CHAPTER-I

1. Introduction

Urinary tract infection is the second most common infectious presentation in community practice. Worldwide, about 50 million people are diagnosed with UTI each year, costing the global economy in excess of 6 billion US dollars. UTI may involve only lower urinary tract or may involve both the upper and lower tract. The term cystitis has been used to describe lower UTI, which is characterized by a syndrome involving dysuria, frequency, urgency and occasionally suprapubic tenderness. However, the presence of symptoms of lower tract infection without upper tract infection does not exclude upper tract infection which is also often present (Akram *et al.*, 2007).

Nepal being a developing country it has about most of the people illiterate, who are not aware of health matters and don't have idea of hygienic living habit, so are always in the threat of infection caused by different types of organisms. According to report (2053/54) published by department of health service, 0.42% (88,037) of total population (21,126,638) was suffered from UTI. The geographical distribution of UTI among Nepalese population was 0.57% (9155) in mountain, 0.47% (44,314) in hill and 0.35% (34,568) in Terai of total population of respective region.

Urinary tract infection is one of the most important causes of morbidity in the general population and is the second most cause of hospital visits. With advancing age, the incidence of UTI increases in men due to prostate enlargement and neurogenic bladder (Das *et al.,* 2006). Recurrent infections are common and can lead to irreversible damage of the kidneys, resulting in renal hypertension and renal failure in severe cases. In the community, women are more prone to develop UTI. Above 20% of women have more than one episode of UTI per year. Pregnancy also makes them more susceptible to infection. Catheter associated UTI is a trenchant problem, with about 5% of catheterized patient developing bacteriuria (Das *et al.,* 2006).

An infection contracted outside of a health care setting is known as community acquired infection where as a hospital acquired infection is defined as infections developing in patients after admission to hospital, which were neither present nor in incubation at the time of hospitalization (Chakraborty, 2001). *E. coli* is the predominant cause of both community and nosocomial urinary tract infection (Bean *et al.,* 2008). Other pathogens include *Proteus mirabilis, Klebsiella spp*, and other members of Enterobactericae family and *Staphylococcous saprophyticus.* In more complicated UTI's, particularly, in recurrent infection, the relative frequency of infection caused *by Proteus spp, Pseudomonas spp, Klebsiella spp* and *Enterobactericae spp.*

Hospitalized patients are more likely to be infected by *E. coli, Klebsiella spp, Proteus mirabilis, Staphylococcus aureus,* other *Enteriobactericae*, *Pseudomonas aerogenosa* and *Enterococci*. In addition, UTI are leading cause of gram negative sepsis in hospitalized patient and are the origin for about half of all nosocomial infection caused by urinary catheters (Forbes *et al.,* 2002).

In human, *E. coli* is the most common cause of urinary tract infection. Approximately 85% of utero cystitis is caused by *E. coli* (Guentzel, 1991). Strains that cause UTI are selected from fecal flora due to their special adaptation of urinary tract epithelial mucosa. Studies with *E. coli* are of particular relevance because this species can occupy multiple niches including human and animal hosts. In addition *E. coli* strains effectively exchange genetic material with pathogens such as *Salmonella spp, Shigella spp, Yersinia spp* and *Vibrio spp* as well as pathogenic *E. coli.* Emerging antimicrobial resistance among uropathogens makes the management of uncomplicated cystitis increasingly challenging. Moreover, the management of UTI is complicated by the increasing prevalence of antibiotic resistant *E. coli.* The antimicrobial resistance is a serious emerging problem throughout the world. Multiple drug resistance (MDR) bacterial isolates have been frequently reported from different parts of the world. The MDR strain is defined as the strain that showed resistance to three or more antibiotic among six commonly prescribed drugs (Tuladhar *et al.*, 2001).The emergence of strains producing extended spectrum of β-lactamase and other exhibiting quinolone resistance now threatens the empirical use of both cephalosporin and ciprofloxacin seriously limiting treatments regimens (Bean *et al.*, 2008).

The present study was conducted with a broad objective to isolate *E. coli* causing UTI and observe susceptibility profile of such organism visiting B and B hospital. To determine current levels of resistance to antibiotics commonly used for empirical treatments of UTI for which we reviewed susceptibility to cephalexin, amoxicillin, cotrimoxazole, chloramphenicol, gentamycin, nalidixic acid, norfloxacin, ofloxacin, ciprofloxacin, cefoperazone, sulbactam, imipenem, meropenem, ceftriaxzone, nitrofurantoin and cefotaxime among all *E. coli* isolates obtained at B and B laboratory over a period of 1 year.

CHAPTER-II

2. Objectives

2.1. General objective:

To isolate *Escherichia coli* (*E. coli*) causing UTI and determine the antibiotic susceptibility profile of the isolates received at B and B Hospital, Lalitpur.

2.2. Specific objectives:

a. To access the antibiotic susceptibility pattern of the isolated *E. coli* from urine.
b. T o determine the status of MDR in the isolates.
c. To access the age and sex distribution of the isolates.
d. To access the distribution of the isolates in terms of nosocomial and community patients.

CHAPTER-III

3. Literature review

3.1 Definition of UTI

Urinary tract infection (UTI) is the one of the commonest of domiciliary and nosocomial bacterial infection, comprising a variety of clinical conditions caused by microbial invasion of tissue lining the urinary tract which extends from renal cortex to urethral maetus (Jha *et al.*, 2005). From a microbiological prospective, UTI exists when pathogenic microorganisms are detected in the urine, urethra, bladder, kidney or prostrate. In most instances, growth of more than 10^5 organisms/ml from a properly collected midstream ‘clean catch’ urine sample indicates infection (Stamm, 2003).

UTI simply means the presence of bacteria undergoing multiplication in urine within the urinary drainage system (Smith and Charles, 1990). According to Lambert and Coulthard, (2002), UTI comprises symptoms of infection together with the presence of pathogenic microorganisms in the urine, urethra, bladder or kidney.

UTI also refers to the presence of bacteria undergoing multiplication in urine within the urinary drainage system (Jha and Bapat, 2005) and the presence of more than 10^5 organisms/ml in the midstream sample of urine.

3.2 Epidemiology of UTI

Acute uncomplicated UTI in woman are a common problem in primary care settings, accounting for approximately 7 million outpatient’s visits each year in United States of America (USA) (Stamm and Hooton, 1993). The highest incidence is found in young, sexually active woman aged between 20-40 years and in post menopausal population (Nicolle, 2001). Approximately one out of three women will require antimicrobial treatment for UTI before age 24, and 40-50% of women will have UTI during their lifetime (Foxman, 2002).

UTI is a common cause of fever in pediatric population. UTI is among the most common cause of serious bacterial infection in children in North America (Dagan *et al.* (1985); Jaskiewicz *et al* (1994)).

UTI is a common problem in children. The incidence varies according to age, race and sex of children. UTI occurs in 1% of boys and 3.5% of girls. It affects male children more than female in the first year of life and females after 1 year of age. 3.5% of febrile children are found to have UTI. Symptoms of UTI may be minimal and non specific in infants and small children (Rai *et al.,* 2008).

Nepal being developing country it has about 61.4% people illiterate who are not aware on health matters and don't have idea of hygienic living habit, so are always in the threat of infections caused by different types of organisms. According to Annual report of fiscal year (2055/2056) published by Department of health services, 0.46% of total out patients suffered from UTI and this was out of the total population of Nepal (2,22,87413). (DoHS, 2000).

Infection of UTI is amongst the most common bacterial infections that prompt patients to seek medical advice second only to infection of respiratory tract. It has been estimated that about six million patients' visits out patients department and about 3000000 are treated in wards every year for UTI world wide. Approximately 10% of human population gets UTI at some stage during their life. The geographical distribution of UTI amongst the Nepal's population is 0.57% in the mountains, 0.45% is estimated to be in planes (Jha and Bapat, 2005).

Urinary tract infection is one of the most important causes of morbidity in general population, and is the second most common cause of hospital visits (Ronald and Pattulo, 1991). UTI is the most common cause of nosocomial infection among the hospitalized patients (Das *et al.,* 2006).

UTI are commonest bacterial infection managed in general practice and are responsible for considerable morbidity particularly in sexually active women. Except in first year of life and after age of 60 years, UTI predominantly affects female (Jha *et al* 2007).

UTI is common in pediatric practice and an important cause of morbidity and mortality in children. UTI are relatively common in children and are a common cause of fever with absence of other urinary symptoms. The exact rates of UTI are unknown as many as 40% of infections in infants and children are asymptomatic. It has been estimated that approximately 3% of girls and

1% of boys have symptomatic UTI before the age of 11 years (Malla *et al.,* 2007). Yet other studies state that up to 7% of girls and 2% of boys will have symptomatic, culture confirmed UTI infection by 6 years of age (Alper and Curry, 2005).

Uncomplicated UTI is among the most common infection in outpatients women, with significant morbidity and health costs. World wide, 150 million people are diagnosed with UTI each year, costing the global economy in excess of 6 billion US dollars (Stamm and Norrby, 2001).

3.3 Pathogenesis

The urinary system is composed of the kidneys, ureters; bladder and urethra (Baron *et al.,* 1990). All play a role in removing waste from body. Urinary tract infections typically occur when bacteria enter the urinary tract through the urethra and begin to multiply in the bladder (Nazar *et al.,* 2009). Although the urinary system is designed to keep out such microscopic invaders, the defenses sometimes fail. When that happens, bacteria may take hold and grow into a full-blown infection in the urinary tract.

The urinary tract can be infected from above (by bacteria entering the kidneys from the bloodstream and traveling downward) or from below (by bacteria entering the urethra and traveling upward) (Chakraborty, 2000).

Infection from above is most often seen in newborns with generalized infection. In older children and adults, infection most often starts from below. In small children still using diapers, stool can sit for some time right at the meatus; the longer it sits there, the more likely it is that bacteria may enter the urethra (Fahimzad *et al.,* 2010). Baby boys are less likely to have this happen than baby girls, because girls' urethrae are much shorter and the head of the penis isn't as likely to sit in stool (Chung *et al.,* 2008). Older girls may become prone to UTI's through wiping back-to-front when they are first toilet-trained, which pulls stool into the vaginal/meatal area. Sexually active teenage and adult women are more prone to UTI's because of friction at the meatus, which tends to push bacteria into the urethra ; the same mechanism may cause UTI's in teenage boys and adult men, although they are again less prone to UTI's than women of the same age.

Among the possible ways, the ability of an organism to produce pilli is important factors that enables the bacteria to attach to the epithelial cells and thereby avoids elimination from urinary tract (Chamberlain, 2002). One theory, called the biofilm mode of growth, suggests that sometimes bacteria form capsules that adhere to the urinary tract, which protects them from many of the normal defenses (Lau *et al.*, 2003).

3.4 Manifestation of UTI

Infection may be expressed predominantly as a single site, Kidney (Pyelonephritis), urinary bladder (cystitis), prostate (prostatitis), urethra (Urethritis), but the entire system is always at risk of invasion by bacteria once anyone of its part is infected. UTI is classified as upper tract and lower tract. The main upper tract infection is pyelonephritis and the main lower tract infection is cystitis.

3.4.1 Urethritis is infection/inflammation of the urethra. This can be due to other things besides the organisms usually involved in UTI's; in particular, many sexually-transmitted diseases (STD's) appear initially as urethritis. However, stool-related bacteria (the most common bacteria on the skin near the meatus) will also often cause urethritis. It is usually sexually transmitted. Pathogen such as *Chlamydia trachomatis*, *Ureaplasma urealyticum*, *Neisseria gonorrhea*, and *Trichomonas vaginalis* are the common cause of urethritis (Chamberlain, 2000).

3.4.2 Cystitis is an infection of the bladder. This is the most common form of UTI. Cystitis is characterized by dysuria, frequency and urgency, with or without suprapubic pain. Causes of cystitis can be infective or non infective. The commonest clinical entity is bacterial cystitis due to common urinary tract pathogen. Bacterial cystitis is usually associated with bacteriuria and pyuria, but both can occur without infection. Bacteriuria may be due to colonization or infection of urinary tract, or contamination of the collected specimen. Pyuria indicates inflammation, which is usually due to bacteria but can be due to other causes (Chung *et al.*, 2008).

3.4.3 Ureteritis is infection of a ureter. This can occur if the bacteria entered the urinary tract from above or if the ureter-to-bladder valves don't work properly and allow urine to "reflux" from the bladder into the ureter.

3.4.4 Pyelonephritis is an ascending urinary tract infection that has reached the pelvis of the kidney. Acute renal impairment in the setting of acute pyelonephritis is often associated with pregnancy, solitary kidney, indwelling catheter, immunocompromised status or use of nonsteroi-dal anti-inflammatory drugs. The kidneys are usually swollen by an interstitial infiltrate and edema, and white cell tubular casts and micro abscesses may be present. Recovery of renal function usually occurs if antibiotics are promptly instituted (Squalli *et al.,* 2010). Acute pyelonephritis during pregnancy is serious illness that can progress to maternal sepsis, preterm labor and premature delivery (Delzell, 2000).

Urinary tract infections are very common in women, and many women will experience more than one. A key reason is their anatomy which can be attributed to urethral length, which provides an effective barrier to bacterial ascent. The female urethra is generally less than 5 cm compared to the male, which is more than 15cm (Chung *et al.*, 2008).

3.5 Risk factor for UTI

3.5.1 Being sexually active: Women who are sexually active tend to have more urinary tract infections than women who aren't sexually active (Baron *et al.*, 1990).

3.5.2 Using certain types of birth control: The proper position of diaphragm, resting against the vaginal walls and depressing the urethra and bladder, may decrease the sensation so that the women fills the urge to urinate less frequently. Further, the diaphragm may cause stasis in the blood flow around the pressure areas enhancing bacterial ability to invade the bladder mucosa. The diaphragm may also cause women to urinate less frequently leaving a pool of residual urine, thus bacteria would have more time to multiply and invade the bladder walls (Foxman and Frerichs, 1985).

3.5.3 Undergoing menopause: Among post menopausal women anatomic or functional defects may be contributing factors of UTI as there is a decrease in Estrogen level that results in colonization of *E. coli* (Rafique *et al.*, 2002).

3.5.4 Having urinary tract abnormalities: Any anatomical barrier to free flow of urine through the urinary tract contributes to the development of UTI; involved are prostatic hypertrophy, neurogenic disorders, tumors and stone (Baron, 1990; Svanborg, 1993).

3.5.5 Having a suppressed immune system: In general, defects in phagocytosis and in humoral or cellular immunity do not appear to predispose to the acquisition of UTI but do influence the clinical manifestations and the severity, microbiology, and complications of infection once it is established. The incidence of UTI in immunosuppressed patients other than diabetics or renal transplant recipients is not higher than the incidence in nonimmunosuppressed individuals. The higher frequencies of infection seen in diabetics and in renal transplant recipients correlate best with the duration of bladder instrumentation rather than with glycosuria or immunosuppressive regimen. Neutropenia blunts the clinical manifestations of UTI and predisposes to bacteremia (Korzeniowski, 1991).

3.5.6 Using a catheter to urinate: Indwelling urinary catheter (IUCs) are routinely cited as the primary cause of nosocomial infections. Approximately 4 million people each year receive an indwelling urinary catheter (IUC) and 5–20% of hospitalized patients who receive an IUC will be diagnosed with a urinary tract infection (UTI). IUC use is thought to be the most significant risk factor for developing nosocomial UTIs, especially in acutely ill elderly patients. Indeed, the leading category of nosocomial infections are UTIs and 80% of these are associated with IUCs (Hazeletl *et al.,* 2006).

3.6 Protective factors

Urine is normally sterile that is, it does not normally contain bacteria. Usually several things keep bacteria out of the urine.There is innate immunity in the lower urinary tract via the flushing out of organisms by urine as well as entrapment of bacteria by the urethral lining. These cells are shed in the urine leading to removal of bacteria from the lower urinary tract. In addition, the normal flora of healthy vaginal mucosa and perineal area contains micro-organisms such as lactobacilli, and an acidic ph environment, which prevent the adherence of uropathogens. Factors that cause urinary stasis and alter the vaginal and perineal environment (spermicides or vaginal atrophy) may alter these protective mechanisms (Chung *et al.,* 2008).

