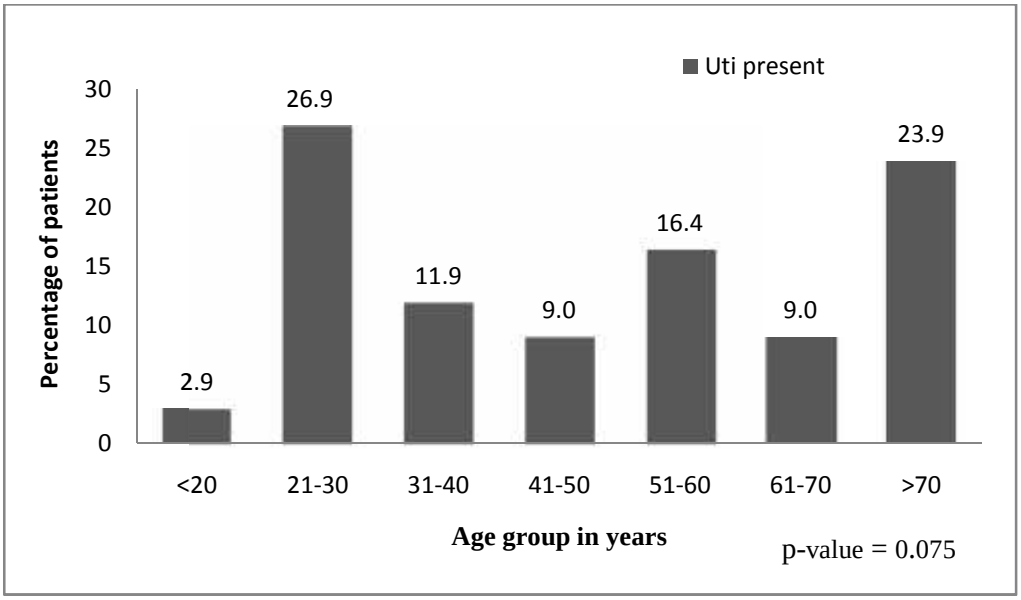

Figure 3: Agewise distribution of UTI in total sample

Among 232 patients, 96 were female and 136 were male. Proportion of infected female was greater (39.6%) than proportion of infected male (21.3%) and the difference was statistically significant ($p<0.05$) (Table 1).

Table 1: Genderwise distribution of UTI

Sex	Presence of UTI		Total (%)	p-value
	Yes (%)	No (%)		
Female	38 (39.6)	58 (60.4)	96 (100.0)	0.003
Male	29 (21.3)	107 (78.7)	136 (100.0)	

Patients belonging to different education level had almost equal prevalence of UTI. The prevalence of UTI was 36.9%, 35.4% and 27.7% in illiterate patients, patients with school level and patients with more than school level education respectively (Table 2).

Table 2: Education level and UTI

Education	Presence of UTI		Total	p-value
	Yes (%)	No (%)		
		27		
Illiterate	24 (36.9)	35 (21.7)	59 (3.0)	0.058
School level	23 (35.4)	73 (45.0)	96 (42.3)	
Above school level	18 (27.7)	54 (33.3)	72 (31.7)	
Total	65 (100.0)	162 (100.0)	227 (100.0)	

Among 191 married patients 61 (31.9%) were infected and among 41 unmarried patients 6 (14.6%) were infected. The higher prevalence of UTI in married individuals than in unmarried ones was statistically significant ($p<0.05$) (Table 3).

Table 3: Marital status and UTI

	Presence of UTI		Total (%)	p-value
	Yes (%)	No (%)		
Married	61 (31.9)	130 (68.1)	191 (100.0)	0.027
Unmarried	6 (14.6)	35 (85.4)	41 (100.0)	

UTI in females with menopause was found to be 43.4% and that in females without menopause was found to be 35.7%. However, this association between the UTI and menopause was not found to be statistically significant ($p>0.05$) (Table 4).

Table 4: Distribution of UTI among female with and without menopause

Menopause	Presence of UTI		Total (%)	p-value
	Yes %)	No (%)		
Yes	23 (43.4)	30 (56.6)	53 (100.0)	0.448
No	15 (35.7)	27 (64.3)	42 (100.0)	

Patients belonging to blood group A+ were found to have high (37.5%) and patients belonging to blood group B+ were found to have least (22.0%) prevalence of UTI (Figure 4). The relationship between the blood group and UTI was statistically insignificant (p-value=0.208).