3.7 Symptoms of UTI

The symptoms a person has with a UTI depend on how old the person is and on where in the urinary tract the infection is located.

Urethritis usually appears as burning on urination. Cystitis may also show up as burning on urination, often in the middle of urination. However, it may have no symptoms other than fever, lower abdominal pain, or even just a funny smell or colour or appearance (cloudy, dark, and even blood-tinged) of urine.

Blood in the urine can be a sign; sometimes the only sign at first - of a urinary tract infection. It can result from microscopic bleeding within the kidneys or from an abscess if the infection is far advanced. Blood can also appear in urine from a bleed anywhere between the kidneys and the urinary meatus; in particular, cystitis can result in bleeding inside the bladder, which will certainly leave blood in the urine, whether as blood-tinging, blood clots in the urine, or something in between.

Since kidneys are located at back, pyelonephritis may appear as pain in back or flank, or in the abdomen. Fever usually (but not always) comes along with the pain. If the kidneys are severely affected, complications due to kidney malfunction may occur.

3.8 Categorization of UTI

3.8.1 Complicated and uncomplicated urinary tract infection

There is a general agreement that for the best management of patients with urinary tract infections, it is important to distinguish between complicated and uncomplicated infections. Complicated infections include those involving the parenchyma (pyelonephritis or prostatitis) and frequently occur in the setting of obstructive uropathy or after instrumentation. The presence of obstruction, stones or high-pressure vesico-ureteric reflux, perinephric abscess, life-threatening septicemia or a combination of these predispose to kidney damage (Huland and Busch, 1984).

Episodes may be refractory to therapy, often resulting in relapses and occasionally leading to significant sequelae such as sepsis, metastatic abscess and rarely acute renal failure. An uncomplicated infection is an episode of cysto-urethritis following bacterial colonization of the ureteral and bladder mucosae. This type of infection is considered to be uncomplicated because sequelae are rare and exclusive due to the morbidity associated with reinfection in a subset of women. A subset of patients with pyelonephritis (acute uncomplicated pyelonephritis), namely, young women who respond well to therapy may also have a low incidence of sequelae.(Nazar *et al.*, 2009).

3.8.2 Recurrent infection-reinfection, relapse and treatment failure

Reinfection is a recurring infection due to a different microorganism that is usually drug susceptible. Most recurring episodes of cysto-uretheritis are due to reinfections that are much more common than relapse and accounts for about 80% of recurrent infections (Nazar *et al.*, 2009).

Unlike relapse, reinfection does not represent failure to eradicate infection from urinary tract but is due to reinvasion of the system. Prophylactic measures must be initiated. Relapse is a return of infection due to the same micro-organism which is often drug resistant. It is defined as the recurrence of bacteriuria with the same organism within three weeks of completing treatment, which during treatment rendered the urine sterile. Relapse implies that there has been a failure to eradicate the infection. This most often occurs in association with renal scars, stones, cystic disease or prostatitis and in patients with chronic interstitial disease or in those who are immune compromised (Nazar *et al.,* 2009).

3.8.3 Asymptomatic bacteriuria

It is defined as the presence of more than 100,000 colony forming unit/ milliliter (cfu/ml) of voided urine in person with no symptoms of urinary tract infection. The largest patient population at risk for asymptomatic bacteriuria is the elderly. Up to 40 percent of elderly men and women may have bacteriuria without symptoms (Orenstein, 1999). Although asymptomatic bacteriuria has been associated with an increase risk of death in the elderly in some studies, a causal link has not been demonstrated (Stamm, 1993).

This is especially common in women as evidenced by a minimum prevalence of 2-4% in young and 10% in elderly women. The cumulative prevalence of asymptomatic bacteriuria in women increases about 1% per decade throughout life regardless of ethnicity and geographic locations.

In contrast to women, the occurrence of asymptomatic bacteriuria in men is rare until after 55 years of age, at which time the prevalence increases per decade and approaches the rate in elderly women. Prostatic hypertrophy and increased likelihood of instrumentation account for the bacteriuria in older men (Nazar *et al.*, 2009).

3.8.4 Symptomatic urinary tract infection

These occur in all age groups. Among newborns and infants, boys are affected more than the girls. When urinary tract is the source of neonatal sepsis, serious underlying congenital anomalies are frequently present. Sexually active women have a markedly increased risk of cystitis. Vast majority of acute symptomatic infections involve young women. A prospective study demonstrated an annual incidence of 0.5-0.7 episodes per patient year in this group(Fihn, 2003). In the absence of prostatitis, bacteriuria and symptomatic UTIs are unusual in men. The risk of cystitis in young men due to uropathogenic E *coli* increases because of lack of circumcision or having a partner with vaginal colonization with such P-fimbriated *E. coli*. At any age, both sexes may develop symptomatic infections in the presence of risk factors that alter urinary flow (Nazar *et al.*, 2009).

3.9 *E. coli* the causative agent of community and nosocomial UTI

In human *E. coli* is the most common cause of UTI. Approximately 85% of urethra cystitis is caused by *E. coli*. UTI caused by *E. coli* usually originates in large intestine as resident or transient members of colon flora and may exist as the dominating or minority strain. Strains that cause UTI are selected from faecal flora due to their special adaptation of urinary tract epithelial mucosa. Moreover, strains of *E. coli* isolated from UTI in immune competent host differ from strain isolated from UTI host compromised by instrumentation (catheter) or by other defect of urinary tract.

UTI are developed in hospitalized patients more frequently than that in the out patients. The difference is due to the general ill conditions of hospitalized patients and higher probability of UTI instrumentation (Rafique *et al.*, 2002).

E. coli is the predominant cause of both community and nosocomial urinary tract infection (Bean *et al.*, 2008). A retrospective study was conducted among out patients and inpatients in Katmandu medical college teaching hospital for 1 month period from 2060/11/15 to 2060/12/15. MSU of 300 patients were cultured. Out of these, urine of 75 patients were found to have significant bacterial growth. Total of five species of bacterial species isolated were *E. coli* 37 (33.3%), *Proteus species* 25 (27.7%), *Klebsiella species* 15 (16.6%), *S. aureus* 8 (8%), *Pseudomonas aeroginosa* 1 (1%) (Karki *et al.*, 2004).

A retrospective study conducted in five hospitals to observe the prevalence of organisms causing UTI and their sensitivity pattern showed that all together six types of organisms were isolated as a causative factors with *E. coli* being the predominant one with 49% followed by *S. aureus* 23%, and *Klebsiella* spp 9.71% (Jha and Bapat 2005).

In a study conducted at Norvic hospital, the urine culture pattern showed that *E. coli* was the most common organism isolated (81.6%) followed by *Proteus spp* (16.5%) (Jha *et al.*, 2007).

In humans *E. coli* is the most common cause of UTI. Approximately 85% of urethrocystitis is caused by *E. coli* (Guentzel, 1991). UTI causing *E. coli* usually originates in large intestine as resident or transient members of colon flora and may exist as the dominating or minority strain. Moreover, strains of *E. coli* isolated from the host compromised by instrumentation or by other defect of urinary tract (Rafique *et al.,* 2002).

In a study pf prevalence pattern of community and nosocomial UTI caused by *E. coli,* a total of 200 urine samples were collected from suspected *E. coli* associated UTI patients. The incidence of *E. coli* was found to be higher in females than in males as out of 200 urine samples, 132 were females and 68 were males. The prevalence of *E. coli* was more in out patients (OPD) than the in patients as there were 141 OPD samples and 95 IPD samples, thereby indicating that this infection also has a community based source of transmission (Rafique *et al.,* 2002).

In one study, a total of 11,865 *E. coli* isolates were cultured form urine samples over a study period. Of these, 10,522 (88.7%) were considered community isolates while 1,344(11.3%) were of nosocomial origin. 10,166 (85.7%) were from women and 1,656 (14.0%) from men (43 sex unknown). 1,227 (10.3%) were from children < 16 years of age (Bean *et al.,* 2008).

In a study carried out at Manipal hospital, UTI was common among females (67.2%) with female to male ratio 2:1. *E. coli* was the most common organism isolated (50%), followed by *Klebsiella* (16.66%), *Proteus* (12.50%) (Malla *et al.,* 2007).

In one study carried out during a period of 1 year, a total of 201 community acquired UTI patients and 253 bacterial isolates were studied. *E. coli* was the leading pathogen and it was significantly more predominant in bacterimic UTI than that in non bacterimic UTI (Lau *et al.,* 2004).

The predominance of Gram negative species, usually Enterobactericae and particularly *E. coli* remain the principal pathogen causing UTI accounting for 75-90% of all urinary tract infections in both inpatients and outpatients (Erb *et al.,* 2007; Foxman *et al.,* 2000). A study was carried out to identify the bacteria causing community acquired UTI; the results showed that of the 912 patients, 34.2% had positive bacterial cultures. The most common isolates were *E. coli* (57.4%), *Klebsiella* pneumonia (9.7%), *S.aureus* 5.8% (Mashouf *et al.,* 2009).

In a test comparing the prevalence of *E. coli* over a ten year period, three hundred specimens were collected of which 187(62.3%) yielded a positive culture of 10^5 cfu/ml. In the age group of

18-54 years, *E. coli* was the most frequently isolated uropathogens (77.5%), followed by *S. saprophyticus* (13.5%) and *Proteus* species 2.7% (Arslan *et al.*, 2005).

3.10 Antibiotic resistance

The impact of antimicrobial agents on public health over past 50 years is unmatched by any other therapeutic class. Precise data on current antibiotic use are difficult to obtain. Such data can be determined in community by the number of prescriptions given by medical officers, health care workers, and medical sales individuals.

Although resistance is reported among antiviral, antifungal and antiparasitic agents and can have a major impact on the management of infected patients, it is the antibacterials, because of the far greater quantity of prescribing and burden of disease, that attract most attention (Barker, 1999).

Resistance is by definition an in vitro phenomenon. Although there is currently no standard methodology for antibiotic susceptibility testing, guidelines do exists in Britain (British society for antimicrobial chemotherapy) and in USA (National committee for clinical laboratory standards).

Disc diffusion testing remains the most commonly employed technique for routine susceptibility testing. The surface of an agar plate is evenly inoculated with the bacterial isolate under investigation and a filter paper disc impregnated with a defined quantity of antibiotic applied to the plate. After incubation, usually overnight, a circular zone of inhibition of growth appears around the discs as a result of diffusion of antibiotic into the agar and inhibition of growth of isolates. The zone size is compared with that obtained with control isolates and a result recorded as sensitive, intermediate or resistant.

A more technically demanding is the determination of an MIC (minimum inhibitory concentration). The MIC is the lowest concentration of an antibiotic that will inhibit visible growth of the isolate under investigation after an appropriate period of incubation. A defined inoculum of bacteria is used in a series of antibiotic dilutions in broth. The method provides a precise measurement which can be compared with a known MIC for a similar (but control) isolate.

Resistance may be either inherent or be acquired by the processes of genetic mutation or gene transfer (Todar, 2002).

Genera of species of bacteria inherently resistant to specific antibiotics (or class of antibiotics) are not uncommon. This may occur at the level of permeability of bacterium to the particular antibiotic or at the target site. Not only is this information predictable and can therefore guide prescribing, it also explains the phenomenon of the 'opportunistic' bacteria where use of an antibiotic favors overgrowth of inherently resistant bacteria and other microorganisms. For example, the penicillin binding protein targets in Enterococcus species have very low affinity for β-lactam antibiotics, particularly the cephalosporins, and their use will favor colonization and, on occasion, subsequent infection with these bacteria. This also explains the development of oral or vaginal candidiasis following antibiotics (Barker, 1999).

The mechanism of acquired resistance falls into one of five categories, although bacteria may employ more than one mechanism:

a. enzymatic modification or destruction of the antibiotic
b. reduced antibiotic uptake into bacterium
c. increased efflux of antibiotic from the bacterium
d. alteration of production of a new target site
e. over expression of the drug

The genetic information that encodes for these mechanisms may emerge by random spontaneous mutation or by gene transfer from other microorganisms. Mutations in particular genes that enable the bacterium to survive in the presence of antibiotic confer an enormous advantage. In the presence of certain antibiotics mutational resistance is more readily selected; examples are listed in table below. Use of these antibiotics should be managed carefully.

Antibiotic-bacteria combination where mutational resistance is more readily selected are:

Antibiotic	**Bacteria likely to develop resistance by mutation**
Rifampicin	All species
Fusidic acid	*Staphylococcus species*

Quinolones	*Staphylococcus species*, *Pseudomonas* species
Cephalosporins	*Enterobacter species, Citrobacter species*

Resistance genes may be present naturally, since many antibiotic classes are natural products and bacteria need to protect themselves. It is the processes of gene transfer coupled with rapid multiplication that disseminate this information; transformation involves transfer of naked DNA following bacterial death into a receptive bacterium; conjugation and transduction gene transfer by cell to cell contact or via bacteriophages, respectively. Besides transduction, transformation and conjugation for most bacteria the most important vector of genetic exchange is the plasmid- a self replicating circular piece of DNA, smaller and separate from the bacterial genome, which transfers into another bacterial strain or species. Smaller still is the transposons, a DNA section of the plasmid which transfers between plasmids and into the genome (Barker, 1999).

3.11 Resistance of *E. coli* in UTI

In a study conducted by Rafique *et al* (2002), the prevalence of *E. coli* was more in out patients (OPD), than in the in patients IPD, thereby indicating that this infection also has a community based source of transmission. *E. coli* isolates were reported to be most sensitive to imipenem and tazobactam, while the greatest resistance of these isolates was against ampicillin and trimethoprim sulfamethoxazole.

A study of London conducted by Bean *et al* (2008) showed that nitrofurantoin was the most effective agent (94% susceptible) followed by gentamycin and cefpodoxime. High rates of resistance to ampicillin (55%) and trimethoprim (40%), often in combination were observed in both sets of isolates. Although isolates exhibiting resistance to multiple drug classes were rare, resistance to cefpodoxime indicative of extended spectrum B lactamase production was observed in 5.7% of community and 21.6% of nosocomial isolates.

In one of the study, out of the various pathogenic organisms isolated, *E. coli* constituted for 93.3%, followed by *Proteus* species, *Klebsiella* species, Citrobacter species, *S. aureus* and others. *E. coli* was found to be most sensitive to amikacin, chlorompehnicol, nitrofurantoin and ofloxacin and least sensitive to most commonly used drugs like cephalexin, nalidixic acid, cotrimoxazole and norfloxacin (Rai *et al.,* 2008).

In a retrospective study conducted among outpatient and inpatient in Katmandu Medical College Teaching Hospital, total five significant species isolated were *E. coli* 33.3%, *Proteus* species 27.7%, *Klebsiella* species 16.6%, *S. aureus* 8.8% and *Pseudomonas* spp 1.1%. *E. coli* was sensitive to nitrofurantoin 83.2%, norfloxacin 67.5%, ofloxacin 81.0%, amoxycillin 43.2%, and nalidixic acid 40.5% (Karki *et al.,* 2004).

In a study conducted at five hospitals of Katmandu valley, the most common organism to cause UTI was found to be *E. coli* (49%), followed by *S. aureus* (23%) and *Klebsiella* (9.71%). The entire organism causing UTI were sensitive to nitrofurantoin, amoxicillin and ciprofloxacin was found to be least effective (Jha and Bapat, 2005).

A study conducted at a tertiary care hospital in Western Nepal showed that *E. coli* was found to be most susceptible to amikacin 98%, followed by gentamycin 87.9%, ceftazidine 80.8%, norfloxacin 98.4% and cotrimoxazole 77.9% (Das *et al.,* 2006).

Mazzulli, (2002), conducted a study in Toronto Canada, found that resistance rates among UTI isolates were highest for ampicillin (39-45%) and trimethoprim-sulfamethoxazole (14-31.4%) and lowest for nitrofurantoin (1.8-16%) and fluroquinolones (0.7-10.0%).

In one study by Jha *et al* (2007), common antibiotic tested were ciprofloxacin, norfloxacin, ofloxacin, nalidixic acid, cefotaxime, ceftriaxzone and cefixime. *E. coli* showed sensitivity to cefotaxime, ceftriaxone and cefixime in most cases. Sensitivity to ciprofloxacin and norfloxacin were less as compared to sensitivity to cephalosporin. *E. coli* was sensitive to cefotaxime in 86% to ceftriaxone in 90.5% and to cefixime in 87.7% cases, where as it was sensitive to ciprofloxacin only in 58.3 % to norfloxacin 46.3% to ofloxacin in 65.5% and to nalidixic acid 29.6%.

The sensitivity pattern of various organisms was studied by Malla *et al* (2007) in which *E. coli* responded better with aminoglycosides, fluoroquinolones and nitrofurantioin but displayed a high resistance to most of the cephalosporins. Resistance was also noticed with ampicillin, amoxicillin and amoxyclav.

One of the studies showed high rate of resistance to ciprofloxacin for *E. coli* which is one of the most recommended drug for treating UTI. This study is similar to the study conducted by Kumari *et al* (2005).

E. coli strain exhibited a high proportion of antimicrobial resistance to ampicillin (80.0%), cephalothin 59%, gentamycin 29%, trimethoprim sulfamethoxazole 56%, and amoxicillin – clavulanic acid 34% which is found in a study conducted by Lau *et al* (2003).

In order to assess multi drug resistance among urinary *E. coli* isolates 11 antimicrobial drugs are tested against 67 isolates form out patient attending in tertiary care teaching hospital and 78 isolates form municipal health unit. 76 % and 22% of the isolates form the tertiary care hospital and municipal unit respectively were resistant to three or more different classes of agents and were considered to present MDR. Among the isolates from the hospital patients 73.0%, 65.0%,58.0% 58.0%, 31.0% were resistant to tetracycline, ampicillin, cephalothin, trimethoprim-sulfamethoxazole and norfloxacin respectively; resistance from the municipal unit patients were 31.0%, 37.0%, 8.0%,29.1% and 12.0% respectively to the same drugs. Altogether the results showed that the rates of resistance found among *E. coli* isolates from the hospital patients reached twice as high resistance as the isolates from the municipal health unit patients (Santo *et al.,* 2007).

Among the β-lactam antibiotics imipenem had the widest coverage against *E. coli* isolates (100%) followed by amikacin (49.0%) and extended spectrum cephalosporin 15-45% (Akram *et al.,* 2007).

Mashouf *et al* (2009) carried out a study on pediatrics. Among antibiotics, imipenem had the widest coverage against *E. coli* isolates. *E. coli* resistance to ampicillin peaked in preteens (76.4%) but was high in teens (65.7%), toddlers (53.4%) and infants 47.6%. resistance to cotrimoxazole peaked in teens (68.3%) but was high in preteens (59.1%), infants 49.4% and toddlers 47.6%.

In a study carried out over a period of 5 year the prevalence of resistance among *E. coli* and all isolates combined was more than 20% for ampicillin, cephalothin and sulfamethoxazole in each year studied. The prevalence fo resistance to trimethoprim and sulfamethoxazole raise from more than 9% in 1992 to more than 18% in 1996 among *E. coli*. In contrast, the prevalence of resistance to nitrofurantoin, gentamycin and ciprofloxacin hydrochloride was 0% to 2% among *E. coli* and less than 10% among all isolated combined, and did not change significantly during the 5 year period (Gupta *et al.,* 1999).

In a comparative study of susceptibility pattern of *E. coli* there was no statistically significant differences when compared with the data from 1996. In 2006, susceptibility of *E. coli* to nitrofurantoin was 100%, to Quinolones 100% to ampicillin 62.8% and to cotrimoxazole 86%, compared with 99.3%, 99.3%, 73.2% and 83.3% respectively in 1996 (Barker, 1999).

The resistance rates of *E. coli* isolates for ampicillin, amoxicillin- clavulonate, cefuroxime, ceftriaxone, floroquinolones, cotrimazole and gentamycin were 55.1%, 37.2%, 32.7%, 23.4%, 15.9%, 25.2%, 41.1%, 6.1% respectively. Floroquinolens were the most common prescribed antibiotics (77.9%), followed by TMP-SMX (10.7%), fosfomycin 9.2%, nitrofurantion 2.1% (Aypak *et al.,* 2009).

A total of 2230 valid *E. coli* strains from female outpatients were isolated and subjected to susceptibility testing. *E. coli* resistance was found to be more common to ampicillin (52.1%), cotrimoxazole 26% and Quinolones 18% where as resistance to amocycillin-clavulanic acid; cefurixime axetil and fosfomycin were below 3% (Gobernado *et al.,* 2007).

Of 176 urine isolates from female students positive for *E. coli*, 29.6% were trimethoprim-sulfamethoxazole resistant and none were nitrofurantoin resistant. Among student with a history of UTI infection resistance to ciprofloxacin was 11.8%, compared to 1.8% among those with prior UTI (Olson *et al.,* 2009).

In a study carried out in Mexico 907 isolates were assessed for susceptibility test, which showed most resistant to ampicillin (74%), sulphamethoprim t(60.1%) and ciprofloxacin 32.6%. the most effective drug was netilmicin (5.1% resistant) and most effective or oral drug was nitrofurantion (7.4%)(Garcia and Cuevas, 2008).

In a study conducted between 2002 to 2006 in Ontario, urinary isolates were resistant to ampicillin (54.4%) and TMP –sulfamethoxozole 40.4%. Patients who had previous admissions for urinary tract infections showed greater resistance to TMP-SMX 70.6%, cefazotin 64.7% and nitrofurantoin 58.8% (Kwan and Onyett, 2008).

In one study on the urinary isolates form symptomatic UTI cases at Santacas University hospital of Saopaulo among 257 children *E. coli* was found in 77%. A high prevalence of resistance was observed against ampicillin and TMP-SMX (55% and 51%). The antibiotic resistance rates for *E.*

coli were Nitrofurantioin (6%), nalidixic acid 14%, 1st generation cephalosporin 13%, 3rd generation cephalosporin 5%, norfloxacin 9% and ciprofloxacin 4%(Guidoni *et al.,* 2008).

A study was carried out to determine the resistance in community and nosocomial *E. coli* urinary isolates in London. Nitrofurantioin was the most active agent (94% susceptible), followed by gentamycin and cefpodoxime. High rate of resistance to ampicillin (55%) and TMP (40.0%) (Bean *et al.,* 2008).

In a study to assess the frequency of resistance to fluorquinolones and to third generation cephalosporin; *E. coli* resistance to fluoroquinolones was 27% with high rate among hospitalized patients. Ten *E. coli* strains were producing extended spectrum beta lactamase and resistant to aminoglycosides and fluoroquinolones (Tagajdid *et al.,* 2010).

A study was conducted among 1838 women in France, 893 of them in patients admitted to the hospital and 945 outpatients in the community. Susceptibility of 1217 *E. coli* isolates to 7 antibiotics were performed. *E. coli* resistance rates in hospitalized and community patients were respectively 47.9% and 39.2% for aminopenicillins, 47.3% and 25.4% for coamoxiclav, 19.2% and 14.1% for cotrimoxazole, 14.3% and 5.7% for first generation Quinolones and 8.9% and 3.7% for fluroquinolones. All these rates were significantly higher among hospitalized patients ($p < 0.05$).

3.12 Community and hospital acquired infection

An infection contracted outside of a health care setting or an infection present on admission is known as community acquired infection where as a hospital acquired infection or nosocomial infection is defined as infections developing in patients after admission to hospital, which were neither present nor in incubation at the time of hospitalization. Such infections may manifest during their stay in the hospital or, sometimes, after the patient is discharged (Chakraborty, 2001).

UTIs are classified as either community acquired or hospital acquired. 70 % of infections are community acquired, usually caused by the bacteria *Escherichia coli* (*E coli*) from the patient's own bowels. Hospital acquired infections are usually *E. coli*, but *Pseudomonas* and *Staphlococci* are important causes, particularly when a surgical instrument such as a catheter is used; instrumentation is the predisposing factor. Hospital infections can often be due to multiple organisms, and antibiotic resistance is a common problem.

The bacteria that cause nosocomial infections may differ from those that cause analogous infections in the community at large. Often they are specialized for survival in the hospital environment where they are subjected to high-use antiseptics and antibiotics. Consequently, nosocomial pathogens may be resistant to a range of antibiotics. Empirical therapy can be difficult for nosocomial infections since it is very difficult to predict the susceptibility of the causative microorganism. Effective treatment regimes can only be employed once the culture and susceptibility results are known. Consequently, blind therapy is often used until laboratory results are available and this is typically of a broad spectrum, to cover the range of possible susceptibilities prevalent within the unit in question. Only after the results of culture and susceptibility testing known are reliable, specific monotherapy begin (Yilmaz *et al.*, 2009).

A look at the causes of urinary tract infections in hospitalized and other patients illustrates the difference between nosocomial and community-acquired infections:

3.13 Causes of Urinary Tract Infections in Hospital Patients:

Escherichia coli: 40%

Proteus mirabilis: 11%

'Other' Gram-negative bacteria: 25%

Coagulase-negative *Staphylococci*: 3%

'Other' Gram-positive bacteria: 16%

Candida albicans: 5%

3.14 Causes of Urinary Tract Infections that is community-acquired:

Escherichia coli: 80%

Coagulase-negative *Staphylococci*: 7%

Proteus mirabilis: 6%

'Other' Gram-negative bacteria: 4%

'Other' Gram-positive bacteria: 3%

The 'other' Gram-negative rods that cause nosocomial infection include members of the family Enterobacteriaceae including *Klebsiella*, *Citrobacter*, *Enterobacter* and *Serratia* species. Other Gram-negative bacteria that are significant causes of nosocomial infection include Pseudomonads and members of the genus *Acinetobacter*.

Urinary tract infections are developed in the hospitalized patients more frequently than outpatients. The difference is due to general ill conditions of the hospitalized patients and the higher probability of UTI instrumentation (Adukauskiene *et al.*, 2006).

The infections in OPD are community based, and their increased numbers are a reflection on the lack of awareness, education and hygienic conditions within our society as a whole. The infection in IPD are hospital acquired, nosocomial infections, which may be due to numerous factors, including poor patient care in hospitals, catheterization and other surgical procedures related to lower abdomen, bowel region(Stamm and Norby, 2001).

In community acquired and nosocomial infections, especially, UTI predispose the causative agents to antibiotics resistance. A major clinical problem is UTI worldwide. Most women experience at least one infection and may suffer multiple recurrences. The most common nosocomial infections are catheter associated UTI that are frequent source of bacterimia (Stamm and Norby, 2001).

There is gradual increasing antibiotic resistance in both community and nosocomial acquired UTI causing uropathogens. Even in women with acute uncomplicated UTI, increasing resistance to ampicillin (30-40%), cephalothin (20-30%) and TMP-SMX (15-20%) has been demonstrated in causative *E. coli* (Gupta *et al.*, 1999).

Higher incidence of community based than nosocomial UTI can be due to high incidence among women, it may be plausible to assume that these women have small children who have not been toilet trained, or during washing may contaminate their hands, nails, etc. Further more another rationale for the high rate of community based infections may be due to the fact in a society, female illness are not taken that seriously, as compared to male illness, which may result in girls and women being asked to stay at home in search for a home remedy for their ailment rather than being sent to hospitals, where treatment may be too costly. Uncomplicated UTI is among the most common infections in out patient’s women with significant morbidity and health cost (Foxman, 2002).

The resistance of *E. coli* against the antibiotic has been increasing gradually and may be attributed to the non compliance of patients, easily availability of antibiotics in market without any prescriptions, irrational use of drugs, wrong dose and dose taken for insufficient length of time.

In a study conducted by Rafique *et al* (2000), the prevalence of *E. coli* was more in outpatients than in inpatients, there by indicating that this infection has a community based source of transmission.

Indiscriminate use of antibiotics has led to development in antibiotics resistance has been shown in many studies. Mean susceptibility of uropathogens was low for cotrimoxazole which often is one of the first line drugs in many countries. The low susceptibility may be due to widespread use of these antibiotics in the community (Das *et al.,* 2006).

Nosocomial isolates were more resistant than community isolates to all the antibiotics: Ampicilllin, Cefalexin, ciprofloxacin, gentamycin, nitrofurantion, trimethoprim, cepfodoxime. The prevalence of gentamycin, ciprofloxacin and Cefpodoxime resistance exhibited the most marked differences (Bean at al, 2008).

In a study a high rate of resistance to commonly used antimicrobial in pathogens isolated from patients with bacterimic and non bacterimic community acquired UTI. Isolates form bacteriemic patients were some what less resistant than those form non bacterimic patients. *E. coli* isolate were predominant in bacterimic patients than in non bacterimic patients (73% vs 49%) $p< 0.01$. The greater concern arising form the results of this study is an increasing resistance of *E. coli* isolates form bacterimic UTI patients to commonly used empirical antimicrobial agents(Lau *et al.,* 2003).

The invitro susceptibility of *E. coli* urinary isolates to the most common antimicrobial agents used for the treatment of patients with UTI varies considerably in different parts of the world. Antibiotics are usually given empirically before laboratory results from urine culture is available. To optimize the use of empirical antibiotic therapy for UTI, it is important for clinician to be aware of the etiological agents and susceptibility pattern of UTI pathogen in their population.

3.15 Diagnosis of UTI

A patient first visits the clinician after feeling some discomfort. Clinical history and examinations is the first step in any diagnosis and the means of identifying patients with suspected UTI. Elements of clinical examination have also been evaluated as diagnostic tests for UTI. Urine tests are commonly used for the diagnosis of UTI.

3.15.1 Collection of Urine Sample

Laboratory urine specimens are classified by the type of collection conducted or by the collection procedure used to obtain the specimen.

3.15.2 Random Specimen: This is the specimen most commonly sent to the laboratory for analysis, primarily because it is the easiest to obtain and is readily available. This specimen is usually submitted for urinalysis and microscopic analysis, although it is not the specimen of choice for either of these tests.

3.15.3 First morning specimen: This is the specimen collected when the patient first wakes up in the morning. The urine is generally more concentrated and therefore, contains relatively higher levels of cellulare elements and analytes such as proteins if present and is considered as a good choice for urine analysis and microscopic analysis. (NCCLS)

3.15.4 Mid stream clean catch specimen: The patient will void first portion into toilet and later in container hence called midstream and is considered as preferred type as there is less incidence of cellular and microbial contaminants. Prevention of contamination by normal vaginal, perenial, and anterior urethral flora is the most important consideration for collection of a clinically relevant urine specimen. The least invasive procedure, the clean catch, mid stream urine specimen collection must be performed carefully for optimal results, especially in females. Uncleaned first void specimens from males were shown to be as sensitive as (but less specific than) MSU specimen (Baron, 1990).

3.15.5 In catheterized patients: Urine for culture should be collected through the sleeve on the tube of the drainage system and not from the drainage bag. An attempt should be made to void the urine present in the catheter, which may show contamination, and collect urine from the bladder.

3.15.6 In infants and children: It is difficult to collect uncontaminated urine via the urethra; a clean catch specimen obtained after careful cleansing of the genitalia is to be preferred, but when this is not practicable a bag specimen may be the only alternative. Suprapubic aspiration of urine is technically easy in the newborn and will provide accurate information. The technique can also be used in adult woman when uncontaminated specimen cannot be obtained by other methods (Smith and Charles, 1990).

3.16 Storage of Urine Sample

NCCLS Guidelines recommend testing urine within two hours of its collection. However, refrigeration or chemical preservation of urine specimens may be utilized if testing or refrigeration within a two-hour window is not possible. A variety of urine preservatives (tartaric and boric acids being the most common) are available that allow urine to be kept at room temperature while still providing results comparable to those of refrigerated urine. Generally, the length of preservation capacity ranges from 24 to 72 hours. Claims for the length of specific analyte preservation should be obtained from the manufacturer. When a specimen is directly transferred from a collection cup into a preservative tube, it provides a stable environment for the specimen until testing can be conducted and reduces the risk of bacterial overgrowth or specimen decomposition. Non-additive tubes (those not containing any chemical preservatives) can be used for urinalysis, but must be handled following strict timing and refrigeration guidelines.

Urine can be preserved with boric acid at a concentration of 1% w/v, bacteria remain viable without multiplying white cells, red cells and casts are also well preserved and there is no interference in the measurement of urinary protein and glucose (Cheesbrough, 1993).