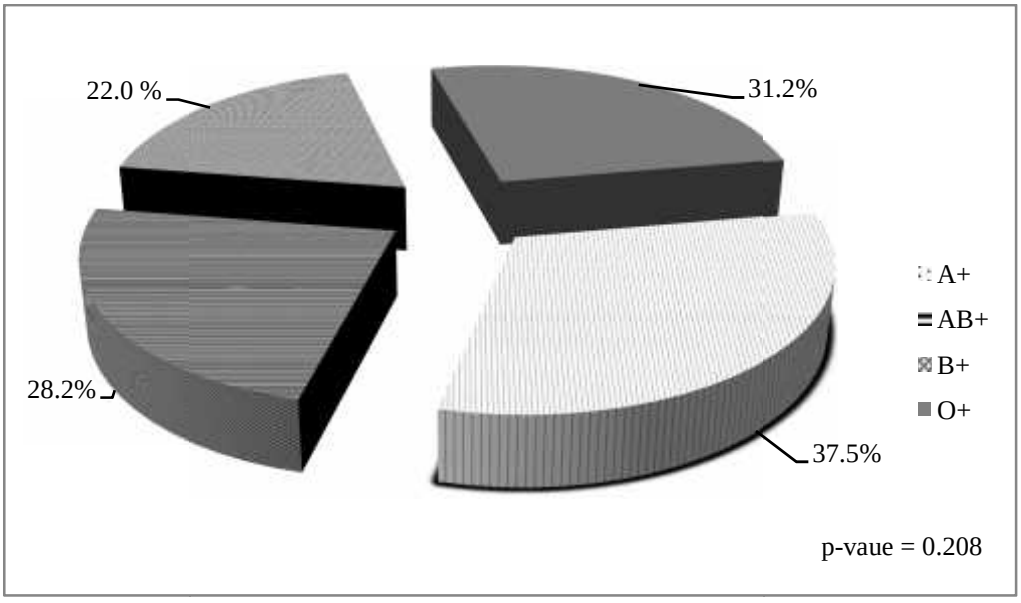

Figure 4: Distribution of UTI among ABO Rh+ve blood group

4.2 DM and UTI

Out of total 113 MSU samples from diabetic patients, 40 (35.4%) samples showed significant growth and out of 119 MSU samples from non diabetic patients 27 (22.7%) samples showed significant growth. The higher percent of UTI infection in diabetic cases than non diabetic cases was statistically significant ($p<0.05$) (Table 5).

Table 5: Distribution of UTI among diabetic and non diabetic patients

Patients	Presence of UTI		Total (%)	p-value
	Yes (%)	No (%)		
Diabetic	40 (35.4)	7 (64.6)	113 (100.0)	0.033
Non diabetic	27 (22.7)	92 (77.3)	119 (100.0)	

Greater proportion of asymptomatic cases was found in diabetic patients 24 (60.0%) than in non diabetic patients 11 (40.7%). The relation between two variables however was insignificant ($p>0.05$) (Table 6).

Table 6: Symptomatic and asymptomatic UTI cases in diabetic and non diabetic patients

UTI Cases	Diabetic	Non Diabetic	p- value
	Yes (%)	No (%)	
Symptomatic	16 (40.0)	16 (59.3)	0.122
Asymptomatic	24 (60.0)	11 (40.7)	
Total	40 (100.0)	27 (100.0)	

Although asymptomatic UTI cases in diabetic female was seem to be greater (66.7%) than the diabetic male (50.0%), statistically this difference was insignificant (Table 7).

Table 7: Distribution of symptomatic and asymptomatic UTI cases among gender in diabetic patients

UTI Cases	Diabetic		p-value	Non diabetic		p-value
	Female (%)	Male (%)		Female (%)	Male (%)	
Symptomatic	8 (33.3)	8 (50.0)	0.292	8 (57.1)	8 (61.5)	0.816
Asymptomatic	16 (66.7)	8 (50.0)		6 (42.9)	5 (38.5)	
Total	**24 (100.0)**	**16 (100.0)**		**14 (100.0)**	**13 (100.0)**	

Among UTI positive diabetic patients, the age group >60 years had the maximum number 20 (50%) of significant bacteriuria followed by age group 51-60 years 9 (22.5%) whereas age group 31-40 years had least number 2(5.0%) of significant bacteriuria. Among UTI positive non diabetic patients, the age group 21-30 had the maximum number 15 (55.6%) of significant bacteriuria followed by age group 31-40 years 6 (22.2%) (Figure 5). Statistically significant association of UTI was found with age group among diabetic (p-value=0.050) and age group among non diabetic patients (p-value=0.041).