3.17 Rapid Diagnosis of UTI (Urinalysis):

The reference standard for the diagnosis of UTI is considered to be any bacterial growth on a culture of urine. Culture has the disadvantage of taking at least 48 hours to give a result. More rapid methods of UTI diagnosis are therefore desirable.

The most widely used rapid tests are dipsticks. Analytes commonly tested by dipstick includes leucocyte esterase, nitrite, blood, glucose and protein.

3.17.1 Nitirte test:

Most species of bacteria that colonize in the urine converts nitrate in urine to nitrite. A positive nitrite test is highly reliable marker for bacterial urinary tract infection. This test depends on the conversion of nitrate to nitrite in the bladder, a process that can be facilitated by bladder incubation (Block, 1986). In order for this to occur, urine must remain in the bladder for a minimum of 4 hours before voiding. This allows time for the pathogen to convert enough nitrates to nitrite. Therefore the specimen of choice for nitrite detection is the first morning specimen.

3.17.2 Leucocyte esterase test:

Leukocyte esterase is an enzyme that suggests the presence of leukocytes. Normal urine contains small amount of glucose. Bacteria metabolise glucose and so this test tests for the absence of glucose. The leukocyte esterase test detects esterase, an enzyme released by white blood cells. In order for the test to be positive, the granulocytic cells must lyse to release esterase. If they do not lyse, the test will show negative. The test is not sensitive to agranulocytic leukocytes, which may also be present in UTI. Granulocytic leukocytes are usually found in urine when infection is present in the urinary system. Esterase activity in urine is therefore considered a reliable means of detecting pyuria (Zorc *et al.* 2005).

3.17.3 Microscopic analysis of Urine:

Non cultural techniques, WBC count, Pus cell count, epithelial cell count have been proposed as an inexpensive method for presumptive diagnosis of UTI. Although a variety of rapid screening methods have been studied for the detection of significant bacteriuria, urine microscopy remains the single most reliable technique (Corman, 1982).

Even though prevalence of UTI may vary in different population, approximately 80% of urine cultures are negative. In an attempt to reduce the cist and time expended in examining these negative cultures, several rapid methods have been developed for characterizing bacteriuria, included microscopic examination, chemical methods and automated systems. Among these non cultural techniques, WBC count and Gram staining have been proposed as sensitive and inexpensive methods. Qualitative urine culture with $\geq 10^5$ CFU/ml remains the standard diagnostic method to diagnose UTI. Presence of RBC’s and epithelial cells are of less significance for UTI diagnosis (Dhakal *et al.*, 2002).

Pyuria, the presence of WBC in urine, is considered significant if more than or equal to 5 WBC or pus cells are seen per high power field in the sediment. Presence of White blood cells in the urine is called pyuria. Microscopical examination of urine is done principally to detect the presence of increased number of polymorphs as an indication of infection in the urinary tract (Collee, 1996).

Bacteriuria without pyuria may occur in early urinary infections, in diabetes, enteric fever and in bacterial endocarditis. Pyuria with a sterile routine culture may be found with renal tuberculosis, gonococcal urethritis and leptospirosis, or when patient with urinary infection has been treated with antimicrobials (Cheesbrough, 1993).

Gram staining of the urine sample is also the presumptive rapid diagnosis of urinary infection and guidance for initial patient treatment. Unspun urine is gram stained to determine the presence of significant bacteriuria. One micro liter drop of well mixed unspun urine is allowed to dry on the surface of slide without being spread out. The presence of one or more bacteria in most oil immersion fields (1000×) is indicative of more than 1000,000 organisms per ml of urine (Baron *et al.,* 1990).

3.17.4 Bacteriological Culture of Urine

Quantitative urine culture with ≥ 10^5 CFU/ml is the standard diagnostic method to diagnose UTI. An inoculating loop of standard dimension in used to take up a small approximately fixed and known volume of mixed uncentrifuged urine and spread over a plate of culture medium. Most often a calibrate loop designed to deliver a known volume, either 0.01 or 0.001 ml urine is used (Baron, 1990).

CHAPTER-IV

4. Materials and Methods

The present study was conducted at B and B hospital Gwarko,Lalitpur. It was carried out for 1 year period of April 2008 to April 2009. During this period, a total of 555 *E. coli* of urine were isolated which were further studied following standard procedures (Cheesbrough, 1984).

4.1 Materials

All the materials required for present work are listed in the Appendix-II**.**

4.2 Methods

4.2.1 Data Collection

All *E. coli* isolates obtained from urine samples submitted for microscopy, culture and sensitivity to the laboratory of B and B Hospital from April 2008 to April 2009 were included. Samples originating from General practice, accident and emergency were considered representative of community isolates(OPD) whilst samples originating from patient hospitalized for ≥48 hours on general or specialized ward were considered as hospital acquired(IPD) (Bean *et al.,* 2008).

The clinical data of the patient requested for urine culture was collected at the time of sample collection for community patients where as the clinical data of the inpatient were collected from the hospital records. The gathered records include name, age, sex, and type of patient i.e. whether the patient is inpatient or outpatient.

4.2.2 Specimen collection

The patients i.e. the community patients were given a clean, dry, sterile and leak proof container and requested for 5-10 ml mid-stream urine sample. Before providing the container, each patient was instructed properly for the collection of sample. The samples from the admitted patient were collected and brought to the laboratory by the laboratory personnel. The samples were processed as soon as possible. Detailed guidelines for collection of clean catch mid-stream urine are mentioned in Appendix-V.

4.2.3 Processing of the specimen

The specimen obtained in laboratory had to undergo many processes such as macroscopic, microscopic, chemical examination and so on as requested by the doctor in the report form. The macroscopic examination is performed so as to observe the color and appearances of the urine. Similarly, microscopic examination may be performed to detect pus cells RBCs and other types of sediments in the urine. In the same way, chemical examination is generally performed to detect the presence of proteins, glucose etc in the urine. The tests are performed as requested by the doctor before urine culture is performed. As we were concerned about the isolation of *E. coli* from urine, the culture of specimen and the antibiotic susceptibility test was of major concern in this research.

4.2.4 Culture of specimen

In order to isolate the bacteria that cause UTI, it is very important to culture the bacteria. Semi-quantitative culture technique was used to culture urine specimens and to detect the presence of significant bacteriuria by standard methods (Cheesbrough, 1984). At first the provided urine sample was mixed properly and then an inoculating loop of standard dimension was used to take up approximately fixed (±10% error was accepted) and known volume (0.001ml) of mixed uncentrifuged urine. The sample was then inoculated on the surface of MacConkey Agar (MA). The inoculated MA plate was aerobically incubated overnight at 37º C.

The bacterial count was reported as:

a) Less than 10^4 organisms/ml, not significant.
b) 10^4-10^5 organisms/ml, doubtful significance (suggest repeat specimen).
c) More than 10^5 organisms/ml, significant bacteriuria.

4.2.5 Identification of isolate as *E. coli*

Identification of significant isolate as *E. coli* was done by using microbiological techniques which involves morphological appearance of the colonies, staining reactions, biochemical properties and serotyping if required in specific cases. Standard protocol provided by Cheesbrough (1984) was followed for identification of *E. coli* from urine specimens.

Obtaining pure culture for identification: Various types of bacteria may be isolated from urine sample. As this research is concerned about the isolation and identification of particularly *E. coli*, only the colonies that are high lactose fermenters on MA are taken into account.The organism was isolated in pure form before performing biochemical and other tests. Gram staining of an isolated colony was done from primary culture (staining procedures are mentioned in the Appendix-IV. A speck of single isolated colony from MA was transferred into the nutrient broth and incubated at 37ºC for 4 hours. It was then subcultured on dried nutrient agar plate and incubated at 37ºC for 24 hours. Thus obtained overnight incubated culture of organism on nutrient agar was used to perform catalase, oxidase, other biochemical and antibiotic susceptibility test.

Biochemical Test: The standard testing procedures for the identification of *E. coli* 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.

The biochemical tests used for the identification of *E. coli* includes 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 identification characteristics of *E. coli* are provided in the Appendix-VII along with composition and preparation of biochemical media and reagents used in the biochemical test. The procedure for performing biochemical tests are mentioned in Appendix-VI.

4.2.6 Antibiotic susceptibility testing

The antimicrobial susceptibility testing of the *E. coli* isolated and identified was done towards 16 antimicrobial disks. The susceptibility testing procedure was performed by modified Kirby-Bauer disk diffusion method as recommended by National Committee for Clinical Laboratory Standards (NCCLS) using Mueller Hinton agar (MHA). The antibiotic susceptibility procedure is performed in order to find out whether the bacteria are susceptible or resistant towards the given antibiotic. The susceptibility is determined by measuring the diameter of zone of inhibition (in mm) for a particular antibiotic and this was compared with standardized zone interpretative chart provided by the company manufacturing antibiotic discs. The susceptibility procedure is as follows:-

a. Mueller Hinton Agar and broth was prepared and sterilized as instructed by the manufacturer.
b. The pH of the medium 7.2-7.4 and the depth of the medium at 4 mm (about 25 ml per plate) were maintained in petri dish.
c. Using a sterile wire loop, a single isolated colony of which the sensitivity pattern is to be determined was touched and inoculated into Mueller Hinton broth tube and was incubated at 37ºC for 2-4 hrs.
d. After incubation, the turbidity of the suspension was matched with the turbidity standard of Mc Farland tube number 0.5(The procedure for the preparation of Mc Farland tube 0.5 is mentioned in the Appendix III.)
e. Using a sterile swab, a plate of MHA was inoculated with the bacterial suspension using carpet culture technique. The plate was left for about 5 minutes to let the agar surface dry.
f. Using sterile forceps, appropriate antimicrobial discs (6 mm diameter) was placed, evenly distributed on the inoculated plates, not more than 6 discs were placed on a 90 mm diameter petri dish.
g. After overnight incubation, the plates were examined to ensure confluent growth and the diameter of each zone of inhibition in mm was measured and compared with standardized zone interpretative chart provided by the company.

The preparation and composition of Mueller Hinton Agar medium is mentioned in the Appendix-III. Details about antibiotic discs used and its interpretative chart are mentioned in the Appendix-II and Appendix-VIII respectively.

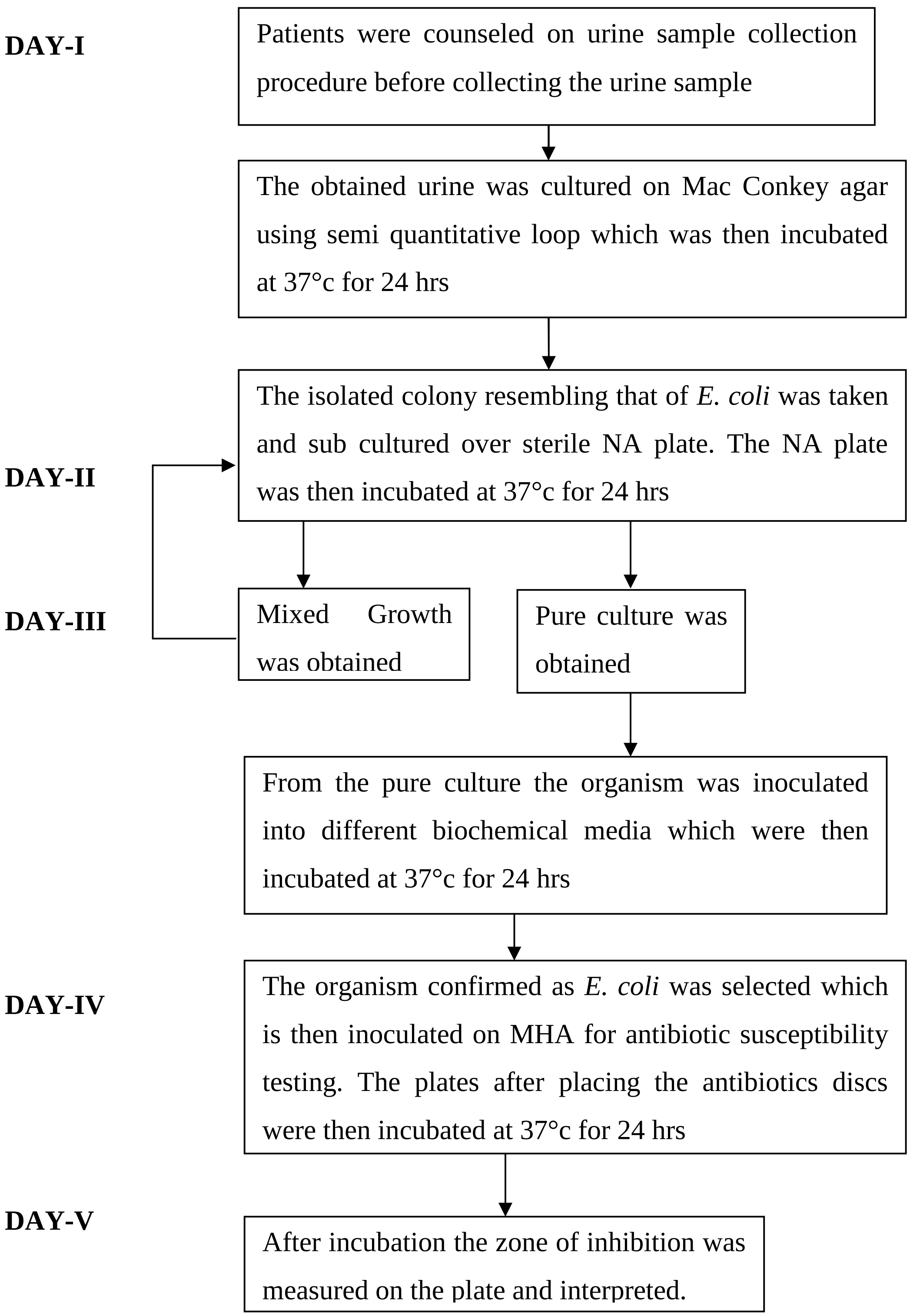
4.2.7 Quality control

By using standard procedures the quality of each test was maintained. The quality of each agar plates prepared was checked by incubating one plate of each lot on the incubator. Control strains of *E. coli* (ATCC 25922) were used for the standardization of the modified Kirby-Bauer test and also for correct interpretation of zone of diameter. Quality of sensitivity tests was maintained by maintaining the thickness of MHA at 4 mm and the pH at 7.2-7.4. Similarly antibiotics discs containing the correct amount as indicated were used. Strict aseptic conditions were maintained while carrying out all the procedures so as to minimize contamination.

4.2.8 Data analysis

The obtained data were analyzed by Chi-square test and odds ratio with the help of computer software Microsoft Excel; 2003.

Flow diagram for processing urine sample



CHAPTER-V

5. Results

A total of 555 *E. coli* isolates were grown from urine samples over the study period of 1 Year (April 2008 to April 2009) at B&B Hospital, Lalitpur Nepal which were further analysed. The bacteria were isolated from urine of in patients (nosocomial) and out patients (community) of B and B hospital.

5.1 Clinical pattern of the result

Among 555 *E. coli* isolates 142 *E. coli* were from inpatient isolates and 413 were from community isolates. Out of total, 209 were from male patient and 346 were from female patient. Similarly, 90 isolates were of nosocomial origin where as 119 isolates were of community origin in males and 52 were from nosocomial origin and 294 were from community origin in case of females.

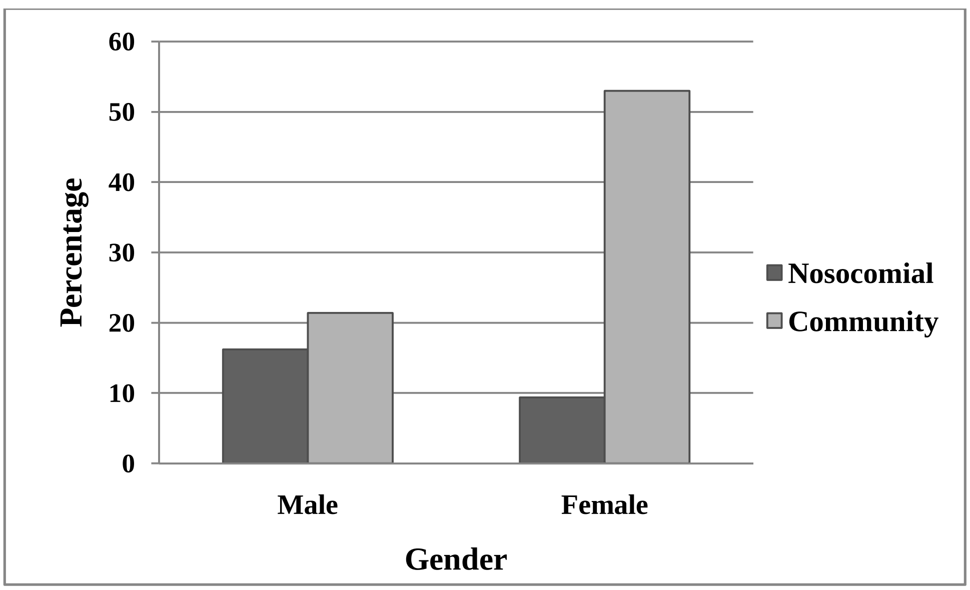


Figure 2: Gender wise distribution of types of patient

As shown in the figure below, out of 555 *E. coli* isolates, 38 isolates were of patients below 16 years (children) of which 11(1.9%) were of nosocomial origin and 27(4.8%) were of community origin. Similarly 517 of those were of above 16 years (adult) out of which 131(23.6%) were nosocomial patients and 386(69.5%) were community patients. This study showed a vast difference of *E. coli* associated UTI in case of children and adults. However in both cases of children and adult the prevalence of community patients were more than twice the nosocomial patients.

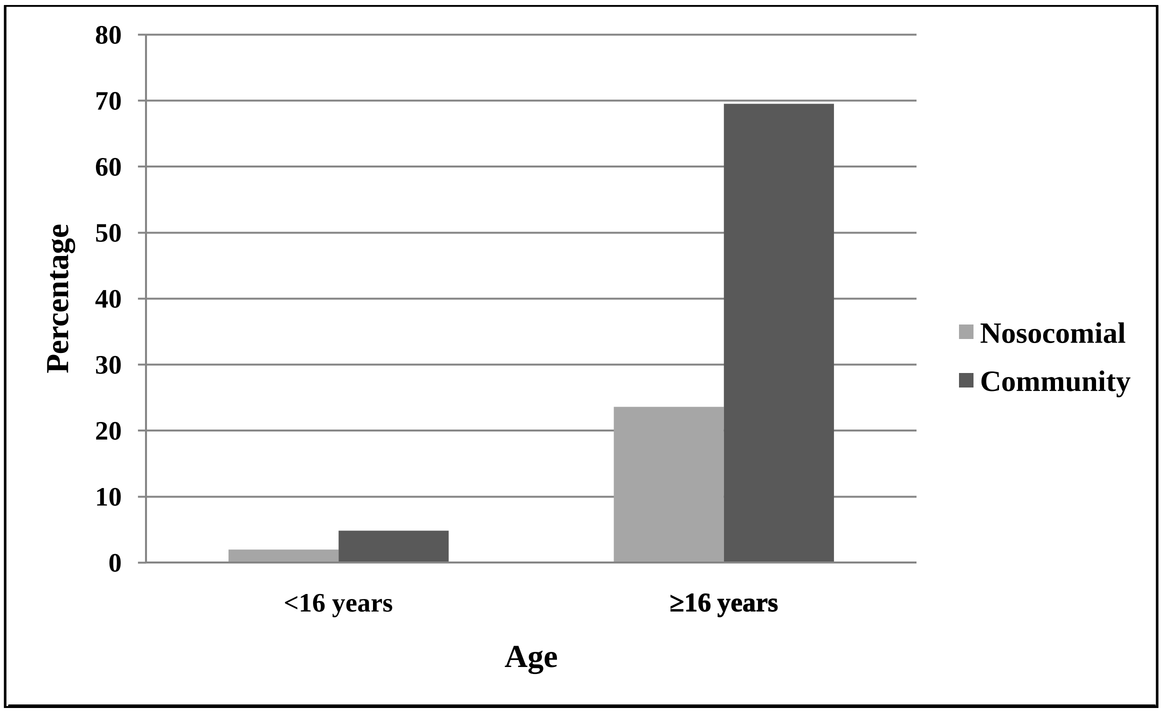


Figure 3: Age wise distribution of *E. coli* isolated from community and nosocomial patients

5.2 Antibiotic susceptibility pattern of *E. coli* isolates.

Antibiotic susceptibility pattern of overall 555 *E. coli* isolates revealed that imipenem, meropenem were the most effective antibiotics in which more than 90% of the isolates were susceptible. After that the next most sensitive antibiotic was cefoperazone sulbactam (84.3%) followed by nitrofurantoin (75.5%) and amikacin (74.1%). As for resistant strains, the isolates of this study were found to be highly resistant to amoxicillin (77.5%) followed by cotrimoxazole (67.2%) and cephalexin (65.2%).This study highlighted on one of the important aspects i.e. the *E. coli* strains possessed high resistance to some of the commonly used antibiotics such as, norfloxacin ofloxacin and ciprofloxacin along with the 3rd generation cephalosporin such as ceftriaxzone and cefotaxime.

Table 1: Antibiogram of *E. coli* isolated from urine samples

S.N	Antibiotic	Sensitive		Resistant		Intermediate	
		N	**%**	**N**	**%**	**n**	**%**
1	Cephalexin	183	33.0	362	65.2	10	1.8
2	Amoxicillin	124	22.3	430	77.5	1	0.2
3	Cotrimoxazole	181	32.6	373	67.2	1	0.2
4	Chloramphenicol	362	65.2	189	34.1	4	0.7
5	Gentamycin	315	56.8	237	42.7	3	0.5
6	Nalidixic acid	156	28.1	396	71.4	3	0.5
7	Norfloxacin	223	40.2	330	59.5	2	0.4
8	Ofloxacin	224	40.4	328	59.1	3	0.5
9	Ciprofloxacin	229	41.3	323	58.2	3	0.5
10	Cefoperazone Sulbactam	468	84.3	81	14.6	6	1.1
11	Imipenem	507	91.4	47	8.5	1	0.2
12	Meropenem	500	90.1	55	9.9	-	-
13	Ceftriaxzone	286	51.5	268	48.3	1	0.2
14	Amikacin	411	74.1	116	20.9	28	5.0
15	Nitrofurantoin	419	75.5	112	20.2	24	4.3
16	Cefotaxime	256	46.1	297	53.5	2	0.4

From the table below, isolates from the males patient were significantly more resistant ($p>0.05$) to all antibiotics except cotrimoxazole and norfloxacin in which no significant association was seen between antibiotic resistance in males and females. In particular, resistance to cefoperazone sulbactam, imipenem, meropenem, amikacin and nitrofurantoin was observed twice as frequently in males than females. In both male and female patients the isolates were more resistant to amoxicillin followed by nalidixic acid. The next most resistant antibiotic was cotrimoxazole in female and cephalexin in male. Regarding most sensitive antibiotic i.e. the antibiotics towards which the *E. coli* was least resistant, were carbapenems. But regarding the empirically used antibiotics such as cotrimoxazole, ciprofloxacin, ofloxacin and norfloxacin the isolates were resistant from approximately 50% to 74%. The odds ratio showed that in all of the antibiotics except amoxicillin and norfloxacin the resistance was twice as greater in female those in males. The highest resistances in males were marked in the antibiotics such as imipenem, meropenem, amikacin and cefotaxime having the odds ratio of more than 3.

Table 2: Antibiotic resistance in relation to gender

S.N	Antibiotic	Male(n=209)		Female(n=346)		p-value	OR
		Resistant		Resistant			
		n	%	n	%		
1	Cephalexin	166	79.43	196	56.65	≤0.05	2.95
2	Amoxicillin	182	87.08	248	71.68	≤0.05	2.66
3	Cotrimoxazole	150	71.77	223	64.45	Ns	1.4
4	Chloramphenicol	95	45.45	94	27.17	≤0.05	2.23
5	Gentamycin	114	54.55	123	35.55	≤0.05	2.18
6	Nalidixic acid	171	81.82	225	65.03	≤0.05	2.42
7	Norfloxacin	135	64.59	195	56.36	Ns	1.41
8	Ofloxacin	155	74.16	173	50	≤0.05	2.87
9	Ciprofloxacin	151	72.25	172	49.71	≤0.05	2.63
10	Cefoperazone Sulbactam	45	21.53	36	10.4	≤0.05	2.36
11	Imipenem	33	15.79	14	4.05	≤0.05	4.45
12	Meropenem	36	17.22	19	5.49	≤0.05	3.58
13	Ceftriaxzone	125	59.81	143	41.33	≤0.05	2.11
14	Amikacin	69	33.01	47	13.58	≤0.05	3.14
15	Nitrofurantoin	64	30.62	48	13.87	≤0.05	2.74
16	Cefotaxime	154	73.68	143	41.33	≤0.05	3.97

Ns: Non significant (for p-value>0.05), OR is calculated taking male as a reference.

The frequency of antibiotic resistance against urinary isolate *E. coli* among two different groups i.e. children (up to 15 years) and adults (≥16 years) is shown below in table 3. Among children, the most powerful antibiotic used was found to be imipenem and meropenem, towards which all the *E. coli* isolated from children was sensitive. Apart from that antibiotics such as cefoperazone sulbactam, nitrofurantoin, amikacin, chloramphenicol and gentamycin also proved to be comparatively sensitive than other antibiotics used. The antibiotics with high resistance were amoxicillin, quinolones and fluoroquinolones, cephalosporin and cotrimoxazole. Similarly, carbapenems and cefoperazone sulbactam proved to be the most sensitive antibiotic even among adults. Besides that, amikacin and nitrofurantoin showed low resistance, however gentamycin being of the same group of amikacin showed higher resistance. The other groups of antibiotics like cephalosporin amoxicillin, quinolones and fluoroquinolones showed higher resistance as always. By the statistical point of view, the association of resistance between children and adult was non-significant in all antibiotics except for amoxicillin and meropenem. A very marked difference was seen in case of amoxycillin in which the odds ratio was 11, that means the resistance of amoxicillin in children was 11 times more than that in adults however no such differences were found with other antibiotics.

Table 3: Antibiotic resistance in relation to age

S.N	Antibiotic	Children(n=38)		Adult(n=517)		p-value	OR
		Resistant		Resistant			
		n	%	n	%		
1.	Cephalexin	27	71.05	335	64.80	Ns	1.33
2.	Amoxicillin	37	97.37	393	76.02	≤0.05	11.67
3.	Cotrimoxazole	22	57.89	349	67.50	Ns	0.66
4.	Chloramphenicol	11	28.95	178	34.43	Ns	0.78
5.	Gentamycin	12	31.58	225	43.52	Ns	0.6
6.	Nalidixic acid	30	78.95	366	70.79	Ns	1.55
7.	Norfloxacin	25	65.79	305	58.99	Ns	1.34
8.	Ofloxacin	24	63.16	304	58.80	Ns	1.2
9.	Ciprofloxacin	22	57.89	301	58.22	Ns	0.99
10.	Cefoperazone Sulbactam	4	10.53	71	13.73	Ns	0.74
11.	Imipenem	0	0.00	47	9.09	Ns	0
12.	Meropenem	0	0.00	55	10.64	≤0.05	0
13.	Amikacin	11	28.95	105	20.31	Ns	1.6
14.	Nitrofurantoin	9	23.68	103	19.92	Ns	1.25
15.	Cefotaxime	24	63.16	273	52.80	Ns	1.53
16.	Ceftriaxzone	18	47.37	250	48.36	Ns	0.96

Children: Patients upto the age of 15 years, Adult: Patients of the age of 16 years or above

OR are calculated taking children as a reference.

The data from table 4 showed that the nosocomial isolates were predominantly more resistant to all the antibiotics than the community isolates as shown in figure 6. Among all 16 antibiotics used, imipenem, meropenem, amikacin, nitrofurantoin, cefoperazone sulbactam were the most sensitive antibiotics in both cases of community and nosocomial isolate. Apart from that, the resistance towards cephalexin, quinolone and fluoroquinolones, amoxicillin and cotrimoxazole was very much higher among the nosocomial isolates i.e. more than 80% of the isolates were resistant towards these antibiotics which in case of community isolates ranged from 49% to 73%, being amoxicillin the most resistant of all in both cases. The resistance percentage seen in gentamycin and chloramphenicol was twice in nosocomial isolate than the community isolates. From the chi-square test, it was determined that association of antibiotic resistant between nosocomial and community isolates was significant for the entire antibiotic except for meropenem (p>0.05). The resistance to all the antibiotics except meropenem in case of nosocomial isolates was observed more than twice as frequently as those from community isolates but the resistance in case of antibiotics such as nitrofurantoin and cefotaxime the resistance in nososcomial isolates was more than five times that those in community isolates.

Table 4: Antibiotic resistance among Community and Nosocomial isolates

		Nosocomial (n=142)		**Community (n=413)**			
		Resistant		**Resistant**			
S.N	**Antibiotic**	**N**	**%**	**N**	**%**	**p-value**	**OR**
1.	Cephalexin	116	81.69	246	59.56	≤0.05	3.03
2.	Amoxicillin	127	89.44	303	73.37	≤0.05	3.07
3.	Cotrimoxazole	116	81.69	257	62.23	≤0.05	2.71
4.	Chloramphenicol	75	52.82	114	27.6	≤0.05	2.94
5.	Gentamycin	100	70.42	137	33.17	≤0.05	4.8
6.	Nalidixic acid	122	85.92	274	66.34	≤0.05	3.09
7.	Norfloxacin	114	80.28	216	52.3	≤0.05	3.71
8.	Ofloxacin	114	80.28	214	51.82	≤0.05	3.79
9.	Ciprofloxacin	117	82.39	206	49.88	≤0.05	4.7
10.	Cefoperazone sulbactam	31	21.83	50	12.11	≤0.05	2.03
11.	Imipenem	19	13.38	28	6.78	≤0.05	2.12
12.	Meropenem	19	13.38	37	8.96	Ns	1.57
13.	Amikacin	63	44.37	53	12.83	≤0.05	4.16
14.	Nitrofurantoin	60	42.25	52	12.59	≤0.05	5.42
15.	Cefotaxime	99	69.72	198	47.94	≤0.05	5.08
16.	Ceftriaxzone	104	73.24	164	39.71	≤0.05	2.5

OR is calculated taking Nosocomial as a reference.

5.3 Analyses of the MDR (Multi Drug Resistant) isolates

From this study, out of total 555 *E. coli* isolates, 389 isolates were found to be multi drug resistant (MDR) i.e. these isolates were resistant to 3 or more than 3 drugs. The isolates resistant to 0 drugs, 1 drug, and 2 drugs were 9.2%, 10.3%, 10.4% respectively which concludes that only 9.2%of the total isolates were sensitive to all the antibiotics used.

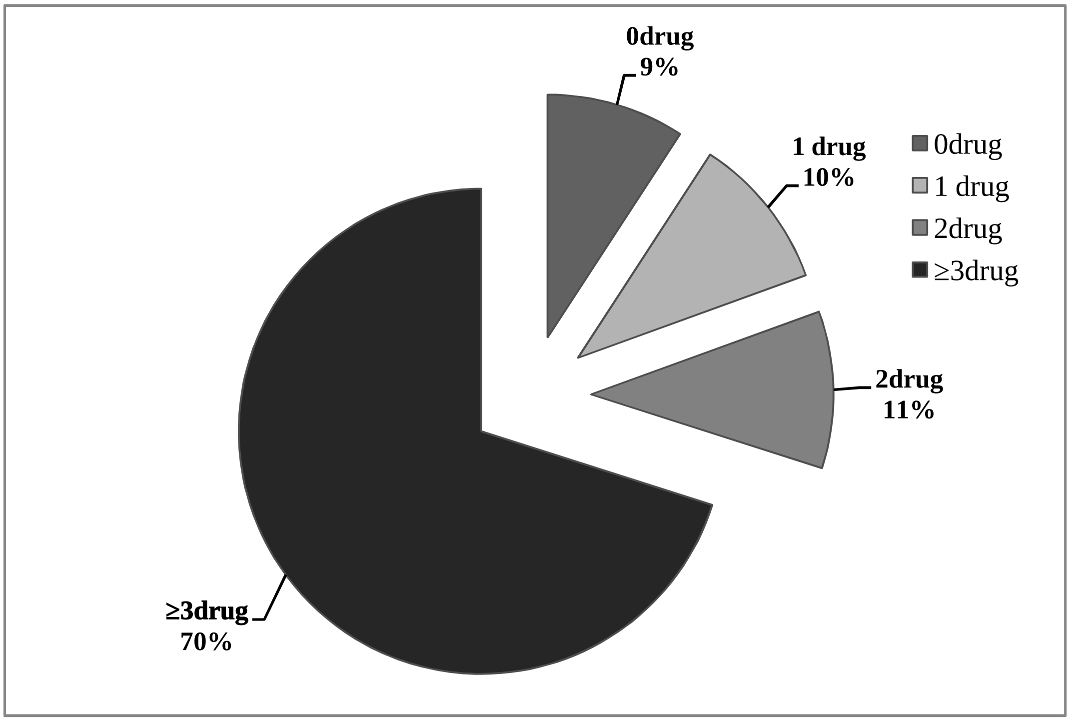


Figure 4: Resistance pattern of total *E. coli* isolates

Higher rate of MDR strains was found in male patients (84.2%) compared to female patients (61.6%).The association of MDR and non MDR strains in male and female was found to be statistically significant. The presence of MDR isolates in male were more than thrice as that in the isolates of female patients (OR=3.33). The result is shown in table 5.