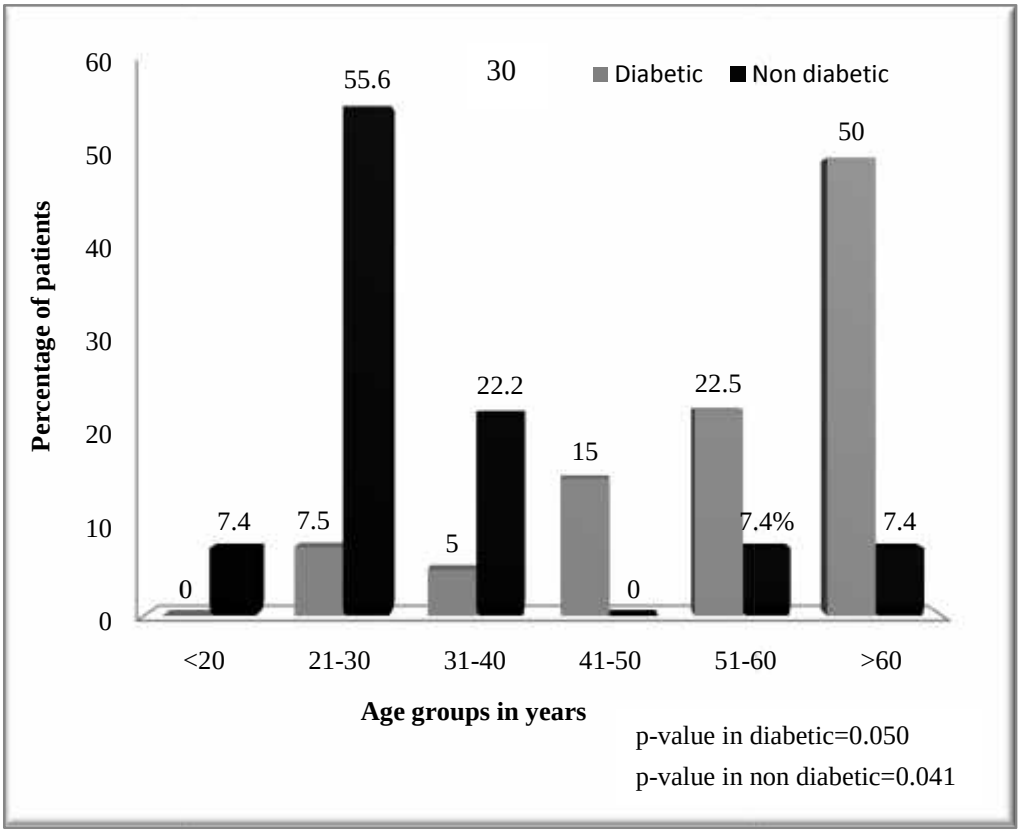

Figure 5: Age wise distribution of growth among diabetic and non diabetic patients

Out of total 66 diabetic patients using insulin 30 (45.5%) had UTI and out of total 47 diabetic patients not using insulin only 10 (21.3%) had UTI. This association between use of insulin and UTI was statistically significant (p<0.05) (Table 8).

Table 8: Use of insulin and UTI

Use of insulin	Presence of UTI		Total (%)	p-value
	Yes (%)	No (%)		

Yes	30 (45.5)	36 (54.5)	66 (100.0)	0.01
No	10 (21.3)	37 (78.7)	47 (100.0)	

Patients afflicted by diabetic for > 20 years were found to be most infected (67.6%) than the patients afflicted by diabetes for 11-20 years and below 10 years (Table 9).

Table 9: Association between duration of being diabetic and UTI

Duration of being diabetes in years	Presence of UTI		Total (%)	p-value
	Yes (%)	No (%)		
≤10	6 (12.8)	41 (87.2)	47 (100.0)	
11-20	9 (31.0)	20 (69.0)	29 (100.0)	0.114
>20	25 (67.6)	12 (32.4)	37 (100.0)	

4.3 Pattern of growth

Monomicrobial growth was found in 65 (28.0%) whereas polymicrobial growth was found only in 2 (0.9%) cases. In this study two significant polymicrobial growths were obtained whereby combination was found between Gram positive and Gram negative bacteria in both cases. *S. aureus* was found with *E. coli* in one case and with *K. pneumoniae* in another case (Table 10).

Table 10: Pattern of growth

Growth	No. (%) (n=232)
Monomicrobial	65 (28.0)
Polymicrobial	2 (0.9)
Insignificant	10 (4.3)
No Growth	155 (66.8)

Gram negative bacteria accounted for 73.8% in diabetic patients and 77.8% in non diabetic patients. Minor isolate, fungi accounted for 5.0% in diabetic patients and 3.7% in non diabetic patients (Table 11).

Table 11: Pattern of bacteria isolated from culture positive urine samples according to Gram's staining

Gram stain	Diabetic patients	Non diabetic patients	Total
	No. (%)	No. (%)	No. (%)

Gram negative	31 (73.8)	21 (77.8)	52 (75.3)
Gram positive	9 (21.4)	5 (18.5)	14 (20.3)
Fungi	2 (5.0)	1 (3.7)	3 (4.4)
Total	**42 (100.0)**	**27 (100.0)**	**69 (100.0)**

Among the total bacterial isolates in MSU of diabetic patients, *E. coli* was the major isolate 17 (40.5%) followed by *Klebsiella pneumoniae* 8 (19.0%). *Citrobacter freundii* and CoNS were minor isolates each accounting for only 1 (2.4%). Similar results were obtained for non diabetic patients whereby major isolate *E. coli* accounted for 10 (37.1%) followed by *Klebsiella pneumoniae* 6 (22.2%) and minor isolates CoNS, non hemolytic *Streptococcus*, *Candida albicans* each accounting for 1 (3.7%) (Table 12).