Table 5: Gender wise distribution of MDR strains

Gender	Total no of *E. coli* isolates	Total no of MDR isolates (%)	p-value	OR
Male	209	176 (84.2%)		
Female	346	213 (61.6%)		
Total	555	389 (70.1%)	<0.05	3.33

91.5% of *E. coli* isolates from nosocomial isolates were MDR where it was only 62.7% in case of community isolates. This data was statistically significant as the p value was calculated to be less than 0.05. The odds ratio of the value of 6.44 proved that the resistances in nosocomial isolates were very high as compared to that in community isolates. Thus we can conclude that MDR isolates were significantly higher in nosocomial isolates than the community isolates which is shown in table 6.

Table 6: Distribution of MDR isolates in terms of types of patient.

Patient type	**Total no of *E. coli* isolates**	**Total no of MDR isolates (%)**	**p-value**	**OR**
Nosocomial	142	130 (91.5%)		
Community	413	259 (62.7%)		
Total	555	389 (70.1%)	<0.05	6.44

The percentage of MDR isolates in Children was found to be significantly higher than that in adult patients. The presence of MDR *E. coli* isolates in children was almost thrice that of Adult (OR=2.98). This result highlights the fact that the urine infection by MDR *E. coli* is prominent in adult than in children as shown in table 7.

Table 7: Age wise distribution of MDR *E. coli* isolates.

Patient's Age	**Total no of *E. coli* isolates**	**Total no of MDR isolates (%)**	**p value**	**OR**
Children	38	33(86.8%)		
Adult	517	356(68.85%)		
Total	555	389 (70.1%)	<0.05	2.98

From the figure below it was shown that out of 389 MDR *E. coli*, 380 i.e. 97.7% of the isolates were found to be resistant to nalidixic acid followed by amoxicillin which was resistant in 96.4%of the cases. The carbapenems and cefoperazone sulbactam were the antibiotics which were least resistant for MDR cases. Antibiotics from cephalosporin and quinolones groups also showed high percentage of resistance while amikacin and nitrofurantoin showed less resistance as compared to others.

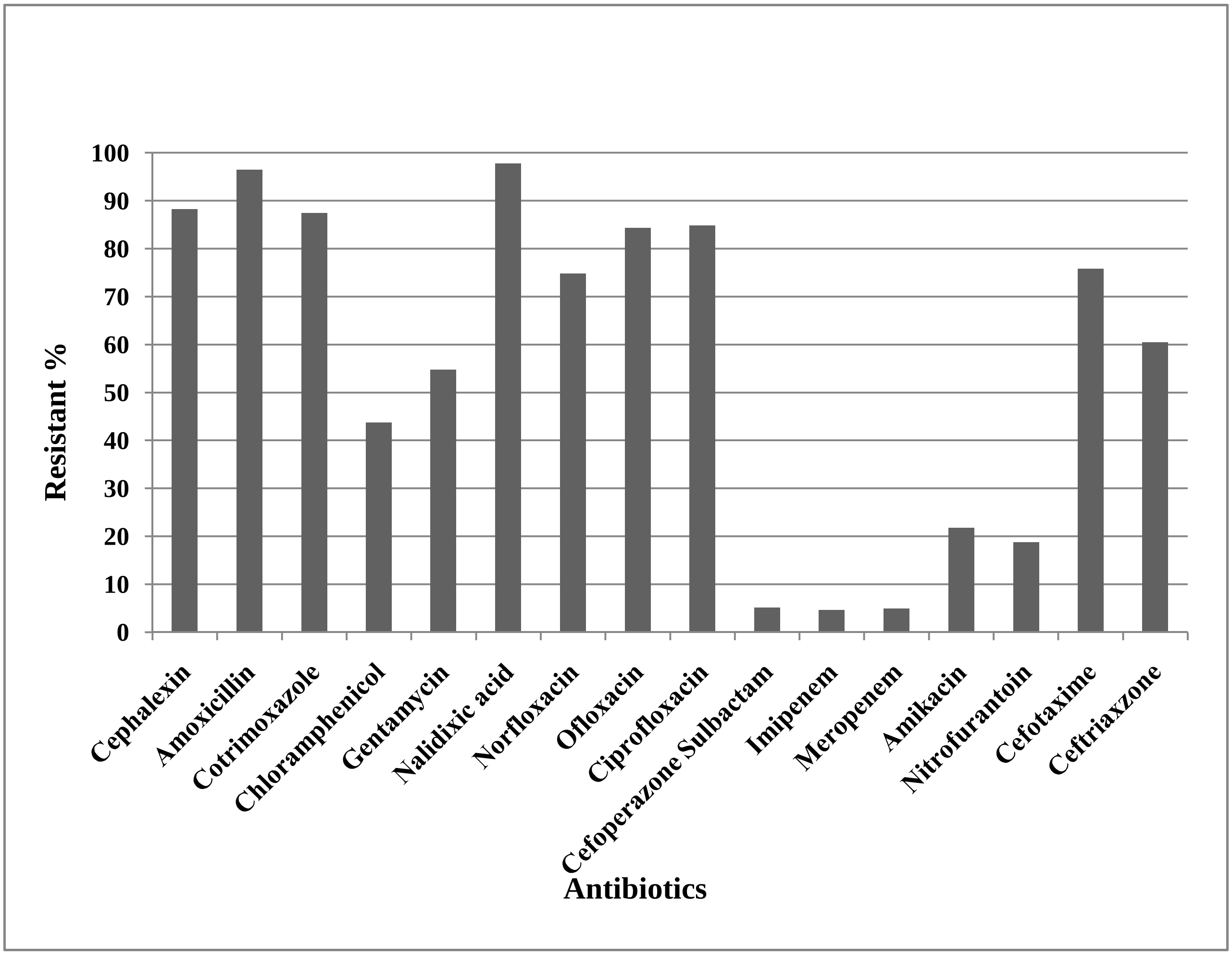


Figure 5: Antibiotic resistance pattern of MDR *E. coli* isolates

Photographs

Photo 1: Pure culture of *E. coli* on MacConkey agar.

Photo 2: Biochemical test for the confirmation of *E. coli.*

Photo 3: Antibiotic susceptibility test of *E. coli* showing inhibition zones on Mueller Hinton Agar

Photo 4: Antibiotic susceptibility test showing MDR *E. coli*

CHAPTER-VI

6. Discussion and Conclusion

6.1 Discussion

Resistance to antibiotics is highly prevalent in bacterial isolates worldwide particularly in developing countries. Routine monitoring of antibiotic resistance provides data for antibiotic therapy and resistance control (Okeke *et al.,* 2000). The increasing prevalence of infections caused by antibiotic resistant bacteria makes the empirical treatment of UTIs more difficult and outcome unpredictable (Kariuike *et al.,* 2008).

The etiology and UTI and the antibiotic resistance of uropathogens have been changing over the past years both in community and nosocomial infection (Akram *et al.,* 2007).*E. coli* is the commonest cause of community and nosocomial UTI. The prevalence of resistance in *E. coli* is a useful indicator of antibiotic resistance in the community. Studies with *E. coli* are of particular relevance because this species can occupy multiple niches including human and animal hosts. In addition *E. coli* effectively exchanges genetic material with pathogen such as *Salmonella, Shigella, Yersinia, Vibrio,* as well as pathogenic *E. coli* (Okeke *et al.,* 2000).

Antibiotic treatment is usually empirical relying on susceptibility data for surveillance studies. This study was therefore set out to determine levels of resistance to 16 antimicrobial agents among *E. coli* isolates obtained a period of 12 months at a laboratory of B and B hospital. Five hundred and fifty five *E. coli* isolates were collected from patients visiting the outpatient department (Community) as well as from the patients admitted to the hospital (nosocomial) for more than 48 hours. The *E. coli* isolates were examined for the antibiotic susceptibility test by using altogether 16 antimicrobial agents. The result obtained were tabulated and analyzed in the previous chapter while the results are compared and discussed with the results of other researchers in this chapter.

Out of the total 555 *E. coli* isolates, the gender wise distribution showed that, 62.3% were from female patient whereas 37.6% were from male patient. This outcome showed that females are mostly affected by UTI than male even in the case of infection caused by *E. coli*. This study is related to almost all studies performed on UTI till now which gives the information about female being as high risk of UTI than males. A similar study carried out by Rafique *et al* (2002) showed a decided inclination toward one sex as 66% *E. coli* isolated were from female patient and 44% were from male patient. In a study conducted by Bean *et al* (2008) similar result was obtained in which out of 11,865 *E. coli* isolates studied, 85% were from female and 14% were from male patient. UTI occur more often in women than men at least particularly because of the short female urethra and its proximity to anus. Additionally, sexual activity serve to increase the chances of bacterial contamination of the female urethra, pregnancy causes anatomical and hormonal changes that favor development of UTIs, and change in the genitourinary tract mucosa related to menopause may play a role. Colonization of the introitus by coliforms is a major background factor for recurrent bladder infection in females. Recent studies have shown that use of contraceptives foams and gels and a diaphragm results in significantly greater colonization of the vagina than sexual intercourse with no such factors (Baron *et al.,* 1990).

In this study, 74.4% of the *E. coli* isolated were found to be of community origin whereas 25.6% were found to be of nosocomial origin. This data suggests the fact provided by Rafique *et al* (2002) in which 70.5% of the total cases were from community and 29.5% of the cases were from nosocomial origin; similar data can be achieved in the study performed by Bean *et al* (2008) the reason for this difference may be due to poor hygienic condition within our community, our house hold and a lack of education and proper personal hygiene practices. Plus with the high incidence among women, it may be plausible to assume that these women have small children who are not toilet trained or during washing may contaminate, their hands, nails etc. which increases the community people to be infected from UTI. In case of nosocomial patient, it is well documented that they have higher probability of UTI which is due to the fact that hospital patient have higher probability of catheterization which makes them susceptible to infection (Das *et al* (2006); Rafique *et al* (2002)).

As with the age wise prevalence pattern of *E. coli* associated UTI, this study showed an interesting pattern, in that there was a much higher incidence of UTI among adults (≥16years) than among children (<16years). Out of total 555 isolates, 38 isolates were from children whereas 517 isolate, were obtained from adult. This difference was seen in both community and nosocomial isolates where greater incidence of *E. coli* associated UTI as seen among adults.

Similar results were obtained in the study conducted by Bean *et al* (2008) in which only 10% of the *E. coli* isolated were from children. The incidence of *E. coli* associated UTI in children may be due to the fact that they are not toilet trained so have a greater chance of infection by *E. coli* (Rai *et al.*, 2007). The study conducted by Rafique *et al* (2002) also showed that out of 200 isolates, only 27 isolates were from children. The reason underlying high infection rate in adult may be due to the onset of reproductive age. In the age of 20-30 years, both males and females are sexually active which may trigger the urinary tract infection in these age groups. Especially in females, sexual activity serves to increase the chance of bacterial contamination (Baron *et al.*, 1990). Also, the anatomic or functional defects in the post menopausal women may be contributing factor to UTI as there is a decrease in the estrogen level that results in the colonization of *E. coli* which has also been reported by other researchers (Ronald and Alfa, 1991; Raz, 2001).

The adult group in this study includes both males and females. The incidence of UTI in male is however low until the age of 60. After that the prostatic hypertrophy contributes to the development of UTI. Bacterial prostatistics is the key background for the problem of recurrent cystitis in males (Baron *et al.,* 1990). This is in agreement with the report of Stamm and Norby, (2009).

Antimicrobial resistance is a growing problem and a cause of great concern throughout the world. Knowledge of antimicrobial resistance trends among isolate of uropathogens is essential to provide clinically appropriate and cost effective therapy (Yilmaz *et al.,* 2009). Periodic evaluation of antimicrobial activity of different antibiotics is essential as their pattern of antibiotic sensitivity may vary over short periods. Increasing antibiotic resistance among urinary pathogens especially *E. coli* to commonly prescribed drugs like cotrimoxazole has become a global reality (Rai *et al.*, 2008).

This study provides an update on *E. coli,* the main cause of UTI in nosocomial and community patients and its antimicrobial susceptibility pattern. The frequency of antimicrobial susceptibility to all isolates to 16 antibiotics is shown in table 1. From the result, it was obtained that carbapenems i.e. imipenem and meropenem were the most sensitive antibiotics out of the 16 antibiotics used. The carbapenems are considered as the most potent of any antibiotic class and are used for serious injection or when resistance compromises all other agents. These antibiotics are stable to ESBLs and usually remain active against stably depressed Gram negative mutants that hyper produce genomic β-lactamases. However certain bacteria of Enterobactericae may produce genomic enzyme that hydrolyses carbapenems (Barker K.F, 1999) is related to the studies conducted by Lau *et al* (2004), Mashouf *et al* (2008) and Akram *et al* (2007) in which the *E. coli* isolated showed high sensitivity to carbapenems.

Similarly, cefoperazone sulbactam was the next sensitive antibiotic apart from carbapenems. Almost 84% of the isolates showed sensitivity towards Cefoperazone sulbactam. cefoperazone sulbactam has affinity to Penicillin binding proteins (PBPs) and inhibition of peptidoglycan (PG) synthesis. It has high affinity towards *E. coli* PBP-3which participate in cell division. Thus inhibits the cross link formation in PG synthesis in *E. coli* which therefore has high antibacterial activity (Matsubara *et al.,* 1980).

Amikacin and nitrofurantoin showed almost similar type of sensitivity of approximately 75% to the *E. coli* isolates. Whereas gentamycin being of the same group of amikacin (Aminoglycosides), differed in their sensitivity. Gentamycin was approximately 57% sensitive where as amikacin was 74% sensitive. According to the study conducted by Barker K.F (1999), resistance is an unusual phenomenon in Gram negatives. They produces the far greatest number of enzymes which can modify gentamycin to a greater extent than amikacin as gentamycin is susceptible to modification by a larger number of enzymes and amikacin is affected by fewer.

Regarding sensitivity of nitrofurantoin, the result of this study in accordance with the studies of Das *et al.* (2006), Jha and Bapat (2005), Karki *et al.* (2004), Bean *et al.* (2006), and Malla *et al* (2007) in which more than75% of *E. coli* isolates were sensitive to nitrofurantoin which extended up to 100% in some studies.

The resistance to quinolones i.e. nalidixic acid and Fluroquinolones i.e. ciprofloxacin, ofloxacin and norfloxacin was found to be much higher approximately 71% of the isolates were resistance to nalidixic acid whereas approximately 60% of the isolates were resistant to Fluroquinolones. This result has been supported by the studies provided by Rai *et al.* (2008). It was also supported by the study of Karki *et al.* (2004); however the resistance towards Fluroquinolone was greater than nalidixic acid. ciprofloxacin is the most frequently prescribed Fluroquinolone for UTIs because of its availability in oral and intravenous formulations (Kariuki *et al.,* 2007).However resistance to Fluroquinolones has increased over the past years and therefore local antimicrobial pattern needs to be monitored regularly.