Table 12: Bacterial isolates among diabetic and non diabetic patients

Bacteria	**Diabetic (%)**	**Non diabetic (%)**	**Total (%)**
Gram Negative			
E. coli	17 (40.5)	10 (37.1)	27 (39.1)
K. pneumoniae	8 (19.0)	6 (22.2)	14 (20.3)
E. aerogenes	2 (4.8)	0 (0.0)	2 (2.9)
Citrobacter freundii	1 (2.4)	2 (7.4)	3 (4.3)
Proteus mirabilis	3 (7.1)	3 (11.1)	6 (8.7)
Gram Positive			
S. aureus	4 (9.5)	3 (11.1)	7 (10.2)
CoNS	1 (2.4)	1 (3.7)	2 (2.9)
Non hemolytic *Streptococcus* spp.	4 (9.5)	1 (3.7)	5 (7.3)
Fungi			
Candida albicans	2 (4.8)	1 (3.7)	3 (4.3)
Total	**42 (100.0)**	**27 (100.0)**	**69 (100.0)**

4.4 Antibiotic susceptibility pattern of organisms

Amikacin and Imipenem were found to be most effective drug for Gram negative bacteria both being effective for 50 (96.1%) isolates, followed by Gentamicin 40 (76.9%) and Cefotaxime 37 (71.1%). Least effective drug was Nalidixic acid which showed to which 35 (67.3%) Gram negative isolates were resistant (Table 13).

Table 13: Antibiotic susceptibility pattern of Gram negative bacteria

Antibiotics used	Susceptibility pattern (n=52)		
	Sensitive (%)	Intermediate (%)	Resistant (%)
Amikacin	50 (96.1)	2 (3.9)	0 (0.0)
Nitrofurantoin	34 (65.4)	8 (15.4)	10 (19.2)
Gentamicin	40 (76.9)	5 (9.6)	7 (13.5)
Cefotaxime	37 (71.1)	7 (13.5)	8 (15.4)
Cefoxitin	24 (46.2)	11 (21.1)	17 (32.7)
Cephalexin	19 (36.5)	8 (15.4)	25 (48.1)
Ofloxacin	29 (55.8)	8 (15.4)	15 (28.8)
Norfloxacin	31 (59.7)	11 (21.1)	10 (19.2)
Cotrimoxazole	29 (55.8)	2 (3.8)	21 (40.4)
Nalidixic acid	8 (15.4)	9 (17.3)	35 (67.3)
Imipenem	50 (96.1)	2 (3.9)	0 (0.0)

Also for Gram positive bacteria, Imipenem was most effective drug being effective to 12 (85.7%) isolates. Cefotaxime stands in second position showing inhibitive action against 10 (71.4%) Gram positive isolates and this was followed by Cotrimoxazole 9 (64.3%) and Amikacin 9 (64.3%). Cephalexin was most ineffective antibiotics with 7 (50.0%) resistant isolates (Table 14).

Table 14: Antibiotic susceptibility pattern of Gram positive bacteria

Antibiotic used	Susceptibility Pattern (n=14)		
	Sensitive (%)	Intermediate (%)	Resistant (%)
Amikacin	9 (64.3)	2 (14.3)	3 (21.4)
Cefotaxime	10 (71.4)	2 (14.3)	2 (14.3)
Cefoxitin	6 (42.8)	0 (0.0)	8 (57.2)
Cephalexin	4 (28.6)	3 (21.4)	7 (50.0)
Cotrimoxazole	9 (64.3)	1 (7.1)	4 (28.6)
Imipenem	12 (85.7)	1 (7.1)	1 (7.1)
Erythromycin	7 (50.0)	3 (21.4)	4 (28.6)
Nitrofurantoin	8 (57.2)	1 (7.1)	5 (35.7)

Amikacin and Imipenem were found to be most effective drugs being effective for 27 (100%) *E. coli* isolates. Similarly other effective drugs were Nitrofurantoin, Gentamicin and Cefotaxime each being effective to 20 (74.1%) *E. coli* isolates. Nalidixic acid was found to have 70.4% and Cephalexin was found to have 40.7% non effectiveness to *E. coli* isolates (Table 15).