In case of cephalosporin, 3 types of cephalosporin have been included in this study. They are cephalexin, a first generation cephalosporin, ceftriaxzone and cefotaxime a 3rd generation cephalosporin. The *E. coli* isolates resistant to cephalexin, ceftriaxzone and cefotaxime were 65%, 48% and 53% respectively. Among these, cephalexin was the most resistant cephalosporin. This phenomenon is supported by the study of Rai *et al.* (2008) and Rajbhandari *et al. (*2002). ceftriaxzone and cefotaxime being the 3rd generation antibiotic are however less resistant than cephalexin. Same result of high resistant percentage to cephalosporin by *E. coli* was found in the study of Malla *et al.* (2007). However this result was in contrary with the study provided by Jha *et al.* (2007) where higher sensitivity toward cephalosporin was observed. Resistance to 3rd generation cephalosporin is caused by the acquisition of plasmids containing gene that encode for ESBL and these plasmids often carry other resistant genes as well. ESBL producing *E. coli* is now relatively common in health care settings and often exhibit multi drug resistance (Malla *et al.,* 2007).

In the remaining 3 antibiotics, i.e. amoxicillin, Chloramphenicol and cotrimoxazole, chloramphenicol was found to be more sensitive than cotrimoxazole and amoxicillin. Amoxycillin was the most resistant antibiotic out of all 16 antibiotics used. *E. coli* isolated showed approximately 75% resistance to this antibiotic. Ampicillin or amoxicillin were once standard therapy for UTI but resistance of *E. coli* to amoxicillin is now very high in most regions of the world (Yilmaz *et al.,* 2009). Similar result is found in the study of Malla *et al.* (2007) in which the *E. coli* isolates were 91.66% resistant to amoxicillin. However in study conducted by Jha and Bapat (2005), amoxicillin was found to be the most sensitive drug in three hospitals of Kathmandu which is in contrast to this result.

In the same way, the next resistance antibiotic apart from amoxicillin was cotrimoxazole (67%), but in the study of Jha and Bapat (2005) and Das *et al.* (2006) cotrimoxazole was found to the sensitive drug. However in most of the other studies of Rai *et al.* (2008), Akram *et al.* (2007) Rafique *et al.* (2002) and Malla *et al.* (2007) high resistance of *E. coli* was seen with cotrimoxazole which is probably due to the fact that this antibiotic has been widely used in treating UTI in many part of the world.

From the susceptibility pattern of *E. coli* with chloramphenicol, it was however more sensitive than fluroquinolones cephalosporin and even gentamycin. 65.25% of the total *E. coli* was susceptible to chloramphenicol whereas only 34% were resistant. This result is in close agreement with the result provided by Rai *et al.* (2008) in which 50% of the *E. coli* were susceptible to chloramphenicol and it was the effective drug after amikacin.

Overall, *E. coli*, was found to be most sensitive to carbapenems, amikacin, nitrofurantoin, cefoperazone sulbactam where as less sensitive to commonly used drugs like cephalexin, nalidixic acid, cotrimoxazole, fluroquinolones etc. This appears to be due to the overuse and misuse of antibiotics (Rai *et al.*, 2008).

Antimicrobial resistance is a global problem. It is now generally accepted as major public health issue and has a 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 of antimicrobial resistance is more troublesome to developing counties. WHO and 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, frequency of antibiotic susceptibility in relation to gender was studied. The urinary isolated from male were significantly more resistant ($p<0.05$) to all the antibiotics except norfloxacin in which the relation was found to be non-significant. The incidence of urinary tract infection was low in male than female however the resistance pattern showed that the *E. coli* isolated from male were significantly more resistant towards the antibiotics than that from female. Similar result can be obtained in a study conducted by Bean *et al.* (2008) in which the *E. coli* from men were significantly more resistant to all the 8 antibiotics than that from women. This study is also supported by the study of Yilmaz *et al.* (2009) which states that despite the fact that women have higher prevalence of UTI because of anatomic and physical factors, male patient have higher resistance rates.

Similarly, the frequency of antibiotic susceptibility in relation to age was studied. Different resistance pattern was observed between children (<16 years) and adults (≥16 years). However the incidence of *E. coli* associated UTI was very low in children as compared to adult which was previously discussed. In children the antibiotics such as carbapenems were found to be extremely potent as 100% of the isolates were sensitive to them. In children *E. coli* responded high resistance to ciprofloxacin, cephalosporin, amoxicillin and nalidixic acid whereas amikacin, nitrofurantoin, chloramphenicol and cefoperazone sulbactam were less resistant. The resistance pattern was almost same in case of the adult patient as well but the difference was that around 10% of the isolates were resistant to carbapenems in adults unlike that in children. From the statistical analysis the association of antibiotic resistance between adult and children was found to be insignificant except for amoxicillin and meropenem in which significant association was observed.

The resistance pattern among nosocomial patient was found significantly greater (except for meropenem) than that among community patient. Higher resistance rates of more than 80% was seen in 7 different antibiotics out of 16 antibiotics used. Almost 70% of the isolates were resistant to gentamycin, ceftriaxzone and cefotaxime. Low resistance was seen in case of carbapenems, cefoperazone sulbactam, amikacin and nitrofurantoin which was however greater as compared to community isolates. This result is supported by the study of Bean *et al.* (2008).

Multiple drug resistance (MDR) is generally defined as resistance to three or more different classes of antibiotics. However, some define MDR as resistance to usually employed drugs. In this study, MDR *E. coli* is defined as the strains resistant to 3 or more than 3 different classes of antibiotics out of 16 antibiotics used. This study defines current occurrence of multi drug resistant *E. coli* among UTI isolates from nosocomial and community patients. In this investigation 389 out of 555 *E. coli* isolates were found to MDR strains i.e. about 70% of the total isolated were triple resistant (resistant to 3 or more antibiotics) isolates which were resistant to 2 drugs (dual resistant) and 1 drug (monoresistant) were approximately 10% where as only 9% of the isolates were not resistant to any of the antibiotics used. A very high percentage of MDR *E. coli* obtained in this study was different from the study of other researches like Shrestha *et al.* (2007) in which only 51.3% of *E. coli* were MDR and Tuladhar *et al.* (2001) in which only 22.2% were MDR. However in a study conducted by Manandhar *et al.* (2005) 61.7% of the total *E. coli* isolates were found to be MDR but the criteria for MDR were organism resistant to 2 or more than 2 antibiotics. Outcome of prevalence of MDR depends on various factors, MDR criterion being the chief followed by the type of antibiotics used in antibiogram and study population. The emergence of MDR is clearly related to the quality of antibiotics and how they are being used. It might be possible that high level of MDR was most probably due to production of ESBL in these isolates (Akram *et al.,* 2007).

In a gender wise distribution of MDR *E. coli*, higher rate of MDR was found in male 84.24 %(176/209) than female 61.6% (213/346). This difference was also found to be statistically significant. This is in agreement with the findings of Manandhar *et al.* (2005) and Shrestha *et al.* (2007) in which all MDR urinary isolates (not particularly *E. coli*) were found to be higher in males than female. This trend likely reflects the tendency for males to present more often with complicated UTI, which may be associated with male resistant pathogen (Sahm *et al.* (2001); Bean *et al.* (2000)).

In a patient wise distribution of MDR species, a very high inclination was seen among nosocomial patient 130/142 (91.5%) than that in community patient 259/413 (62.7%). In an investigation performed by Santo *et al.* (2007) similar results were seen in which the percentage of isolates showing MDR was high in hospital patients (76.0%) than in the municipal samples (22%).This is because antibiotic resistance is more prevalent in inpatient that than in outpatient setting (Miller and Tang, 2004) It is quite alarming that high number of MDR isolate were seen among hospitalized patient. Antibiotic resistance is becoming a big problem for public health which has threaten the lives of hospitalized individual, as well as those with chronic conditions and add considerably to health care cost (Akram *et al.*, 2007). This difference of MDR isolates among community and nosocomial patient were also statistically significant.

High percentage of MDR isolate was present among both the pediatric (<16years) and adult (≥16 years) patient which was 86.8% and 68.25% respectively. However, the MDR isolate among pediatric patient was seen to be higher than that in the adult patient which was also statistically significant ($p>0.05$). This may one of the reasons for the mortality in children in Nepal where complicated UTI and subsequent renal failure still continue to be one of the major causes (Malla *et al.,* 2007).The higher rate of MDR in adult might be related to antibiotic resistance in elder groups which is also shown in a study of Gobernado *et al.* (2007).

The antibiotics resistance pattern MDR *E. coli* isolates to the 16 antimicrobials was shown in figure 5. The result revealed that nalidixic acid; amoxicillin, cephalexin and cotrimoxazole respectively were the top most antibiotics showing the resistance to the MDR *E. coli* isolates. They showed the resistance in 97.7%, 96.4% 88.2% and 87.4% of the isolates respectively. Similarly, the isolates were resistant to fluoquinolones to a greater extent i.e. approximately 84% of the MDR isolates were resistant to fluroquinolones except norfloxacin which was resistant in approximately 75% of the cases. Among cephalosporins, 2nd generation cephalosporin i.e. cefotaxime and ceftriaxzone were 75.8 % and 60.4% resistant respectively. Comparatively low resistance was seen amongst gentamycin (54.7%) and chloramphenicol (43.7%) whereas even lower resistance was showed by amikacin and nitrofurantoin i.e. of 21.8% and 18.7% respectively. Lowest resistance rates were performed by carbapenems and cefoperazone sulbactamwhich was their characteristic feature even in case of the total *E. coli* isolates. Since the carbapenems are the potent antibiotics and are used in case of serious infectious only they are less used in general and this might be the reason for their lower resistance towards MDR *E. coli* (Barker, 1999).

The resistance of *E. coli* against the antibiotic has been increasing gradually which may be attributed to non-compliance of patients, easily availability of antibiotics in market without any prescription. Furthermore this study has highlighted the clinicians to perform culture sensitivity test before prescribing any antibiotic to ensure proper and effective therapy and curb the chances of spread of resistance.

The infections in OPD are community based and their increased numbers are reflections of awareness, education and hygienic conditions within our society as a whole. The infections in IPD are hospital acquired nosocomial infections, which may be due to numerous factors including poor patient care in hospitals, catheterization and other surgical procedures related to lower abdomen and bowel region (Ronald and Alfa (1991) and Stamn and Norby (2001)). The usefulness of gradually every new antibacterial drug introduced into medicine has sooner or later been threatened by the emergency of resistant strains. The contribution of laboratory research to the control of resistance has been highly significant but nevertheless limited.

Effective management of UTIs in both inpatient and outpatient settings has been complicated by the fact that many uropathogenic strains has developed resistance to antimicrobials (Shrestha *et al.,* 2007). Therefore as a general rule, to control the spread of community based infections, awareness of UTI should be spread though the use of print and electronic media, school going children should be educated about personal hygiene and so on. With regards to nosocomial infections, better patient case must be emphasized in hospitals through stricter regimes and administration (Rafique *et al.,* 2002).

6.2 Conclusion

This study was basically carried out to determine the prevalence of *E. coli* as a urinary pathogen among nosocomial and community patients.

The conclusion of this research findings are that there is high number of nosocomial patient than the community patient and regarding the gender females were present in greater number than males. Similarly, out of total samples very large samples were from adult patient i.e. of ≥16 years and very small number were of children of <16 years. The overall drug of choice was Carbapenems after which 2nd drug of choice was amikacin and nitrofurantoin but the empirically used antibiotics such as fluroquinolones and cotrimoxazole were poor in their action. From the susceptibility profile between genders, males were significantly more resistant ($p<0.05$) than females except in the case of norfloxacin where $p \geq 0.05$.

In the same way, there was no significant difference in the résistance of antibiotic except for amoxicillin and meropenem in case of children and adult whereas for community and nosocomial patient, isolates from nosocomial patient showed significantly high resistance pattern than that of community patient. This indicates that the antibiotics commonly used for the treatment of nosocomial UTI's are less effective. From the analysis of MDR strains it was quite alarming to note that more than 70% of the isolates were found resistant to 3 or more than 3 different classes of antibiotics. The distribution of MDR strains between genders, types of patient and between adult and children was found to be statistically significant ($p<0.05$).

Antibiotic resistance is becoming a big problem for public health which threatens the lives of hospitalized patient as well as community patient. Therefore a formulation of strict antibiotic prescription policy is an important issue to be addressed by the policy makers in all country. Moreover, this study concludes that the carbapenems, cefoperazone sulbactam, amikacin and nitrofurantoin were the most effective drugs to *E. coli* compared to the antibiotics tested which therefore may be the drugs of choice for the treatment of UTI by *E. coli* in our region.

CHAPTER-VII

7. Summary and recommendations

7.1 Summary

1. Out of 555 *E. coli* isolates obtained from urine samples, 209 (37.66%) were from males and 346(62.34%) were of females. Similarly, out of total, 143(25.6%) were of nosocomial origin and 413 (74.4%) were of community origin.
2. Among nosocomial patients 90 were male and 52 were female and from community patient, 119 were males and 294 were females.
3. The distribution of adult and children patient among nosocomial and community groups showed that 11 isolates were of children and 131were of adults among nosocomial isolates. Similarly, 27 were of children and 386 were of adults among community isolates.
4. The Antibiogram of the total isolates towards 16 antibiotic showed that the most effective drug was imipenem (19.3%) and meropenem (90.1%) followed by cefoperazone sulbactam(84.3%) where as amoxicillin and nalidixic acid (which were 77.4% and 71.4% resistant respectively) were found to be the least effective drug.
5. In gender wise distribution, carbapenems were the most effective drug while amoxicillin was the least effective drug in both male and female, the resistance in male was statistically higher than that in females except for norfloxacin.
6. The two different group of children and adult showed variation in antibiotic susceptibility although carbapenems were the most effective drug in both cases, the isolates from children were 100% susceptible to carbapenems where as that from adult were approximately 90% susceptible. In the same way, amoxicillin was the least active drug in both the cases from the statistical point of view, these was significant difference in the antibiotic resistance to all the antibiotics except for cephalexin, cotrimoxazole and ceftriaxzone where the association was insignificant.

7. The antibiotic resistances in nosocomial isolates were significantly greater than that in community isolates except for meropenem. High resistance percentage of more than 80% was seen in most of the antibiotics in case of nosocomial patients.
8. The prevalence of MDR strains was found to be more than 70%.approximately 9%, 10%, &10% of the strains were resistant to 0 drug 1 drug and 2 drugs respectively.
9. The distribution of MDR strains was found to be significantly higher in males (84.2%) than in females (61.6%).
10. High prevalence of MDR *E. coli* was seen in nosocomial patient (91.5%) than that in community patient (62.7%) which was statistically significant.
11. The MDR isolates in children (86.8%) were significantly greater than that in adult (68.85%).
12. Among the total MDR *E. coli* 91.7% were resistant to nalidixic acid followed by amoxicillin (96.4%) , cephalexin (88.2%) and so on .The MDR isolates were again least resistant to imipenem, meropenem and cefoperazone sulbactam.

7.2 Recommendations

1. Continued surveillance of resistance rates among uropathogens is needed to ensure appropriate recommendations for the treatment of infections.
2. Strict rules and regulations of antibiotic policy should be established in our country to check selling of antibiotics without prescription, and thus check the development of resistance to some extent.
3. The present findings are the suggestive of need of periodic monitoring of antibiotic susceptibility pattern to provide effective treatment and thereby to make it more cost effective particularly in the impoverished countries like ours and elsewhere.
4. *E. coli* was found to be most sensitive towards imipenem, meropenem and cefoperazone sulbactam compared to other antibiotic tested and therefore these drugs may be the drug of choice for the treatment of UTI in our region.

REFRENCES

Adukauskiene D, Kinderyte A, Tarasevicius R, Vitkauskiene A.(2006) Etiology, risk factor and out come of urinary tract infection, *Medicina (Kaunas)* 42(10):805-9

Akram M, Shahid M, Khan UA (2007) Etiology and antibiotic resistance pattern of community-acquired urinary tract infections in JNMC Hospital Aligarh, India. *Ann Clin Microbiol Antimicrob* 6:4

Arslan H, Azap OK, Ergonul O and Timuskaynat F (2005)Risk factors for ciprofloxacin resistance among E coli strains isolated from community acquired UTI in Turkey. *J Antimicrobe Chemother*; 56(5): 914-18

Aypak C, Altunsoy A, Duzgun N (2009) Emperic antibiotic therapy in acute uncomplicated urinary tract infections and fluoroquinolone resistance: a prospective observational study. *Ann Clin Microbiol Antimicrobe*; 8:27.