Table 15: Antibiotic susceptibility pattern of *E. coli*

Antibiotics used	**Susceptibility Pattern (n=27)**		
	Sensitive (%)	**Intermediate (%)**	**Resistance (%)**
Amikacin	27 (100.0)	0 (0.0)	0 (0.0)
Nitrofurantoin	20 (74.1)	3 (11.1)	4 (14.8)
Gentamicin	20 (74.1)	4 (14.8)	3 (11.1)
Cefotaxime	20 (74.1)	3 (11.1)	4 (14.8)
Cefoxitin	15 (55.6)	7 (25.9)	5 (18.5)
Cephalexin	13 (48.2)	3 (11.1)	11 (40.7)
Ofloxacin	16 (59.3)	5 (18.5)	6 (22.2)
Norfloxacin	17 (63.0)	7 (25.9)	3 (11.1)
Cotrimoxazole	16 (59.3)	1 (3.7)	10 (37.0)
Nalidixic acid	4 (14.8)	4 (14.8)	19 (70.4)
Imipenem	27 (100.0)	0 (0.0)	0 (0.0)

Maximum isolates i.e. 13 (92.9%) of *K. pneumoniae* were sensitive to Amikacin and Imipenem. Immediately after these lie two drugs Gentamicin and Cefotaxime which were effective to 10 (71.5%) and 9 (64.3%) isolates of *K. pneumoniae* respectively. Nalidixic acid didnot inhibit the growth of 9 (64.3%) and Cephalexin, Norfloxacin and Cotrimoxazole each didn't inhibit the growth 7 (50.0%) *K. pneumoniae* isolates (Table 16).

Table 16: Antibiotic susceptibility pattern of *Klebsiella pneumoniae*

Antibiotics used	Susceptibility Pattern (n=14)		
	Sensitive (%)	Intermediate (%)	Resistance (%)
Amikacin	13 (92.9)	1 (7.1)	0 (0.0)
Nitrofurantoin	8 (57.2)	3 (21.4)	3 (21.4)
Gentamicin	10 (71.5)	1 (7.1)	3 (21.4)
Cefotaxime	9 (64.3)	3 (21.4)	2 (14.3)
Cefoxitin	6 (43.0)	3 (21.4)	5 (35.6)
Cephalexin	4 (28.6)	3 (21.4)	7 (50.0)
Ofloxacin	5 (35.6)	3 (21.4)	6 (43.0)
Norfloxacin	5 (35.6)	2 (14.3)	7 (50.0)
Cotrimoxazole	6 (43.0)	1 (28.6)	7 (50.0)
Nalidixic acid	2 (14.3)	3 (21.4)	9 (64.3)
Imipenem	13 (92.9)	1 (7.1)	0 (0.0)

Hundred percent sensitivity was shown by isolated strains of *Proteus mirabilis* to antibiotics Ofloxacin, Norfloxacin and Imipenem. Also 83.3% isolates of *Proteus mirabilis* showed sensitivity to antibiotics Amikacin and Gentamicin whereas maximum isolates i.e. 66.6% were resistance to Cephalexin and Nalidixic acid (Table 17).

Table 17: Antibiotic susceptibility pattern of *Proteus mirabilis*

Antibiotics used	Susceptibility Pattern (n=6)		
	Sensitive (%)	Intermediate (%)	Resistance (%)
Amikacin	5 (83.3)	1 (16.7)	0 (0.0)
Nitrofurantoin	3 (50.0)	0 (0.0)	3 (50.0)
Gentamicin	5 (83.3)	0 (0.0)	1 (16.7)
Cefotaxime	4 (66.6)	1 (16.7)	1 (16.7)
Cefoxitin	2 (33.3)	1 (16.7)	3 (50.0)
Cephalexin	2 (33.3)	0 (0.0)	4 (66.6)
Ofloxacin	6 (100.0)	0 (0.0)	0 (0.0)
Norfloxacin	6 (100.0)	0 (0.0)	0 (0.0)
Cotrimoxazole	3 (50.0)	0 (0.0)	3 (50.0)
Nalidixic acid	1 (16.7)	1 (16.7)	4 (66.6)
Imipenem	6 (100.0)	0(0.0)	0 (0.0)

The drug with maximum effectiveness for *Staphylococcus aureus* was Imipenem (85.7%) followed by Cefotaxime, Amikacin and Cotrimoxazole (each 71.4%). Least effective drugs were Erythromycin and Cephalexin (Table 18).

Table 18: Antibiotic susceptibility pattern of *Staphylococcus aureus*

Antibiotics used	Susceptibility pattern (n=7)		
	Sensitivity (%)	Intermediate (%)	Resistance (%)
Amikacin	5 (71.4)	0 (0.0)	2 (28.6)
Cefotaxime	5 (71.4)	0 (0.0)	2 (28.6)
Cefoxitin	3 (42.8)	0 (0.0)	4 (57.1)
Cephalexin	2 (28.6)	0 (0.0)	5 (71.4)
Cotrimoxazole	5 (71.4)	1 (14.3)	1 (14.3)
Imipenem	6 (85.7)	0 (0.0)	1 (14.3)
Erythromycin	2 (28.6)	3 (42.8)	2 (28.6)
Nirofurantoin	4 (57.1)	1 (14.3)	2 (28.6)

Among five isolates of non hemolytic *Streptococcus* spp. 4 (80%) were sensitive to Cefotaxime and Imipenem (Table 19).

Table 19: Antibiotic susceptibility pattern of non hemolytic *Streptococcus* spp.

Antibiotics used	Susceptibility patterns (n=5)		
	Sensitivity (%)	Intermediate (%)	Resistance (%)
Amikacin	3 (60.0)	1 (20.0)	1 (20.0)
Cefotaxime	4 (80.0)	1 (20.0)	0 (0.0)
Cefoxitin	2 (40.0)	0 (0.0)	3 (60.0)
Cephalexin	1 (20.0)	2 (40.0)	2 (40.0)
Cotrimoxazole	2 (40.0)	0 (0.0)	3 (60.0)
Imipenem	4 (80.0)	1 (20.0)	0 (0.0)
Erythromycin	3 (60.0)	2 (40.0)	0 (0.0)
Nitrofurantoin	3 (60.0)	0 (0.0)	2 (40.0)

4.5 Antibiotic susceptibility pattern of *Enterobacter* spp.

All the two isolates of *Enterobacter* spp. were sensitive to Amikacin, Imipenem, Gentamicin, Cefotaxime and Cotrimoxazole while all of them were resistance to Cephalexin and Nalidixic Acid. Only one isolate was sensitive to Cefoxitin, Nitrofurantoin, Norfloxacin and Ofloxacin.

4.6 Antibiotic susceptibility pattern of *Citrobacter freundii*

All the three isolates of *Citrobacter freundii* were sensitive to Amikacin and Gentamicin, two isolates were sensitive to Nitrofurantoin, Cefotaxime, Norfloxacin, Cotrimoxazole and Imipenem, only one isolate was sensitive to drugs Ofloxacin and Nalidixic acid and no isolates were sensitive to remaining drugs Cefoxitin and Cephalexin.

4.7 Antibiotic susceptibility pattern of CoNS

Only two isolates of CoNS were obtained in this study. Cotrimoxazole, Imipenem and Erythromycin were the drugs effective to both the isolates. Remaining drugs Cefotaxime, Cefoxitin, Cephalexin, Amikacin and Nitrofurantoin were effective to only one isolate.

CHAPTER-V
DISCUSSION

UTI is still not given appropriate concern although it responsible for large number of morbidity especially in developing countries like Nepal. Majority of the people both educated and uneducated are still unknown about UTI and thus are afflicted by this disease although it can be prevented by simply applying hygienic behaviour.

Consequences of UTI seems to be trivial in comparison to other major health problems but one should not forget that if not given timely concern simple case of UTI may lead to complex cases like kidney infection which are difficult to manage. The condition may even be more exacerbated if patients are immune-compromised

with disease like DM. Thus, this study was designed primarily to identify risk factor for UTI especially in diabetic patients and its causative agents.

The study was conducted among both in and out patients of the hospital. Data for various risk were collected through questionnaire and urine samples were processed in the Microbiology laboratory.

Among 232 patients 113 (48.7%) patients were diabetic and 119 (51.3%) were non diabetic. Among diabetic patients the duration of being diabetes ranged from <1 years to above 20 years. In this study association between various risk factors (age, sex, education, marital status, and use of insulin, menopause and duration of being diabetes) were studied.

Prevalence of UTI in total patient was 28.9%. In this study the age group 21-30 years had higher prevalence of UTI whereas age group <20 had lowest prevalence of UTI. Thus, UTI cases have been found to be more common in sexually active age group 21-30. Bankable *et al*. (2011); Gupta (2012); Rajbhandari and Shrestha (2002) and Salih (2011) reported similar results. The women in this age group are most prone to sexual activity and pregnancy which is common cause of UTI as such activity can increase the introduction of bacteria into the urethra.

Almost double proportion of female were afflicted by UTI in comparison to male. This indicates that females are more prone to have UTI than males. Sharma *et al*., 2011 in Nepal reported 65% female and 35% male were infected with UTI. Also in other studies by Mohanty *et al*., 2003 and Raz *et al*., 2011 females were more infected than the males by UTI. Urethra in female is much shorter and closer to the anus so that intestinal flora has easy access to urinary tract. Other risk factor for UTI in females includes sexual intercourse and family history.

In this study among patients with different education level, UTI was present in almost same proportion. Mazokopakis *et al*., (2012) concluded from a study that there were no statistically significant differences between the recurrent UTI and educational level. Baerheim *et al*., (2003), Ghenghesh *et al*., (2009) and Lafi (2010) also concluded that there is no association between level of education and UTI infection. The reason behind this result may be the fact that even the educated people were unaware of UTI and were not taking appropriate precautions.

The proportion of UTI positive cases among married patients were almost two times more than that of unmarried cases. This indicates that marriage is also one of the independent risk factor for UTI. Astal and Sharif (2002) and Salih (2011) also found higher prevalence of UTI in married patients. Married patients are at higher risk of UTI due to their regular involvement in the sexual activity.