Barker KF (1999) Antibiotics resistance a current perspective. Blackwell science ltd Br . *J Clin Pharmacol*, 48, 109-124

Baron EJ, Peterson LR, Finegold SM (1990) Bailey and Scotts, Diagnostic Microbiology, 9th edition, *The C.V. Mosby Company*

Bean CD, Krahe D and Wareham WD (2008) Antimicrobial resistance in community and nosocomial *Escherichia coli* urinary tract isolates, London 2005-2006. *Ann Clin Microbiol Antimicrobe;* 7:13

Block B (1986) Urinary Tract Infections. *American Family Physician*; 33 (6): 172-85.

Blondeau JM (2008) Current issues in the management of UTIs: extended release ciprofloxacin as a novel treatment option. *Drugs 64* (6): 611-628.

Brooks GF, Butel JS and Morse SA (2004) Jawetz, Melnick and Adelberg's Medical Microbiology, 23rd ed. McGraw Hill, USA.

BSAC (1998) Standardized disc sensitivity tesing method.

Chakraborty P (2001) A Textbook of Micobiology, 1st ed. New Central book Agency (P) ltd, Calcutta.

Chamberlain NR (2002) Urinary Tract Infections (Urethritis, Cystitis, Pyelonephritis). *Kirksvile College of Osteopathic Medicine*.

Chattopadhya AK and Mandal AK (1993) Microbes associated with urinary tract infection in Burdwan, West Bengal. *Ind J Physiology and Allied Sciences*; 47(2): 67-72

Cheesbrough M (1984) Medical Laboratory Manual for Tropical Countries Microbiology, 1st ESBL ed. Cambridge University Press.

Cheesbrough M (2000) District Laboratory Practice in Tropical Countries. Cambridge University Press.

Chhetri PK, Rai SK, Pathak UN, Thapa JB, Devkota KC, Shrestha BO and Shrestha RR (2003) Retrospective study on urinary tract infection at Nepal Medical College Teaching Hospital, Kathmandu. *Nep Med College* J 3:83-85

Chung A, Arianayagam M, Rashid P (2008) Bacterial cystitis in women, *Amfam Physician* 39(5) 295-8

Colgan R, Johnson RJ, Kuskowski M, Gupta K (2008) Risk factors for Trimethoprim-Sulfamethoxazole Resistance in patients with acute uncomplicated cystitis. *J Antimicrobe Chemother*; p 846-851.

Collee JE, Fraser AG, Marmion BP, Simmons A (1996) Mackie and Mc Cartney, Practical Medical Microbiology, *Churchill Livingstone of Pearson Professional Limited*, 14th edition.

Corman LTCLI, Foshee LCWS, Kotchmer MGS, Harbison MRW (1982) Simplified Urinary Microscopy to Detect Significant Bacteriuria. *J Pediatr* 70: 133-135

Dagan R, Powell KR, Hall CB, Menegus MA (1985) Identification of infants unlikely to have serious bacterial infection although hopitalaized for suspected sepsis. *J Pediatr;* 107 :855-60.

Das RN, Chandrashekhar TS, Joshi HS, Gurung M, Shrestha N, Shivananda PG (2006) Frequency and susceptibility profile of pathogens causing urinary tract infections at tertiary care hospital in western Nepal. *Singa Med J* 47(4): 281-5

Datta SK (2003) A study of Antimicrobial activity on *E. coli* isolated from urine samples. *J Ins Med* 5:17-18.

Delzell JE, Lefevre ML (2000) Urinary Tract Infections During Pregnancy. Amfam *Physician* 61: 713-19.

Dhakal BK (1999) A prospective study of urinary tract infection based on culture and direct microscopy of urine along with the antibiotic sensitivity test of urinary pathogen. A dissertation submitted to central department of Microbiology, T.U. Kathmandu.

Dhakal BK, Pokhrel BM, Ahnn J (2002) Microscopic detection of Urinary tract infection in Nepalese patients. *The journal of microbiology* 4: 267-73

DoHS, Annual report of 2055/56 Department of health service HMG Nepal, 2000.

Emmerson AM, Jones MA (2001) The quinolones: decades of development and use. *J Antimicrob Chemother* 51: 13-20.

Erb A, Sturmer T, Marre R, Brenner H (2007) Prevalence of antibiotic resistance in Escherchia coli: Overview of geographical, temporal, and methodological variations. *Eur J Clin Microbial Infect Dis* 26:83-90

Fahimzad A, Taherian M, Dalivani R, Shamshiri (2010) A Daiper Type as a risk factor in Urinary tract infection in childrens. *Iran J Pead*; 1: 97-100

Forbes BA, Sahm DF and Weissfeld AS (2002) Bailey and Scott's Diagnostic Microbiology, 11th ed. Mosby Inc, USA.

Foxman B and Frerichs RR (1985), Epidemiology of urinary tract infection:Diapharagm use and sexual intercourse. *Am J Public Health*; 75(11):1308-1313

Foxman B, Barlow R *et al.* (2000) Urinary tract infection: Self reported incidence and associated costs, *Ann Epidemiol* 10: 509-515

Foxman B (2002) Epidemiology of urinary tract infections: incidence, morbidity and economic costs. *Am J Med*; 113 (Suppl. 1A):5-13

Ghimire P and Sharma AP (1995) Prevalence of bacteriuria and UTI in pregnant women of Nepal *J Nep Med Assoc;* 33: 232-5

Gobernado M, Valdes L, Alos IJ (2007) Antimicrobial susceptibility of clinical Escherichia coli isolates from uncomplicated cystitis in women over a 1 year period in Spain. *Rev Esp Quimioterap, Enero*; 20:68-76.

Godkar PB (2001) Textbook of Medical Laboratory Technology, 1st ed. Balani publishing House, Mumbai, India.

Greenwood D, Slack R and Peutherer J (2001) Medical Microbiology, A guide to Microbial Infections: Pathogenesis, Immunity, Laboratory Diagnosis and Control, 15th ed. Churchill Livingstone, USA .

Guentzel, M.N., (1991) Escherichia, Klebsiella, Enterobacter, Serratia, Citrobacter and Proteus. In: Baron, S.(Ed), Medical microbiology (3rd ed.). Churchill livingstone, Newyork. pp :377-388.

Guidoni EB, Berezin EN, Nigro S, Santiago NA, Benini V, Toporovski J (2008) Antibiotic resistance patterns of pediatric community acquired urinary infections. *Braz J Infect Dis*; 12(4):321-3.

Gupta K, Schloles D and StammWE (1999) Increasing prevalence of antimicrobial resistance among uropathogens causing acute uncomplicated cystitis in women. *JAMA*; 281: 736-738.

Hazelett SE, Tsai M, Gareri M, Allen K (2006) The association between indwelling urinary catheter use in the elderly and urinary tract infection in acute care. *BMC Geriatr*; 12; 6:15.

Huland H, Busch R (1984) Pyelonephritic scarring in 213 patients with upper and lower urinary tract infections: long-term followup**.** *J Urol;* 132(5): 936-9**.**

Jaskeiwicz JA, Mc Carthy CA, Richardson AC (1994) Febrile infants at low risk for serious bacterial infection- An appraisal of the Rochester criteria and implications for management. Febrile infants collaborative study group. *J Pediatr;* 94:390-6

Jha N, Bapat SK (2005) A study of sensitivity and resistance of pathogenic microorganisms causing UTI in Kathmandu valley. *Kathmandu University Medical Journal;* Vol 3, No 2, Issue 10, 123-129.

Jha R, Sijapati K, Pant AD (2007) Urine culture pattern in norvic international hospital. *Journal of Institute of Medicine*; 29:3 40-43.

Kariuki S, Revathi G, Corkill J, Kiiru J and Mwituria J (2007) *Escherichia coli* from community acquired urinary tract infections resistant to fluoroquinolones and extended spectrum beta lactams. *J infect developing countries*; 1(3): 257-262.

Karki A, Tiwari BR, Pradhan SB (2004) Study of bacteria isolated from urinary tract infection and their sensitivity pattern. *J Nep Med Assoc*; 43: 200-203.

Karlowsky JA, Kelly LJ, Thornsberry C, Jones ME and Sahm DF (2002) Trends in antimicrobial resistance among urinary tract infection isolates of *E. coli* from female outpatients in the United States *J Antimicrob Chemother*; 46(8):2540-45.

Korzeniowski OM (1991) Urinary tract infection in the impaired host. *Med Clin North Am*.;75(2):391-395

Kumari N, Ghimire G, Magar JK, Mohapatra TM and Rai A (2005) Antibiogram of isolates from UTI cases in eastern part of Nepal. *Nepal Med Coll J*; 7:116-118.

Kwan WC, Onyett H (2008) Community acquired urinary tract pathogens and their resistance patterns in hospitalized children in southeastern Ontario between 2002 and 2006. *Paediatr child health* Vol 13 No 9

Lambert HJ, Coulthard MG (2002) The Child with Urinary Tract Infection. Clinical Pediatric Nephrology. 3rd edition, *Oxford University Press*.

Lau MS, Peng YM, Chang YF (2004) Resistance rates to commonly used antibicrobials among pathogens of both bacteremic and non-bacteremic community-acquired urinary tract infections. *J microbial Immunol Infect*; 37; 185-191.

Lobel B, Valot A, Cattoir V, Lemenand O, Gaillot O (2008) Comparison of antimicrobial susceptibility of 1,217 Escherichia coli isolates from women with hospital and community-acquired urinary tract infections. *Presse Med*; 37:746-750

Malla KK, Sharma MS, Malla T, Thapalia A (2007) Clinical Profile, Bacterial isolates and antibiotic susceptibility patterns in urinary tract infection in children- Hospital based study. *J. Nepal Paediatr. 28(2)52-61*

Manandhar S (1996) Microbiology of urinary tract infection:A hospital based study, A dissertation submitted to central department of Microbiology, T.U. Kathmandu.

Manandhar T (2005) Antibiotic susceptibility profile of bacterial pathogens in urinary tract infection with special reference to extended spectrum beta lactamase (ESBL) producing strains, A dissertation submitted to central department of Microbiology, T.U. Kathmandu.

Manges AR, Johnson JR, Foxman B, Obryan TT, Fullerton KE, Riley LW (2001) Widespread distribution of urinary tract infections caused by a multidrug resistant *Escherichia coli* clonal group. *N Engl J Med;* 345(14):1007-13.

Mashouf YR, Babalhavaeji H, Yousef J (2009) Urinary tract infections: Bacteriology and Antibiotic Resistance Patterns. *Short communication Indian pediatrics* .

Matsubara N, Minami S, Matsuhashi M, Takaoka M, Mitsuhashi S (1980) Affinity of cefoperazone for Penicillin binding proteins. *J Antimicrobe Chemother;* 18 (1):195-199.

Mazzulli T (2002) Resistance trends in urinary tract pathogens and impact on management. *J Urol*; 168: 1720-2.

Miller GL, Tang WA (2004) Treatment of uncomplicated urinary tract infections in an Era of increasing antimicrobial resistance. *Mayo clin proc* ; 79(8): 1048-1054.

National Committee for Clinical Laboratory standards dilution procedures for susceptibility testing of aerobic bacteria. Approved standards NCCLS publication M 7-A3. Villanova, PA. NCCLS 1993

NCCLS GP-16A2, Volume 21, No. 19, Urinalysis and Collection, Transportation, and Preservation of Urine Specimens; Approved Guideline-Second Edition, p. 4-21.

Nicolle LE (2001) Epidemiology of urinary tract infections. *Infect Med*;18:153-162

Okeke NI, Fayinka TS, Lamikanra A (2000) Antibiotic resistance in *Escherichia coli* form Nigerian students, *Emerg Infect Dis* 6(4): 393-5

Olson PR, Harrell JH, Kaye SK (2009) Antibiotic resistance in urinary isolates of *Escherichia coli* from college women with urinary tract infections. *J Antimicrobe Chemother;* 53(3): 1285-1286.

Pokharel BM (2004) A hand book of clinical bacteriology, 1st ed. Gorakhnath desktop and printers, Katmandu.

Rafique S, Mehmood A, Qayyum M and Qazibash AA (2002) Prevalence patterns of community based nosocomial urinary tract infection caused by Escherichia coli. *Pakistan J Biol Sci* 5(4): 494-496.

Rai CK, Pokharel BM and Sharma AP (2001) A prospective study on antibiotic sensitivity profile of the organisms associated with clinical infections among the patients attending TUTH. *J Nep Med Assoc*; 3:13-16.

Rai GK, Upreti HC, Rai SK, Shah KP and Shrestha RM (2008) Causative agents of urinary tract infections in children and their antibiotic sensitivity pattern: a hospital based study. *Nepal Med coll J*; 10(2): 86-90.

Rajbhandari R and Shrestha J (2002) Bacteriological Study of urinary tract infection and its antibiotic sensitivity test (Hospital based study). *J Nepal Med Assoc* 4(4):26-32.

Ronald AR, Pattulo MS(1991) The natural history of urinary infections in adults. *Med clin North Am*; 75:299-312

Sahm DF, Thornsberry C, Mayfield DC, Jones ME and Karlowsky JA (2001) Multidrug-Resistant Urinary Tract Isolates of *E. coli*: Prevalence and Patient Demographics in the United States in. *J Antimicrob Chemother;* 45:1402-1406.

Santo E, Mendoca M, Salvador and Marin MJ (2007) Multidrug resistant Urinary tract isolates of Eschericha coli from Ribeirao Preto, Sao Paulo, Brazil. *The Braz J Infect Dis*; 11(6):575-578.

Shrestha P (2007) Study of bacteria causing urinary tract infection and their antimicrobial resistance trend at National public health Laboratory. A dissertation submitted to central department of Microbiology, T.U. Kathmandu.

Smith GR and Charles SF Easmon Ed (1990) Principles of Bacteriology Virology and Immunity, 8^{th} edition, volume 3, *Edward Arnold Publication*, UK.

Stamm WE, Norrby RS (2001) Urinary tract infections: Disease panorama and challenges. J Infect Dis; 183: 1-4

Stamm WE, Hooton, TM (1993) Management of urinary tract infections in adults. *N Engl J Med*; 329: 1328-1334

Svanborg C (1993) Resistance to Urinary Tract Infection. *N Engl J Med*; 329: 802-803.

Tagajdid MR, Boumhil L, Iken M, Adnaoui M, Benouda A (2010) Resisance to fluoroquinolones and third generation cephalosporin of *Escherchia coli* isolated from urines. *Med Mal Infec.***;** 40(2):70-3.

Todar K (2002) Todar's Online text book of bacteriology Cited: http://www.textbook of bacteriology.net

Tseng MH, Lin WJ, Teng CS, Chu ML, Wang CC (2008) Changing trend in antimicrobial resistance of pediatric uropathogens in Taiwan. *Pediatr Int;* 50(6):797-800.

Tuladhar NR (2001) A Report: Surveillance of Multiple Drug Resistant (MDR) bacterial infection among the patients attending to different Out Patient Departments (OPD) and Hospitalized Patients in Tribhuvan University Teaching Hospital.

Yengkokpam C, Ingudam D, Yengkokpam IS and Jha BK (2009) Antibiotic susceptibility pattern os urinary isolates in Imphal(Manipur) India; *Nep Med Collage J:*9(3):170-2

Yilmaz N, Agus Neval, Yurtsever GS, Pullukcu H, Gulay Z, Coskuner A, Kose S, Aydemir S, Gulenc N and Ozgenc O (2009) Prevalence and antimicrobial susceptibility of Escherichia coli in outpatient urinary isolates in Izmir, Turkey. *Med Sci Monit*; 159(11): 61-65.

Zorc JJ, Kiddoo DA, Shaw KN (2005) Diagnosis and management of pediatric urinary tract infection. Clin Microbio Rev: 18(2): 417-422