Higher proportions of female with menopause were found to be UTI positive in comparison to female without menopause. However, the relationship between gender and UTI was statistically insignificant. The study by Akinjogunla (2010) unveiled that 39.6 % of postmenopausal women had UTI. Hormone estrogen in female reduces the vaginal pH and stimulates the growth of vaginal normal flora, *Lactobacillus* which protects the region from other pathogenic bacteria. In post-menopausal women, since the level of estrogen is abated, they are more susceptible to UTI.

Among ABO blood group, high rate of infection was found in blood group A +ve. This result correlates with the study conducted by Sakallioglu *et al*., 2007 where 36% case with A positive blood group were revealed UTI positive. Adhesion of the bacteria to the cells of urinary tract greatly depends on the genetic markers such as blood groups.

The prevalence of UTI among diabetic patient was higher than that of non diabetic patients by more than 10%. The diabetic patients are more vulnerable to different types of infections including urinary tract infections than non diabetic ones. The data obtained was closely related to the study conducted by Adeyeba *et al*., 2007. In their study the prevalence of UTI in DM was 21%. In another study conducted in Bangladesh by Saber *et al*., 2010 the proportion of UTI in diabetes was higher (43.8%). There are slew of reasons for this result which includes urinary incontinency, retention of urine in bladder, exacerbated defense system etc.

Asymptomatic bacteriuria in diabetic patients was slightly higher by about 20% than in non diabetic patients. This divulges that asymptomatic cases are more in diabetic patients than in non diabetic patients. The association between the presence of diabetes and asymptomatic bacteriuria was statistically insignificant. Sibi ***et al*., 2011 presented** 68% of the diabetic patients had asymptomatic UTI. Jha *et al*., 2009 observed 9.43% prevalence of UTI among elderly diabetic patients. Asymptomatic bacteriuria represents complicated form of UTI which is more common in patients with special risk factors such as diabetes.

In case of female in both diabetic (66.7%) and non diabetic (42.9%) patients asymptomatic bacteriuria was higher than in male diabetic (50%) and non diabetic (38.5%). In diabetic female 72.34% asymptomatic UTI cases was found in a study by Ophori *et al*., 2010. Also, in another study by Frank-Peterside and Wokoma, 2009 asymptomatic bacteriuria in female was 60% and that in male was 40%.Since female are at high risk of UTI than male due to short urethra, prevalence of asymptomatic UTI is also higher in female. So routine screening of UTI should be done in female even if the symptoms are not present.

Exactly half the diabetic patients belonging to age group >60 years were the UTI infected. Among non diabetic cases however high rate of infection was in age group 21-30. Also in a study by Saber *et al*., 2010, 42.1% infection was found in diabetic patients above 60 years. Similar data was also found in a study by Janifer *et al*., 2009 where prevalence of UTI in diabetic patients >55 years was 67.8% and 61% in male and female respectively. Higher prevalence of UTI is detected in this study during adolescent in diabetic patients can be justified by the fact that diabetic patients during their old age are immunologically more dilapidated and physically very less capable to give attention to their health and hygiene than their normal counterparts. Sexually active age group have high chances of having UTI among non diabetic patients.

More than double proportion of diabetic patients receiving insulin than the diabetic patients without receiving insulin were suffered from UTI. Thus, patients with complicated diabetes are at more risk of UTI infection than those with uncomplicated diabetes. Such risk was also obtained in a study by Khalid *et al*., 2012. But in another study by Ghenghesh *et al*., 2009 no significant association was found in the rate of uropathogens isolated in DM patients using oral medications when compared to those taking insulin. Insulin users among diabetic are more infected because they represent more complicated form of diabetes.

More than half proportion of patients who have been bearing diabetes for >20 years were infected followed by 11-20 years. While those who have been bearing diabetes for ≤10 years had lowest prevalence of UTI. The study conducted by Janifer *et al*., 2009 showed 58.2% and 57.6% prevalence of UTI among male and female patients respectively with ≥10 years of diabetes. But Boyko *et al*., 2002 concluded no significant difference for UTI in women with diabetes of <10 years or ≥10 years. Patients with higher duration of diabetes have more complicated form of diabetes, more age and also are more immunologically suppressed and thus have higher chances getting UTI.

Gram negative bacteria were the major isolates in both diabetic and non diabetic cases. Also, two fungi were isolated from diabetic and one fungus from non diabetic cases. The other studies carried out by Jha *et al*. (2009), Cruz *et al*. (2009) and Janifer *et al*. (2009) also concluded Gram negative organisms as major isolates of urine sample. Shrestha *et al*., 2007 isolated 93.8 % Gram negative organisms and 6.3% Gram positive organisms.

Out of 8 different bacterial isolates, *E. coli* was the dominant organism. Adeyeba *et al*., 2007; Jha *et al*., 2009; Mansour *et al*., 2009 and Papazafiropoulou *et al*., 2010 also reported *E. coli* as major UTI causing organism. In our study, *E. coli* was followed by *K. pneumoniae*, and *S. aureus*. The same result was reported by Ghenghesh *et al*. (2009), Ophori *et al*. (2010), Bano *et al*. (2012) and Rijal *et al*. (2012). Other bacterial isolates in the order of their isolated proportion were *P. mirabilis*, non hemolytic *Streptococcus* spp, *C. freundii*, *E. aerogens* and CoNS. The fungi isolated was *Candida albicans*. The result of a study conducted by Behzadi *et al*., 2010 showed 6.8% *Candida albicans* and that by Bano *et al*., 2011 showed 4.8%.

There was no difference between the organisms isolated in diabetic and non diabetic patients and *E. coli* was major isolates in both cases. Similarly, the proportion of other bacterial isolates in diabetic and non diabetic patients were *K. pneumoniae* 8 (19.0%) and 6 (22.2%); *S. aureus* 4 (9.5%) 3 (11.1%); *Proteus mirabilis* 3 (7.1%) 3 (11.1%); non hemolytic *Streptococcus* 4 (9.5%) 1 (3.7%); *Enterobacter aerogens* 2 (4.8%) 0 (0.0%); *C. freundii* 1 (2.4%) 2 (7.4%) and CoNS 1 (2.4%) 1 (3.7%) respectively. 2 (4.8%) and 1 (3.7%) was the proportion of *Candida albicans* in diabetic and non diabetic patients. In a study by Boyko *et al*., (2005) the uropathogens isolated did not differ by diabetic status. *E. coli* and *K. pneumoniae* since belong to Enterobacteriaceae are main cause of UTI. The source of Gram positive uropathogens especially is other than large bowel such as previous catheterization.

Amikacin and Imipenem was the most potent drug for the treatment of Gram negative bacteria both being effective for 96.1% isolates. This high efficacy of Amikacin was also found by Mutate *et al*. (2004) and Raza *et al*. (2011) Mansour *et al*., 2009 found efficacy of Amikacin as 90.5—100%. Amikacin was followed by Gentamicin (76.9%) and then by Cefotaxime (71.1%). Nitrofurantoin was also effective for more than half (65.4%) of the isolates. Of all the antimicrobial agents tested, Nalidixic acid showed most disappointing result since only 8 isolates were sensitive out of 52 Gram negative isolates. Also first generation Cephalosporin (Cephalexin) and second generation Cephalosporin (Cefoxitin) were insignificantly effective.

Imipenem (85.7%) was the most effective drug for Gram positive organisms followed by Cefotaxime (71.4%). Cotrimoxazole, Gentamicin and Nitrofurantoin were also effective for more than half proportion of isolates. Almost half of the isolates were resistant to Cefoxitin and Cephalexin rendering them comparatively ineffective than above mentioned drugs.

All the isolates of the *E. coli* were sensitive to drugs Amikacin and Imipenem. Other drugs of choice for *E. coli* were Nitrifurantion, Gentamicin and Cefotaxime. With only 14.8% effectiveness, the Nalidixic acid was the drug of no potential for *E. coli.* Similar pattern of susceptibility was obtained for *Klebsiella* with highest sensitivity to Amikacin and Imipenem and lowest sensitivity to Nalidixic acid.

Of all the antibiotics tested for *S. aureus,* Imipenem followed by Cefotaxime, Cotrimoxazole and Amikacin were effective drugs. Cephalexin, Erythromycin and Cefoxitin were the drugs that should be avoided to treat the UTI caused by *S. aureus.* The best drugs to treat CoNS caused UTI were Cotrimoxazole, Erythromycin and Imipenem. Among five isolates of non hemolytic *Streptococcus* spp. four were sensitive to Cefotaxime and Imipenem and only one was sensitive to Cephalexin.

CHAPTER-VI
CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The most infected age group with UTI was 21-30 years in total patients and >60 in diabetic patients**.** Statistically significant association of UTI was found with gender, marital status, presence of diabetes and use of insulin. The infection rate was high in patients with A +ve blood group. Age, Education level, menopause, blood group and duration of being diabetic were not found to be statistically associated with UTI infection. Insignificant association was found between asymptomatic UTI and diabetes as well as asymptomatic UTI and female diabetes. *E. coli* was the most prevailing organism causing UTI. Imipenem was the most potent drug for both Gram positive and Gram negative organisms. These results intimate that sexually active age group, female gender and diabetic are risk groups for UTI and should be very cautious to so as to prevent it.

6.2 Recommendations

1. Sexually active age groups and patients with complicated diabetes should take special measures to prevent UTI.
2. The high rate of UTI among educated people put forth that knowledge about UTI should be disseminated as other popularly known diseases.
3. Since *E. coli*, organism normally present in bowel is the prevailing cause of UTI we should give high attention to personal hygiene.
4. Imipenem is the drug of choice for both Gram positive and Gram negative bacteria.

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